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Effect of Chitin-Glucan Hydrogel Coating on Shelf-Life of Kashar Cheese

Running title: Hydrogel Production from Fungal Chitin-Glucan

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SUMMARY

Research background. Study investigates the application of chitin-glucan-based hydrogel (CGH), obtained from *Aspergillus niger* mycelia grown in biological waste, to extend the shelf life of fresh Kashar cheese by mitigating biochemical and microbiological degradation during storage.

Experimental approach. Biological waste to be used as a medium for obtaining mycelium from *A. niger* was collected weekly for four weeks from a hotel, chitin-glucan nanofibers were produced from the mycelium using an alkaline method. The nanofibers were then freeze-thawed in an alkaline solvent system to form the hydrogel. To investigate the effect of hydrogel on the shelf life of fresh Kashar cheese, hydrogel-coated cheese samples were analyzed.

Results and conclusions. Fourier transform infrared spectroscopy (FT-IR) analysis confirmed the hydrogel's chitin-glucan composition, while scanning electron microscope (SEM) images demonstrated its successful application as a surface coating. Coating with CGH significantly increased the pH and mass loss of cheese samples compared to the control (distilled water, $p \leq 0.05$). Moisture loss rates were 8, 18 and 14 % for samples treated with water, KOH-CGH, and NaOH-CGH,

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respectively. Although the hydrogel didn't significantly inhibit mold and yeast, ($p \geq 0.05$), the KOH-CGH coating effectively reduced lactic acid bacteria (LAB) proliferation ($p \leq 0.05$), which is associated with souring defects. Additionally, peroxide value (PV) reduction in coated samples ($p \leq 0.05$) suggests improvements in oxidative stability. Hydrogel coatings also influenced the texture properties of the cheese: hardness, chewiness, adhesiveness, and cohesiveness increased, while resilience and gumminess decreased ($p \leq 0.05$). Using zero-order kinetics, the shelf life of cheese was calculated based on peroxide formation, with deterioration defined at 2 mmol O_2/kg fat. The shelf life of uncoated cheese was estimated at 155 days, whereas it extended significantly to 555 days for cheese coated with either KOH-CGH or NaOH-CGH. These findings demonstrate the hydrogels' capacity to reduce oxidative and microbial spoilage, thereby prolonging the cheese's usability.

Novelty and scientific contribution. Study highlights that CGH is sustainable, innovative edible coating with antioxidant properties, offering a promising approach for improving the quality, extending the shelf life of Kashar cheese. Future research could further optimize hydrogel formulations to enhance antimicrobial efficacy and explore their application in other high-moisture food products.

Keywords: chitin-glucan, hydrogels, edible coating, Kashar cheese, antioxidant effect

INTRODUCTION

Kashar cheese, like many dairy products, is highly susceptible to microbiological and biochemical degradation, which shortens its shelf life and results in economic losses. To mitigate these issues, edible coatings have emerged as effective solutions for extending product shelf life and maintaining quality after packaging (*1*). These coatings are typically made from biodegradable materials such as polysaccharides, lipids, and proteins, and they function by reducing mass loss, controlling oxygen and carbon dioxide permeability, and minimizing rancidity and spoilage caused by microbial growth (*2-4*). Among these, hydrogels have gained attention due to their unique physical properties. Comprising a three-dimensional porous network, hydrogels can absorb significant volume of water due to their hydrophilic composition, with water content often exceeding 90 % (*5-7*). Recent studies have demonstrated the superior efficacy of hydrogels in preventing food spoilage and enhancing shelf life compared to conventional packaging systems (*8-10*).

Chitin-glucan complex, a polysaccharide present in the cell walls of fungi, has been identified by Ordoñez *et al*. (*11*). Despite its desirable antifungal and antioxidant properties, the insolubility of chitin-glucan in most solvents has limited its direct application as a food coating. To overcome this, hydrogels can be synthesized from insoluble polysaccharides through processes such as freeze-thaw

cycles in suitable solvent systems, followed by crosslinking (*12,13).* Recent advancements have shown that alkaline solvents like NaOH and KOH, used in combination with freeze-thaw methods, are effective in producing hydrogels from chitin and its derivatives (*14,15*).

