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Research Article

Sustainable and Reusable Gelatin-Based Hydrogel “Jelly Ice Cubes” as Food Coolant. I: Feasibilities and Challenges

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ABSTRACT: Microbial cross-contamination caused by meltwater has been a serious food safety and quality concern. A new type of food coolant, “jelly ice cubes” (JICs), based on gelatin hydrogels, was designed and tested in this study to diminish meltwater while inheriting the high cooling efficiency of traditional ice. The new zero-plastic JICs can prevent meltwater-caused cross-contamination with high affordability, recyclability, sustainability, and biodegradability. JICs based on 10% gelatin hydrogels achieved 265.35 J/g latent heat of fusion and cooling efficiency comparable with traditional ice. JICs survived the normal pressure equivalent to a food load as tall as 1 m on top throughout the repeated freeze−thaw cycles. After each freeze−thaw cycle, JICs could be effectively rehydrated, cleaned, or sanitized with brief water or diluted bleach rinse. The application of JICs can potentially reduce water consumption in the food supply chain and food waste by controlling microbial contaminations. Since the feasibility was proven, future expectations for JICs in improved stability and heat-absorbing ability were proposed.

KEYWORDS: *Cross-contamination, Food safety and quality, Freeze−thaw cycles, Freezable water, Latent heat of fusion*

■ INTRODUCTION

Temperature abuse is one of the critical reasons that causes deficiencies in food safety and food quality.[1] Perishable food requires strict cooling conditions to prevent the rapid growth of pathogenic organisms.[2] According to the United States Department of Agriculture (USDA), cold food, cooked or uncooked, should be stored at temperatures below 4.4 °C (40 °F) to avoid the food safety “danger zone” (40−140 °F).[3] Unfortunately, unexpected temperature abuses frequently occur at retailers’ and customers’ levels. The temperature abuses accelerate bacteria growth and spoilage of foods, ultimately resulting in increased health concerns and food waste.[4−7] Currently, to control temperature, traditional ice in different shapes (slabs, cubes, or slurries) is the most approachable and common cooling media for customers and retailers. The general preference toward ice is due to its affordability, cooling efficiency, and convenience of use. The high cooling efficiency of ice comes from its considerable heat capacity and latent enthalpy. However, the meltwater of ice has drawn concerns over food cross-contamination. As shown in Figure 1a, a small piece of bacterial-contaminated food may cause the contamination of a widespread area through meltwater. There have been some efforts with the substitution of traditional ice with antimicrobial ice cubes.[8−11] Acidic electrolyzed water (AEW) or essential oil has been used as the antimicrobial agent in the traditional ice. Antibacterial effects were shown with controlled bacterial levels in the preserved food. However, concerns arise about the toxicity and impact on food flavors of these chemicals. Commercially available icepacks are also widely used and available in the market. However, the thick plastic shell decreases the cooling efficiency and introduces environmental burdens. Thus, the demand is high for efficient, reusable, and safe coolant substitutes for ice.

Here, we propose a new type of “jelly ice cubes” (JICs) to replace the traditional ice as food coolants. The ideal model of JICs is shown in Figure 1b. Critical features of the proposed JICs include no meltwater, reduced cross-contamination, hypo-allergy, zero plastic, affordability, recyclability, sustainability, and biodegradability.

In a hydrogel, water, as the solvent in the precursor solution systems, is associated in different ways with matrix materials through sol−gel transformation and various forms of free water, freezable bound water, and nonfreezable bound water.[12−14] Bound water is formed through the H-bonding between water molecules and the gelatin hydrophilic sites, including amino acid side groups, carboxyl groups on the C-terminus, amino groups on the N-terminus, and peptide bonds.

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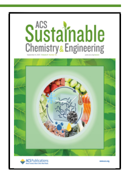

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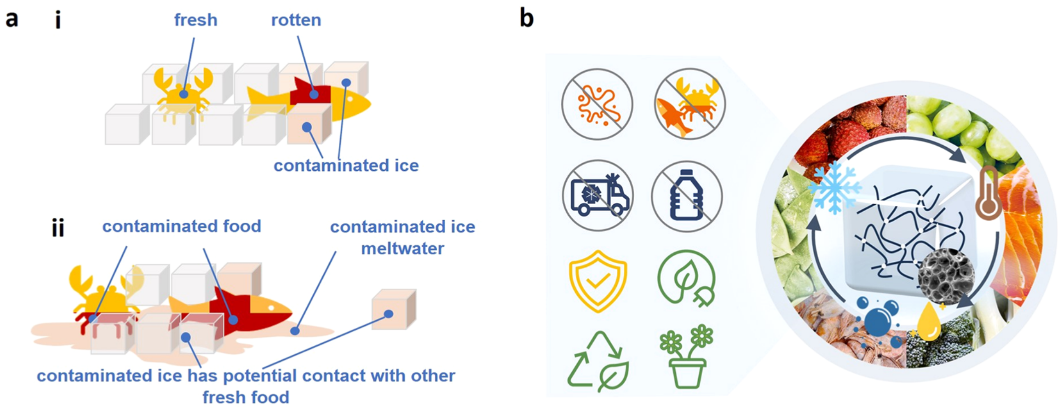

Figure 1. (a) Schematic drawings of the cross-contamination caused by ice meltwater: (i) rotten foods in red contaminate the surrounding ice (into orange color); (ii) the contaminated ice melt, and the meltwater contaminates the rest of the food. (b) The schematic structure, potential using cycles, and characteristics of JICs.

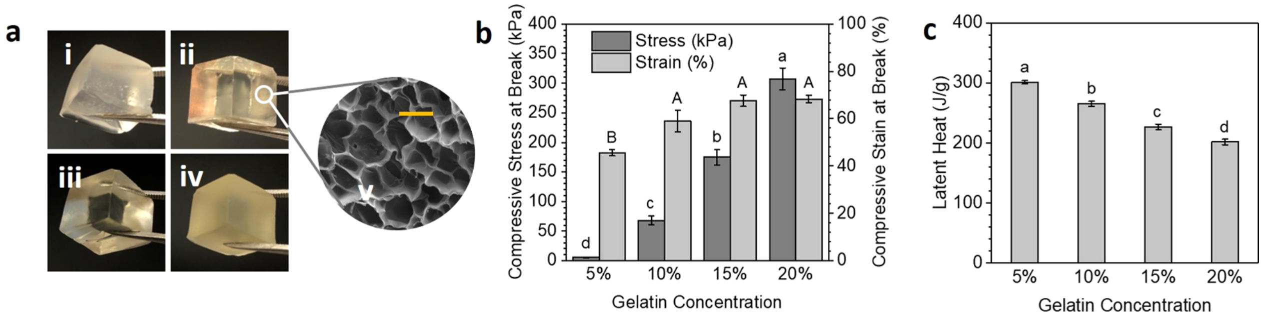

Figure 2. (a) Appearances of hydrogels with 5% (i), 10% (ii), 15% (iii), and 20% (iv) gelatin under room temperature. (v) SEM image of lyophilized JICs (10% gelatin). (b) Static compressive stresses and strains at the break of hydrogels with different gelatin concentrations. (c) Latent heat of prepared hydrogels with various gelatin concentrations. Data are expressed as mean ± SD of at least three replicates. Means with different letters on the bars (A−B or a−d) differ significantly at the $p \leq 0.05$ level.

The freezable bound water has weaker bounding toward the matrix structure through H-bonds compared to the non-freezable bound water. Free water and freezable bound water, undergoing phase change at or slightly below 0 °C in the freeze−thaw cycles, are referred together as freezable water in the later discussions of this study. The thermal behavior of hydrogels is critical to the feasibility of applying hydrogels as coolants. Around the food coolant functioning temperature range, −20 to 4 °C, ice formed by freezable water is the principal heat-absorbing component in hydrogels due to its ultrahigh latent heat (334 J/g near 0 °C) and high heat capacity in a broad range. Moreover, the latent heat of free water and freezable bound water in a hydrogel is predominant over the heat absorbed by ice without phase change. The dominating factor in designing JICs is to increase the ratio of freezable water composition in the total water content.

Gelatin has been widely used as the matrix material of hydrogels because of its high hydrophilicity. The generic advantages, including sustainability, biodegradability, and high biocompatibility, made gelatin hydrogels great candidates in biomedical materials and tissue engineering.[15−18] However, no study has been conducted to apply protein-based hydrogels as food cooling media. In this paper, the feasibilities and challenges of applying gelatin-based hydrogels as a new type of food cooling media, JICs, are tested and discussed.

■ RESULTS AND DISCUSSION

Necessary Gelatin Concentration in JICs. The gelatin-water ratio in JICs was the first factor to be determined. Two critical evaluating factors should be considered throughout the feasibility analysis of JICs − freezable water content and mechanical strength. Freezable water content should be as high as possible for promising heat-absorbing ability, which requires high total water content. Meanwhile, the mechanical strength of JICs is contributed by the gelatin matrix structures and should be strong enough to bear the pressure from food loads without use of any plastic shell. As shown in the Supporting Information, Figure S1a, if JIC survives a normal pressure of 10 kPa, it will not be crushed by a food load as tall as 1 m. Hence, desired JICs should be made of an appropriate gelatin content that provides a compressive strength higher than 10 kPa with the highest possible water content. The hydrogels made of 5% (i), 10% (ii), 15% (iii), and 20% (iv) gelatin were prepared and tested under ambient conditions, as shown in Figure 2a. The 5% hydrogel was very soft and easy to be peeled off by sharp edges, whereas the hydrogels with 10%, 15%, and 20% gelatin were firm and could be readily released from the silicon mold. As shown in Figure 2b and Figure S1b, 10% gelatin hydrogels demonstrated an average compressive stress at break of 67.95 kPa with a strain of 58.95%, which was higher than the required 10 kPa with allowed redundancy for possible strength losses of the materials during repeated using cycles, making 10% gelatin hydrogels a good base material of JICs. For the rest of the discussion, the hydrogels prepared, tested, and