Previous studies on edible coatings have largely focused on polysaccharide-based systems such as chitosan or alginate, with well-documented antifungal and moisture-retention properties. However, the use of chitin-glucan as a coating material remains underexplored, particularly for high-moisture dairy products like Kashar cheese. Moreover, while research exists on hydrogels synthesized from chitin (*14*), the integration of chitin-glucan hydrogels for food preservation applications has not been sufficiently studied.

In addition, most studies evaluating hydrogel performance have not addressed the role of solvent systems (*e.g*. NaOH *vs*. KOH) in tailoring hydrogel properties for specific food applications. This study addresses these gaps by developing a novel hydrogel system based on chitin-glucan nanofibers derived from organic waste sources, aligning with sustainability goals, comparing the effects of different solvent systems (NaOH *vs*. KOH) on hydrogel properties, such as antifungal activity, oxidative stability, and textural impact, and testing hydrogel coatings on Kashar cheese, providing insights into their practical application in preservation.

In our previous research, chitin-glucan nanofibers with antifungal properties were extracted from *A. niger* mycelium cultured on organic waste-derived media (*16*). However, these nanofibers were found unsuitable for food surface applications due to their dense intermolecular hydrogen bonding and water insolubility. To address these limitations, this study aimed to convert water-insoluble chitin-glucan nanofibers into hydrogels with potential antifungal and antioxidant properties for food surface applications. Specifically, we investigated the effects of NaOH and KOH solvents on hydrogel production and evaluated the applicability of these hydrogels as coatings for Kashar cheese, assessing their impact on shelf life and quality preservation.

MATERIALS AND METHODS

Materials

A. niger MRC 200806 used in this study was obtained from the stock culture of Prof. Dr. Arzu Çağrı Mehmetoğlu. *A. niger* was activated in Tryptic Soy Broth (TSB) (Merck, Germany) containing 0.6 % (*m*/*V*) yeast extract (Merck, Germany) at 30 °C for 24 h. For later use, a stock culture was created and stored at -18 °C by adding 15 % glycerol (*VV*) (Sigma-Aldrich, Germany). Before the experiment, the test microorganisms were activated in Nutrient Broth (Sigma-Aldrich, Germany). The Kashar cheese was produced by a local company (Yelken Gıda, Türkiye), purchased from a local grocery store, and stored at 4 °C until use. Oxytetracyclin-Glucose-Yeast Extract Agar (OGYE)

(Merck, Germany), de Man, Rogosa and Sharpe Agar (MRS) (Merck, Germany) and Plate Count Agar (PCA) (Merck, Germany) were used for microbiological analysis of Kashar cheese samples.

Preparation of media for the growth and cultivation of A. niger from food waste

Biological waste to be used as a medium for the growth and cultivation of *A. niger* was collected weekly for four weeks from a hotel kitchen in Sakarya. The content and chemical composition of biological wastes in the medium composition are given in Table S1 and Table S2, respectively. The data obtained in our previous study was used in the preparation of the medium (*16*). For this purpose, a mixture was prepared from the groups classified according to their content because of chemical analysis in the ratios determined according to preliminary trials, water was added to this waste mixture at a ratio of 1:2 (*m*/*V*), homogenized with the help of a blender (Philips HR2695/00 5000, Türkiye) and the particles were removed by filtering through cheesecloth. The pH of the medium was adjusted to 5.00 using 1 M NaOH and 1 M HCl and the medium was stored at -18 °C until use.

Production of chitin-glucan nanofiber from A. niger

A 100 µL aliquot of culture from *A. niger* (activated at 30 °C for 72 h in TSB with 0.6 % yeast extract) was inoculated into 0.015 L of prepared waste medium and incubated at 30 °C for 96 h (the optimization results of the study pending publication were used) (*16*). At the end of the incubation period, the biomass produced by *A. niger* was dried and used for chitin extraction. In chitin-glucan nanofiber extraction, the fungal biomass was mixed with water at a ratio of 1/30 (*m*/*V*) and kept in a hot water bath (Daıhan, WiseBath WSB-30, Türkiye) at 85 °C for 30 min and then the mixture was centrifuged (Hettich Zentrifugen EBA 21, Germany) at 9000 rpm for 15 min at 4 ºC (*17*). To remove the alkali-soluble fraction, the pellet was mixed with 1/30 (*m*/*V*) of 1 M NaOH and kept in a water bath at 65 °C for 3 h and centrifuged again at 9000 rpm for 15 min. The remaining pellet formed chitin-glucan complex insoluble in alkali and was mixed with distilled water at a ratio of 1/30 (*m*/*V*) and subjected to ultrasound (Sonics, VCX750, USA) treatment for 2 min at a frequency of 20 kHz using 60 W power. The resulting suspension was centrifuged at 9000 rpm for 15 min; the precipitation was washed with distilled water and centrifuged again. The chitin-glucan nanofibers remaining in the pellet were placed in a 1/1 (*m*/*V*) solution of ethyl alcohol (96 % *V*/*V*) and dried in an oven at 60 °C for 24 h to prevent hydrogen bond formation during drying (*17,18*). The obtained chitin-glucan nanofibers were stored at 4 °C until they were used for hydrogel production and characterization experiments.

Preparation of chitin-glucan based hydrogel

Hydrogel formation was achieved by subjecting chitin-glucan nanofibers to a freeze-thaw process in an alkaline solvent system. In this study, a 2 % (*m*/*V*) concentration of chitin-glucan nanofibers was dissolved in 25 g of NaOH and KOH solutions, each prepared at a concentration of 5 mol/L, by stirring (M Tops, MS300HS, Türkiye) at 500 rpm for 1 h. The suspensions were then frozen at -20 °C for 18 h. During the dissolution process, the suspension was continuously stirred at 500 rpm at ambient temperature for 1 h. Following this, the suspension was centrifuged (20 000×*g*, 30 min at 4 °C) to separate the insoluble fraction from the dissolved components. The soluble fraction was then dialyzed against deionized water using a 12 kDa molecular mass cut-off (MMCO) membrane for 48 h at ambient temperature, with stirring at 200 rpm on a magnetic stirrer (*19*). Fourier transform infrared spectroscopy (FT-IR) (Shimadzu IRAffinity-1S, Japan) was employed to characterize the hydrogels obtained. FT-IR measurements were conducted within the wavelength range of 4000 cm^{-1} to 400 cm^{-1}, and the data analysis was performed using LabSolutions IR software (*20*).

Coating of chitin-glucan nanofiber-based hydrogels on Kashar cheese

To evaluate the suitability of the hydrogels as food coating materials, fresh Kashar cheese obtained from the local market was sliced into pieces weighing (10±2) g, with dimensions of 2 cm×2 cm×0.5 cm, under aseptic conditions. Each analysis was planned to be performed in triplicate, and cheese slices were dipped into 0.05 L of chitin-based hydrogel prepared with either KOH or NaOH for 10 seconds, then dried at room temperature for 1 h on a wire rack in a sterile cabinet. The same procedure was applied to the control samples, using an equivalent volume of sterile water. After drying, all samples were individually packaged in sterile polyamide-polyethylene bags (90 μm thick, with an oxygen permeability of 160 $cm^3/(m^2 \cdot day)$ at 23 °C and 0 % RH, and a water vapor permeability of 8.5 $g/(m^2 \cdot day)$ at 38 °C and 90 % RH) for subsequent analysis.