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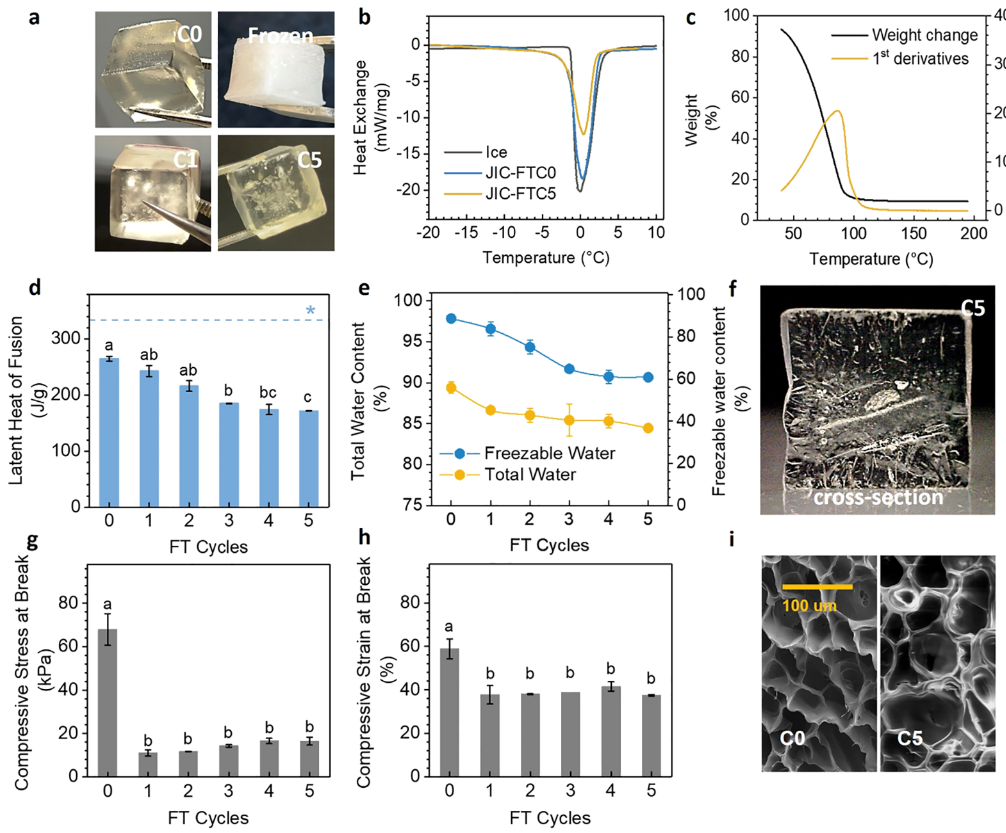

Figure 3. (a) Appearances of gelatin hydrogel JICs under ambient conditions (C0, no FT treatments; C1, after one FTC; C5, after five FTCs) and frozen status. (b) The representative DSC curve of traditional ice, JIC at FTC0 and FTC 5. (c) The representative TGA curve (JIC-FTC0) and its first derivative. (d) The change of latent heat of the fusion of JICs after up to five FTCs. The dashed line with a star mark is the fusion heat of traditional ice. (e) The change of total water content and freezable water content in JICs after FT treatments. (f) The inner cavities caused by FT treatments (photo by Dino-Lite microscope). The static compressive stress at break (g) and strain at break (h) of prepared JICs after up to five FTCs. (i) The SEM image of lyophilized JIC specimens, with a scale bar of 100 μm. Data are expressed as mean ± SD of at least three replicates. Means with different letters on the bars (a - c) differ significantly at the $p \leq 0.05$ level.

analyzed were JICs based on 10% gelatin hydrogels. Figure 2c confirms that the higher water content ensures higher heat absorbing abilities. The average latent heat of fusion of 10% gelatin hydrogels was 265.4 J/g, 79.3% of the latent heat of traditional ice. Although it may require a larger amount of JICs to achieve the cooling effect equivalent to traditional ice, with multiple using cycles, the use of JICs can still reduce the overall water usage and significantly increase food safety by reducing cross-contamination caused by ice meltwater.

Properties and Reusability of JICs. The reusability of JICs was evaluated through repeated freeze−thaw (FT) treatments, as shown in Supporting Information, Figure S2. For each freeze−thaw cycle (FTC), JICs were frozen (F) at −20 °C for 18 h and thawed (T) at ambient conditions for 6 h. After each FTC, a minor amount of water formed on the surfaces of hydrogels, coming from either the condensed water or the water loss of JICs. The water was wiped dry gently with a Kimwipe. The appearances of JICs before freezing (C0), under frozen status (Frozen), after one FTC (C1), and after five FTCs (C5) are presented in Figure 3a. Before FT treatment, the gelatin hydrogel was transparent and uniform, whereas under a frozen status, JICs formed a large number of opaque aggregations of ice crystals. Differential scanning calorimetry (DSC) was used to study the changes of latent heat of fusion of JICs after each FTC, shown in Figure 3b. The fusion heat near 0 °C (ice−water transition) was used to evaluate the heat-absorbing abilities of JICs since it contributes the most significant part in keeping the food below critical temperatures. To study the possible water content changes and explain the fusion heat changes in detail, JICs were analyzed with thermogravimetric analysis (TGA). As shown in Figure 3c, TGA curves accompanied by their first derivative curves were obtained. Three characteristics can be observed by combining the results of two thermal analyzing methods. First, multiple FTCs reduced the latent heat of fusion of JICs and decreased its heat-absorbing abilities. As shown in Figure 3d, the average latent heat of JICs near 0 °C was initially 265.35 J/g and gradually dropped to an average of 171.93 J/g after five FTCs. Second, from Figure 3b, the heat exchange tendency of JICs and traditional ice aligned well around 0 °C, except that the phase change of JICs started at a slightly lower temperature than traditional ice. The broad endothermic areas at lower temperatures could be contributed by the freezable bound water in hydrogels.[12] Interestingly, by comparing the thawing curves of FTC0 and FTC5 in Figure 3b, the heat absorbed in the ice-melting region reduced significantly after 5 FTCs, while the heat absorbed at the lower temperature stayed steady, which indicates that the loss of heat-absorbing ability along FTCs was mainly due to the loss of free water in hydrogel structures.[19−21] Also, a small amount of sweat appeared on the surface of JICs and at the bottom of the containers after each FTC, confirming the free water loss occurred during the FT

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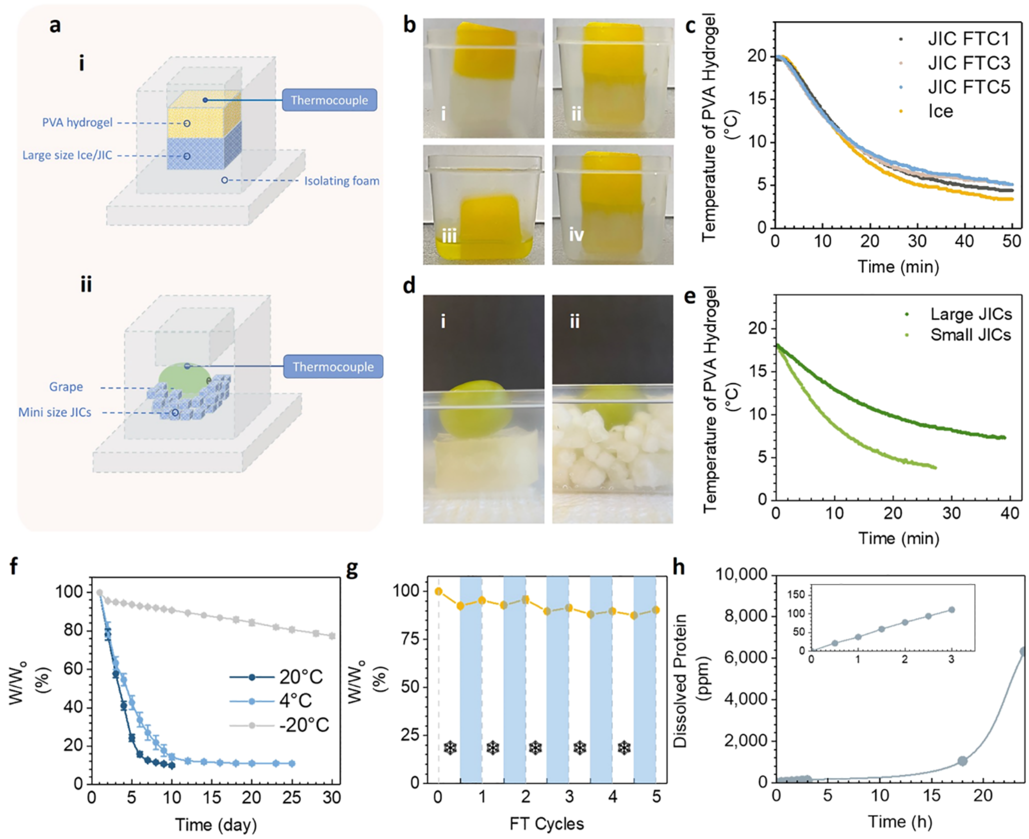

Figure 4. (a) Schematic drawings of a prepared cooling efficiency test device: (i) cooling a large PVA hydrogel cube with a large piece ice cube or JIC; (ii) cooling an irregular shape of fruit using mini size JICs. (b) Photos of using ice cubes i and iii) or JICs (ii and iv) to chill a large PVA hydrogel cube: the beginning (i, ice cube at −20 °C; iii, JIC at −20 °C) and the end (ii, ice cube melted; iv, JIC thawed) of the tests. (c) Cooling curves of PVA hydrogel cubes cooled by a large ice cube or a large JIC. (d) Photos of grapes chilled by mini-sized JICs (i) and large-sized JICs (ii). (e) The cooling curves of grape cooled by various sized JICs. (f) Water loss of JICs under 20 °C, 4 °C and −20 °C. (g) Water loss-rehydrate cycles of JICs (10 × 10 × 10 mm) along FTCs. The snow mark represents one FTC, and the blue ribbon represents a 20 min rehydration in 10 mL of DI water at ambient conditions. (h) The loss of proteins in JIC when immersing and shaking in water. Data are expressed as mean ± SD of at least three replicates.

processes.[10,22−24] Third, in Figure 3c, it is fair to consider the weight loss from ambient conditions to 110 °C represented the total water content in JICs since the dominant peak of the first derivative curve ends at 110 °C.[25] The overall water loss and the change in freezable water content were calculated accordingly, shown in Figure 3e. Consistent with the earlier discussion, the average total water content decreased from 89.38% to 84.46% after five FTCs. The average freezable water content, defined as the ratio of freezable water over the total water content, decreased from 88.76% to 60.86%, which indicates the increasing ratio of nonfreezable bound water to freezable water during repeating FTCs, agreeing with the results reported by Liu et al.[19] When the freezable water formed ice crystals during the freezing step, the polymer chains were packed and oriented due to the growth and expansion of ice, which may have caused increased H-bond cross-linking of the polymer chains. Once thawed, the physical cross-linking points could form H-bonding with water molecules and change the free water-bound water content ratio.