Analyses applied to coated Kashar cheese samples

The coated samples were examined under a scanning electron microscope (SEM) (Tescan Vega II, Czech Republic) to determine the surface morphology. The pH of the samples was measured using a digital pH meter (Mettler-Toledo Seven Compact S210, Switzerland) (*21*). The mass of the samples (Radwag - AS 220.R2, Poland) was recorded on a precision scale on day 0, 14th and 28th days of storage. Differences between *m* were calculated as % mass loss according to the equation below:

$$\text{Mass loss} = (m_i - m_s / m_i) \cdot 100 \qquad /1/$$

where m_i is initial sample mass and m_s is sample mass at the end of the 14th or 28th days.

The moisture was determined at a constant temperature of 105 ºC using the AND MX-50 (USA) moisture analyzer. The counts of yeast/mold and lactic acid bacteria in Kashar cheese samples were assessed using OGYE, MRS, and PCA media, respectively, on days 0, 14, and 28 of storage at 4 ºC (*22,23*). For the preparation of samples for microbiological analysis, samples of the Kashar cheese prepared and coated at 10 g were placed in a sterile stomacher bag and homogenized (Interscience, Bagmixer 400, France) for 2 min with the addition of 90 mL of 0.1 % peptone water (Merck, Germany). Then 1 mL was taken from the samples diluted at a 1:10 ratio and transferred to a test tube containing 9 mL of peptone water that had been sterilized beforehand. After vortexing the test tube, a decimal dilution series was prepared from the sample. In mold and yeast counts, samples were taken from prepared dilutions and inoculated using the spread plate method after adding selective OGYE (Merck, Germany). Samples were incubated at 25 °C for 2 days for mold counting and 5 days for yeast counting (*22*).

For lactic acid bacteria counting, inoculation was performed using the pour plate method with MRS (Merck, Germany) from appropriate dilutions, and plates containing colonies were counted after incubation at (42±1) °C for 48 h (*23*).

The peroxide value (PV) in the samples during the storage was determined according to the official AOCS method (*24*) and the values obtained were expressed as mmol O_2 per kg oil using the following equation:

$$PV=(V\cdot M)/m \qquad /2/$$

where V is the volume of sodium thiosulfate (L), M is sodium thiosulfate molarity (0.01 M), and m is the sample mass (g).

CT3 Texture Analyzer (Brookfield, Middleboro, MA, USA) device was used to determine texture properties of the samples. Hardness, Adhesiveness, Cohesiveness, Resilience, Gumminess Measurement was performed using a cylindrical probe (Numbered TA39, USA). The analysis measurement conditions were set at a speed of 10 mm/s, and the penetration distance was determined as 5 mm. Color values (*L*, a*, b**) of Kashar cheese samples were determined with the (Lovibond RT 300 Series Reflectance Tintometer, England) color analyzer. The color analysis calculation was carried out by measuring the inner section of the sample at five different points and taking the average of these measurements.

Shelf-life of the Kashar cheese

In shelf-life studies, determining the most suitable kinetic model—zero-order, first-order, or second order—is essential for accurate predictions. This decision is based on analyzing the raw data

in various forms. For zero-order kinetics, the data are plotted directly against time; for first-order kinetics, the logarithm of the data is plotted; and for second-order kinetics, the reciprocal of the logarithmically transformed data is used. The model with the highest regression coefficient (R^2) was selected to represent the data and used for subsequent shelf-life calculations.

The shelf life of cheese samples was calculated using the following equation:

$$\text{Shelf-life}=(PV_e–PV_0)/k \quad /3/$$

where PV_0 is the initial peroxide value (0 mmol O_2/kg fat), PV_e is the peroxide value at the onset of deterioration (of 2 mmol O_2/kg fat), and k is the kinetic constant of the reaction.

Statistical analysis

Statistical analyses were performed using Minitab statistical software v. 18.0 (*25*). The data were analyzed by analysis of variance (one-way ANOVA), and consistent variability was determined at the $p \leq 0.05$ level from Tukey's multiple comparison test. The experiment design was applied with 3 samples (control, KOH-CGH, NaOH-CGH), 3 storage times (0th, 14th, and 28th days) and 3 repetitions.