It is not surprising to see numerous cavities in the cross-section photos of JICs after five FTCs in Figure 3f. In Figure 3a, the inner structure of JICs after C1 and C5 was intensely destroyed by the ice crystals formed during the freezing process. Morelle et al.[26] studied the effect of freezing on hydrogel structures and found a large number of microsized cavities distributed throughout the specimens once frozen. Here, the mechanical properties of JICs after multiple FTCs were also tested to show the impact of freezing to the strength of JICs, as shown in Figure 3g,h. Before FT treatments, JICs had average compressive stress at break of 67.95 kPa. After one FTC, due to the generated cavities, the stress at break dropped to 11.03 kPa with a reduction in the strain. However, with more FTCs, the strength of JICs showed a slight increase, which could be attributed to the physical cross-linking effect generated by the multiple FT treatments.[21] Besides, scattered crystallites formed and started to grow into large crystals in the cavities.[19,20] The rise in the number of H-bonding increased the stiffness of the polymer chains. Figure 3i showed the SEM images of lyophilized JICs after C0 and C5. The increase in apparent cell size and unevenness of the sponge-like structures after five FTCs can be observed from the image, validating the destructive effect of ice crystals on the polymer framework during FT treatments. The oblate surface of JICs after C5 agrees with the above analysis, showing that the expansion of ice crystals exerted a laminating effect on the polymer matrix structures.

The above analyses indicate that current research still faces several challenges to make JICs more competitive to traditional ice: (1) Increase of the total freezable water content in JICs to bring the heat-absorbing ability higher and closer to traditional

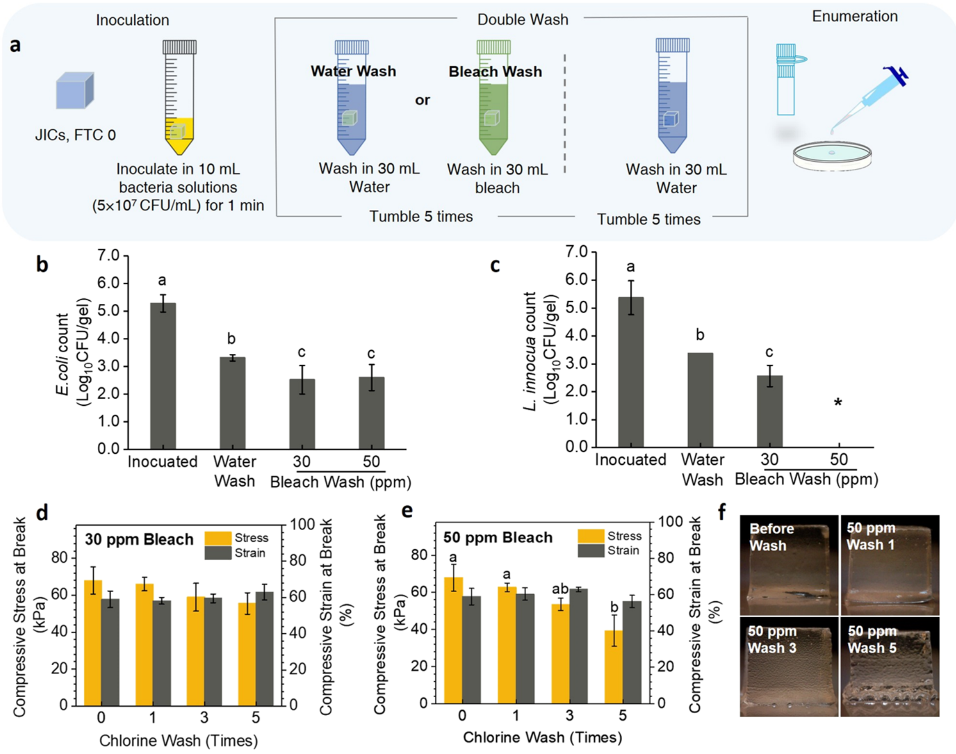

Figure 5. Surface cleaning efficiency with simple water wash or bleach wash. Schematic drawings of bacterial inoculation and surface washing steps, (a). Bacterial (b: *E. coli*; c: *L. innocua*) concentrations on the surface of JICs after inoculation or after water or bleach wash. Star mark means that the bacterial concentration was below the detection limit of 2.0 log CFU/gel. The compressive properties of JICs after one to five times of bleach wash: (d), 35 ppm bleach; (e), 50 ppm bleach. (f) Images of JICs before and after 50 ppm bleach wash. Data are expressed as mean ± SD of at least three replicates. Means with different letters on the bars (a−c) differ significantly at the $p \leq 0.05$ level.

ice; (2) improvement of the reusability of JICs by stabilizing matrix-water interactions to prevent the loss of freezable water content; (3) the further strengthening of the hydrogel matrix structures to reduce the destructive impact of temperature variations and ice crystal formations, which may be achieved by chemically cross-linking the hydrogel matrix.

Cooling Efficiencies and Water Retention. As shown in Figure 4a,i, a testing device was prepared to mimic the practical situation and compare the overall cooling performance of JICs with traditional ice. The thermal insulation well is made of Styrofoam with a top cover designed to reduce air circulation and heat exchange between inner and outer environments. Inside the well, PVA hydrogels (30 × 30 × 20 mm) photo-cross-linked by excessive amount of riboflavin 5′-phosphate sodium (RBPS) were used to simulate a piece of food to be chilled, and the RBPS, used as a photosensitizer to cross-link proteins, also served as a yellow colorant to simulate the microorganisms on food. The migration of yellow colorants showed the possible cross-contamination during the food cooling process. Underneath the PVA hydrogel, large-sized traditional ice or JICs (30 × 30 × 20 mm) served as the coolant. A thermocouple was used to record the temperature change at the top center point of the PVA hydrogel. Figure 4b shows the actual status of the inner objects at the start (i and iii) and the end point (ii and iv) of the cooling test. For (i) and (iii), the PVA hydrogel was chilled by traditional ice, whereas in (ii) and (iv), the cooling medium was JICs. The meltwater of traditional ice was conspicuous, as well as the spread of yellow colorant in water. In contrast, there was no visible water leak from JICs, none as the spread of the colorant. After the cooling test, only the contact surface of JICs with the PVA hydrogels was stained with the yellow colorant, which shows a substantial effect of controlling cross-contamination across the food surface by eliminating meltwater from ice. Figure 4c is the cooling curves obtained by the thermometer buried in the center of the top surface of PVA hydrogels. Here, to simplify the situation, we studied the cooling efficiency of different coolants by comparing the temperature changes in "food" over the cooling period. At 0 min, the PVA hydrogels were placed in contact with the cooling media. Ice, as predicted, showed the best cooling efficiency with the fastest cooling speed and the lowest ending temperature of the "food". In comparison, the cooling efficiency of JICs during FTC1, FTC3 and FTC5 was slightly lower than ice but still promising. At 50 min, the final temperature of cooling objects was 3.4 °C (cooled by ice), 4.4 °C (cooled by JIC FTC1), and 5.1 °C (cooled by JIC FTC3 and FTC5). The repeated FTCs slightly reduced the cooling ability of JICs, as discussed earlier, while the impact was acceptable and could be compensated with excessive coolants.

Compared with commercially available icepacks, besides the sustainable features with no plastic shells, another distinctive advantage of JICs is that the coolant's size can be adjusted flexibly per demand. For example, as shown in Figure 4a,ii, to fit the shape of a grape, which has no flat contacting surface with the cooling media, JICs can be customized into mini sizes (5 × 5 × 5 mm), thus increasing the contacting surface area with objects and elevating the overall cooling efficiency. An experimental setup is shown in Figure 4d. Grapes (around 9 g) at ambient conditions were placed either on top of a large chunk JIC (17.5 g) or surrounded by mini JIC pieces of the same mass. Temperatures of the central point at the upper surface of the grape were recorded with a thermocouple, and

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the cooling curves are shown in Figure 4e. The distinction in the efficiency of heat absorptions between different sizes of JICs was obvious. It took the large JIC 10 min, while it took the mini-sized JICs only 5 min to decrease the grape's temperature by 5.2 °C. To bring the grape's temperature to below 4 °C (critical temperature), it took the mini-sized JICs 26 min. In contrast, the large JIC chunk could not chill the grape to a temperature below 7.3 °C after 39 min. Reduction in the size of JICs significantly boosted their cooling efficiency. Size flexibility of JICs allows customization of coolants toward food in different shapes.

Since JICs are designed with no plastic shells, it is a significant challenge to maintain their water content for a satisfactory cooling performance. The percent weight change represents the water loss rate of JICs under various working conditions. In Figure 4f, large JIC chunks were tested under 20, 4, and −20 °C, to demonstrate dehydration performance under various working conditions over an extended period. When stored at −20 °C, JICs have 89.7% weight left after 10 days and 77.5% weight left after 30 days. At 4 °C, around 19.4% weight was lost after 1 day of storage, and 43.0% remained after the first 5 days. When stored at 20 °C, 22.9% of the weight loss was observed after the first day of storage, and 75.0% of the weight loss occurred in 5 days. As expected, without plastic shells, the dehydration of JICs was rapid, especially under ambient conditions. Evaporation of JICs' water content damages their cooling performance for the subsequent FTCs, as discussed earlier. Thus, once thawed, it is better to handle JICs with a minimum exposure time under a temperature above 0 °C. After each use, freezing temperature, i.e., −20 °C, is preferable for storage to retain water content in JICs.

As mentioned earlier, less than 8% of water might be lost during each FTC. Thus, a quick rehydration process may compensate for the water loss and maintain the performance of JICs at a satisfactory level. Figure 4g elaborates the FT−rehydration repeat using cycles of JICs. Each FTC caused around 2.5−7.5% of weight drop, and the rehydration process could restore the weight by approximately 3%. After five FT−rehydration cycles, the weight only decreased by around 10%, which means that the overall cooling performance of JICs can be mostly preserved by quick rehydration.

In a JIC piece, the stability of matrix structures constructed by proteins are also vital components for coolant performance. Since gelatin is water-soluble, protein dissolution was also studied. With the Bradford test, the concentration of protein dissolved in water was tested and plotted in Figure 4h. In the initial 3 h, the dissolved protein concentration showed a positive linear pattern. However, no visible collapse of the hydrogels, but only slight swelling was observed in the first 3 h. Within 18 h on the shaking bed, materials broke up into small irregular pieces. Also, it should be mentioned that without continuous shaking, JICs can maintain their structures in water for a much more significant amount of time. Overall, if JICs need to work in a water-rich environment, their physical stability within 3 h is reliable.

Surface Cleaning and Disinfection. As a reusable coolant, it is vital to control microorganisms present on the surface of materials to ensure food safety. The surface cleaning efficacy of JICs through a water or diluted bleach rinse was tested following the steps shown in Figure 5a, as described in Materials and Methods. Two types of bacteria, *Escherichia coli* (*E. coli*, Gram-negative) and *Listeria innocua* (*L. innocua*, Gram-positive), were tested. As shown in Figure 5b,c, a water rinse decreased the concentration of *E. coli* and *L. innocua* by 2.0 log CFU/gel. In comparison, the concentration of *E. coli* was reduced by around 2.8 log CFU/gel with the rinse of a diluted bleach solution. The difference in bacterial reductions between bleach solutions containing 30 and 50 ppm active chlorine (Cl^+) was not significant, which might be due to the contact time not being long enough to disinfect the Gram-negative bacteria. For *L. innocua,* 30 ppm of Cl^+ bleach rinse reduced the bacteria concentration by 2.8 log CFU/gel, whereas 50 ppm of Cl^+ bleach solution rinse resulted only in a reduction of 2.0 log CFU/gel. The results proved the effectiveness of surface cleaning with water or bleach rinse. It is worth noting that the water or bleach rise can also rehydrate JICs during the washing.