RESULTS AND DISCUSSION

FT-IR spectra of chitin-glucan-based hydrogels

FT-IR analysis was conducted to investigate the impact of NaOH and KOH solvent systems on the chemical structure of chitin-glucan-based hydrogels (CGHs). The results, shown in Fig. 1a and Fig. 1b, confirm the successful synthesis of hydrogels with characteristic features of chitin-glucan.

The FT-IR spectra of CGHs prepared in NaOH and KOH solutions exhibited broad absorption bands in the 3000–3500 cm^{-1} range, indicative of hydroxyl (-OH) stretching vibrations, which are characteristic of chitin-glucan's polysaccharide structure (*19*). A strong peak at 3375 cm^{-1} was observed for NaOH-CGH (Fig. 1a), while KOH-CGH exhibited two distinct peaks at 3375 and 3275 cm^{-1} (Fig. 1b), suggesting slight differences in the chemical environment of hydroxyl groups due to the solvent system used. Additionally, peaks at 2875–2860 cm^{-1}, attributed to C-H stretching vibrations, confirm the presence of typical chitin-glucan structures (*19*). Further, the spectra revealed β-1,3 and β-1,6 glycosidic linkages characteristic of chitin-glucan. These were represented by absorption peaks at 891, 922, 1154, 1372 and 1730 cm^{-1}, consistent with previous studies (*19,26,27*). The observed differences between NaOH- and KOH-CGH spectra suggest that the choice of alkaline solvent impacts the degree of hydrogen bonding and crosslinking in the hydrogel matrix. KOH-CGH

exhibited additional spectral features, including a more prominent secondary peak at 3275 cm^{-1}, which may reflect increased structural complexity or variability in hydroxyl group interactions.

These FT-IR results align with previous research highlighting the structural characteristics of chitin-glucan hydrogels. Similar studies have reported the effects of solvent systems on hydrogen bond formation, with KOH often promoting greater disruption of intermolecular hydrogen bonds due to its ionic radius and stronger basicity compared to NaOH (*13,14*). This structural variation may influence the physical properties of hydrogels, including their mechanical strength, porosity, and functional performance as food coatings.

The findings contribute to the growing body of literature on polysaccharide-based hydrogels, addressing gaps in the understanding of solvent-specific impacts on chitin-glucan hydrogel synthesis. This study is among the first to systematically compare NaOH and KOH solvent systems for chitin-glucan hydrogels in the context of food preservation, offering insights into optimizing hydrogel properties for specific applications.

Morphological characteristics of Kashar cheese samples

The morphological structures of cheese samples coated with chitin-glucan-based hydrogels (CGHs), prepared using KOH and NaOH solvent systems, were examined through scanning electron microscopy (SEM) (Fig. 2a and Fig. 2b). The SEM images show that both coatings effectively covered the surface of Kashar cheese. Surface roughness, characterized by white ridges, is likely due to variations in coating thickness, which resulted from the non-homogeneous distribution of the composite material. In previous studies, similar morphological characteristics were observed for chitin-glucan-based hydrogels prepared using similar methods (*28*). These studies indicated that NaOH-CGH exhibited a more heterogeneous microstructure with larger pores, while KOH-CGH displayed a smaller pore size and a more homogeneous network structure. Consistent with these findings, the greater moisture loss in NaOH-CGH-coated samples compared to KOH-CGH-coated ones can be attributed to differences in pore size and the heterogeneity of the coating distribution.

pH of Kashar cheese

The initial pH values of the Kashar cheese samples increased from 5.96 (control) to 6.11 and 6.18 for the KOH-CGH and NaOH-CGH coatings, respectively ($p \leq 0.05$) (Table 1). During storage, the pH decreased by 0.15 in the control samples (distilled water) and by 0.21 in NaOH-CGH-coated samples. In contrast, at the end of storage the pH increased slightly by 0.01 in KOH-CGH-coated samples ($p \leq 0.05$). The growth of LAB are known to lower pH by converting lactose into lactic acid

during cheese ripening (*29*). In this study, the growth of LAB was inhibited in KOH-CGH-coated samples, which is thought to have contributed to the prevention of the pH decline, thereby delaying the development of sour flavour.

Mass loss

On the final day of storage, mass loss was recorded as 0.06, 0.09 and 0.15 % in the cheese samples coated with distilled water (the control), KOH-CGH, and NaOH-CGH, respectively (Table 1). No significant mass loss was observed in either the control samples or those coated with NaOH-CGH during the 14-day storage period ($p \geq 0.05$). The coating treatments significantly increased the mass loss of the cheese samples compared to the control, with the effect of the coating treatments on mass loss being statistically significant (28-day storage period) ($p \leq 0.05$). Previous studies have indicated that coatings containing polysaccharides may lead to mass loss due to their low resistance to water penetration (*30*). The higher mass loss observed in hydrogel-coated samples may be attributed to the hydrogels losing more water and drying more rapidly than the cheese during storage. Therefore, it is postulated that the observed mass loss is primarily due to the dehydration of the hydrogel rather than the cheese itself.

Moisture loss

Moisture loss during storage was calculated to be 8, 18 and 14 % in the control, KOH-CGH, and NaOH-CGH-coated cheese samples, respectively (Table 1). The moisture loss was greater in the hydrogel-coated cheeses compared to the control, with the NaOH-CGH coating demonstrating better moisture retention than the KOH-CGH coating. Water loss in control cheese samples coated with pure water is thought to be caused by the drying of the cheese, while water loss in cheese samples coated with hydrogels is thought to be caused by the drying of the hydrogels. The hydrogel was produced using chitin-glucan, which is a polysaccharide-based hydrophilic compound. Although not as rapid as in the control samples, the higher moisture loss in cheese samples coated with hydrogel is associated with this situation. It is believed that the initial water loss observed in the control cheese samples, which were dipped in distilled water, resulted from the evaporation of water from the cheese surface during the early days of storage, while subsequent drying was attributed to moisture loss from the cheese itself. Additionally, the water loss in the coated cheese samples may primarily stem from the hydrogels losing moisture rather than from the cheese.

Microbiological analyses

The growth of yeasts and mold

The initial yeast and mold count in all Kashar cheese samples was 1.95 log CFU/g, which increased by approximately 4 $\log_{10}$ during 28 days of storage (Fig. 3). On day 14, the growth of mold and yeast was significantly inhibited ($p \leq 0.05$) in the coated cheese samples, particularly those coated with NaOH-CGH. However, by day 28, the coatings did not significantly inhibit mold and yeast growth compared to the control ($p \geq 0.05$). While previous studies have demonstrated that chitin-glucan inhibits fungal growth (*31*), this effect was not observed when chitin-glucan was applied in hydrogel form to Kashar cheese. This discrepancy could be attributed to the concentration of chitin-glucan used, which was optimized for hydrogel formation rather than antifungal properties. Higher concentrations of chitin-glucan might enhance its antifungal effect but were not tested in this study to preserve the hydrogel structure. Chitin-glucan antimicrobial properties are primarily attributed to its ability to interact with microbial cell membranes (*32*). Chitin derivatives, such as chitosan, can disrupt cell wall integrity, inhibit cell membrane functions, and interact with intracellular components like nucleic acids and proteins. These mechanisms hinder microbial growth by increasing cell permeability or by interfering with cellular processes. However, when chitin-glucan is converted into a hydrogel form, the concentration and structure of the biopolymer play a crucial role in its effectiveness (*32*). In our study, the hydrogel's lower chitin-glucan concentration may have limited its antifungal effect, as higher concentrations are generally more effective in combating microbial growth (*33*).

The growth of lactic acid bacteria

The number of LAB increased from 3.39 log CFU/g to 6.25 and 7.10 log CFU/g in Kashar cheese samples coated with KOH-CGH and NaOH-CGH at the end of the storage, respectively (Fig. 4). Coating with KOH-CGH inhibited LAB growth in cheese samples by 1 log compared to the control and NaOH-CGH coating. The rise in the population of LAB is a crucial factor contributing to increased acidity in the environment, leading to the development of undesirable sour flavor in cheese. The inhibitory effect of the KOH-CGH coating on LAB may have delayed the initiation of the sour flavor in cheeses, thereby contributing to an extension in the product's shelf life.