The oxidizing properties of chlorine solutions might cause structural damages to JICs during the cleaning process. Static compression tests were applied toward JICs after up to five cleaning cycles with bleach solutions. As shown in Figure 5d, with 30 ppm bleach solutions, the mechanical strength of JICs was not significantly damaged. After five cleaning cycles, 80% of the compressive strength was maintained. However, with 50 ppm bleach solutions, as shown in Figure 5e, the structural damage was apparent. With only three bleach rinse cycles, 20% of the strength was lost, while only half of the strength was left after five cycles. The surface of JICs was significantly damaged after five cleaning cycles with 50 ppm bleach solutions, as shown in Figure 5f and Figure S3. Chlorine bleach could convert peptide bonds in proteins to N-chloramine structures, which reduced hydrogen bond interactions of JICs. However, the chloramine structures in JICs may help the self-cleaning of the cubes.[27] From the above tests, a water or bleach rinse may be applied in practice to clean the surface of JICs after each use. Meanwhile, to build a more robust coolant, the structural stability of JICs in different environments is essential for further improvement.

■ CONCLUSIONS

The feasibility of applying renewable and environmentally friendly JICs as a new type of food coolants was proven through lab-scale tests and analysis. The cross-contamination concerns caused by meltwater from traditional ice could be reduced with the use of the novel JICs. Meanwhile, the overall cooling efficiency was maintained by the JICs at high levels with a stable solid structure and a freezable water content of over 85%. The properties of JICs were kept in a relatively stable status for at least five reusing cycles, which guarantees its sustainability and reduces the total water demand in food supply chains. After each using cycle, a brief rinse with water or diluted bleach solutions proved to be an effective way to rehydrate JICs and reduce surface bacteria concentrations. The biodegradable and plastic-free material minimizes the environmental burden. However, there are still problems and challenges left for future improvements of the JICs. There should be better control over the hydrogel matrix structures and matrix-water interactions during manufacturing and service phases. Robust hydrogel structures with higher stability against temperature variations or phase changes of water can be potentially achieved through chemical cross-linking proteins. Most importantly, the increase in freezable water content in JICs is also expected to bring their heat-absorbing ability closer to traditional ice.

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■ MATERIALS AND METHODS

Materials. Gelatin (Type A, 225 bloom food grade) was purchased from MP Biomedicals, LLC (Solon, OH). Poly(vinyl alcohol) (PVA, Mn 85000−146000, 99+%, hydrolyzed) was purchased from Sigma-Aldrich (Milwaukee, WI). Riboflavin 5′-phosphate sodium (dihydrate, RBPS) was purchased from Spectrum Chemical (Gardena, CA). Coomassie (Bradford) assay, Luria−Bertani (LB) broth, and Tryptic soy broth (TSB) were purchased from Thermo Scientific (Rockford, IL). SuperPremix Miller's Luria−Bertani (LB Agar) was purchased from U.S. Biotech Sources, LLC (Richmond, CA). Tryptic Soy Agar (TSA) was purchased from Bioworld (Dublin, OH). Bleach solution was a product of Clorox Co., Ltd. (Oakland, CA). Deionized (DI) water was used to prepare the gelatin solutions.

JICs Preparation and Freeze−thaw Treatment. Homogeneous gelatin solutions in various concentrations (5, 10, 15, 20 wt %) were prepared by dissolving gelatin in DI water in a 70 °C water bath overnight with continuous stirring. The homogeneous solutions were then settled in silicon molds (mostly in 10 × 10 × 10 mm mold unless specified) and completed the sol−gel transition under 4 °C overnight. Once ejected from the mold, JICs with 10% gelatin concentration were treated with freeze−thaw treatments to mimic their multiple usage cycles. Each freeze−thaw cycle consisted of 18 h of freezing at −20 °C and 6 h of thawing at ambient conditions (21 °C) in the air. Up to five freeze−thaw cycles (FTC0, FTC1, FTC2, FTC3, FTC4, FTC5) were performed and tested. Samples were stored at 4 °C after treatments and subject to ambient conditions before tests.

Characterization of JICs. Gelatin hydrogels at different concentrations and JICs after various FTCs were tested with an Instron 5566 tester (Norwood, MA) for the static compressive properties. Static loading cells of 10 kN, 5 kN, and 10 N were used according to the specific sample strength. Samples in size of 10 × 10 × 10 mm were tested with a rate of 10%/min. The stress and strain at break were calculated and plotted as results.

The latent heat of different gelatin-based hydrogels and JICs after various FTCs was measured using a differential scanning calorimeter (DSC-60, Shimadzu Corporation, Pleasanton, CA). The heat absorbing-releasing profiles of JICs were obtained from −30 °C, supported by liquid nitrogen, to 10 °C with a 1 °C/min heating rate under a 50 mL/min protective nitrogen flow. The latent heat of fusion around 0 °C of JICs was calculated by integrating the heat flow (W/g)/time (s) curve. Latent heat of fusion of traditional ice at 334.5 J/g was used as the reference. The freeze−thaw process during the DSC test was not counted as an FTC since both freezing and thawing conditions were different from the FT treatment defined earlier.

The water contents of JICs were tested using the thermogravimetric analysis (TGA, SDT-Q600, TA Instrument, New Castle, DE). The TGA curve's first derivative was taken to assist the identification of water loss. As shown in Figure 3d, the prominent peak of TGA curves for water ends around 110 °C. The total water loss was calculated according to equation 1, and the freezable water content was calculated according to equation 2, where w is the weight of JICs at 110 °C, w_0 is the initial weight of JICs in TGA tests, and H is the fusion heat of JICs at the specific FTC, in unit of J/g.

$$Total\ water\ content(\%) = \frac{w_0 - w}{w_0} \times 100\% \quad (1)$$

$$Freezable\ water\ content(\%) = \left(\frac{H}{334.5}\right) \div \left(\frac{w_0 - w}{w_0}\right) \times 100\% \quad (2)$$

The cross-section of JICs was observed using a Dino-Lite digital microscope (Dunwell Tech. Inc., Torrance, CA). The internal microscopic structures of lyophilized JICs were observed using a Quattro environmental scanning electron microscope (ESEM, Thermo Fisher Scientific, USA). Samples were first sliced into small pieces with disposable scalpels and lyophilized using a Benchtop K lyophilizer (VirTis, Los Angeles, CA).

The overall cooling efficiency of ice and JICs was tested with a designed device shown in Figure 4a. The isolating foams generated a 35 × 35 × 80 mm well with a flexible cap. Two types of comparisons were delivered. The first was to compare the cooling efficiency of ice and JICs. Large ice chunks or JIC chunks (30 × 30 × 20 mm) were prepared and conditioned at −20 °C. Large PVA hydrogels (15%) prepared with 0.5% RBPS were used as the cooling objects (30 × 30 × 20 mm). The PVA-RBPS hydrogel was photo-cross-linked via a Spectrolinker XL-1000 equipped with five 8 W UVA (365 nm) light tubes (Spectronics Corporation, Lumberton, NJ). The second group of comparisons was on the size effect of JICs. Mini JICs (0.1 g/piece) in a total of 15 g and a large JIC chunk (single piece, 15 g) were prepared and conditioned at −20 °C. Grapes (9.0 ± 0.5 g) were used as irregular-shaped cooling objects. In both comparisons, a thermocouple (type K, Omega Engineering Inc., Stamford, CT) with a 4-channel hand-held data logger (Omega Engineering Inc., Stamford, CT) was attached to the top surface's central point of the objects (either PVA-RBPS hydrogel or grapes) to record temperature changes from the moment the object touched the coolant.

The water-withholding ability of prepared JICs was tested by measuring the mass change of JICs along time under different temperatures. Their weight changes under 20, 4, and −20 °C were monitored with analytical balances (0.1 mg detection limit). The initial samples were around 15.000 ± 1.000 g.

The rehydration tests were conducted with cubic samples around 1 g. Weight change after each complete FTC (snow marks) or rehydration in the excess amount of water (30 mL, 20 min, with minor stirrings, blue ribbons) was tested and calculated for the weight change ratios.

Protein dissolution of JICs was tested by immersing 5 g (5 cubes) of JICs in 25 mL of DI water with continuous shaking (200 r/min) at ambient conditions. The concentration of proteins dissolved in water was tested using the Bradford micro method (working range 1 to 25 μg/mL). A 1 mL portion of diluted (1:1, 1:10 or 1:100 diluting ratio) sample was added into 1 mL of the Coomassie reagent. The absorbance signal at 595 nm under a UV spectrophotometer (Evolution 600, Thermo) was recorded to calculate the protein concentration according to the standard curved constructed with bovine serum albumin (BSA).

To characterize the surface cleaning efficiency of regular water wash or bleach (sodium hypochlorite) wash on JICs, antimicrobial assays were applied toward generic *E. coli* LJH1247 (Gram-negative) and *L. innocua* (Gram-positive). Diluted bleach solutions (30 and 50 ppm of Cl^+) were prepared from diluted Clorox without additional pH adjustment. The detailed procedure is shown in Figure 5a. A JIC piece (10 × 10 × 10 mm) was first inoculated in bacteria solutions (5×10^7 CFU/mL) for 1 min. After inoculation, the artificially contaminated JICs were washed with water or bleach. For the water rinse group, contaminated JICs were washed twice in 30 mL of DI water with five times of tumbling (gently turn the tube upside-down twice, total around 10 s). For the bleach rinse group, JICs were first washed with diluted bleach solutions (either 30 or 50 ppm of Cl^+) for five tumbles, transferred into 30 mL of DI water, and then tumbled for an additional five times to rinse off the extra chlorine residue and avoid extra contact time. The surviving bacteria on the surface of JICs were recovered by the rinsing of the "washed" JICs with 10 mL of sterile Phosphate Buffered Saline (PBS) buffer and enumerated by being plated onto plate count agar (detection limit = 2.0 log CFU/gel).

Statistical Methods. All experiments were performed at least three times, and the results are presented as mean value ± standard deviation. Statistical analyses were conducted using one-way analysis of variance (ANOVA). Mean values were compared using Tukey's test to identify significant differences between each treatment group ($P \leq 0.05$).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.1c02853.

The normal pressure produced by the food load on top of JICs, representative compressive curve of 10% gelatin hydrogel (JIC-FTC0), schematic drawings of the freeze−thaw treatment JICs, photos of JICs after five times of 50 ppm bleach wash (PDF)

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Notes

The authors declare no competing financial interest.

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