Peroxide analysis

On the first days of storage, the peroxide value remained at zero for all cheese samples (Fig. 5), however, on the 14th day of storage, both KOH-CGH and NaOH-CGH coatings exhibited a significant inhibition of peroxide formation in the cheese samples compared to the control group ($p \leq 0.05$) (Table 1). No statistically significant difference was found between the effects of NaOH-CGH and KOH-CGH coatings on peroxide levels in the cheeses ($p \geq 0.05$).

Peroxide serves as the primary indicator of rancidity resulting from the hydrolysis of fats (*34*). With prolonged storage, the PV typically increases due to enhanced oxidation. Similar studies have demonstrated that the application of hydrophilic coatings can delay fat hydrolysis in foods by slowing down oxygen transfer (*35-37*). Consistent with these findings, the hydrogels used in the present study may have slowed oxygen transfer and delayed hydrolysis during the storage of Kashar cheese.

Chitin-glucan has demonstrated significant antioxidant properties, which are attributed to its ability to scavenge free radicals and inhibit oxidative processes (*38*). Studies show that β-glucans, which are part of the chitin-glucan complex, can prevent oxidative damage by reducing the effects of reactive oxygen species in biological systems. This antioxidant activity can help protect food products, such as Kashar cheese, from lipid oxidation and rancidity. The hydrophilic nature of chitin-glucan hydrogels likely contributes to delaying oxidative degradation, preserving the quality and extending the shelf life of food products (*32,38*).

Texture analysis

The coating treatment significantly increased the hardness, adhesiveness and gumminess properties and decreased the resilience ($p \leq 0.05$) of the cheese samples compared to the control (Table 2). It was observed that the hydrogel coating prepared with NaOH significantly increased the cohesiveness value of the cheese compared to the control, but this value decreased in the hydrogel coating prepared with KOH ($p \leq 0.05$). The coated cheeses showed a higher hardness value, a decrease in resilience and a difference in adhesive value compared to the control, all of which were associated with higher moisture loss in the coated-cheese samples. In similar studies, as in the present study, the relatively higher hardness and stickiness value and the decrease in resilience in coated samples compared to the control were explained by the loss of moisture in the products with hydrogel (*30,39*).

Color

L, a** and *b** values of Kashar cheese samples coated with the hydrogels obtained using chitin-glucan nanofiber are given in Table 3. *L** value indicates the color transition from light to opaque, *a** value from green to red and *b** value from blue to yellow. The highest *L** value was 87.37±0.90 in the control samples and the lowest value was 72.01±0.09 in KOH-CGH coated cheese samples during storage. The coating application significantly decreased the *L** value of the cheese samples ($p \leq 0.05$). During color measurement, based on the values read by the device, the *L** value approaches 0 for dark and matte colors and 100 for white and bright colors. In this study, since the natural color

of chitin-glucan, which was used as a polymer in the coating, is close to black, it plays an important role in making the color appear slightly darker and more matte.

The coating application significantly increased *a* value ($p \leq 0.05$); however, the highest value was measured in the samples coated with NaOH-CGH at baseline (Table 3). The lowest value during storage was measured in the control (0.43±0.15).

The coating application did not cause a significant change in *b* value ($p \geq 0.05$). Although there was a decreasing trend in the *b* value of the coated samples during storage, the difference between them was found to be insignificant ($p \geq 0.05$). In the previous studies with Kashar cheese, it was found that different coating types or packaging materials did not have a significant effect on the *b* value during storage (*40,41*).

Shelf-life of the Kashar cheese

In this study, zero-order kinetics was chosen for shelf-life estimation at various temperatures, as it provided the highest R^2 value compared to other models (*21*). A single regression curve (R^2) was applied to both KOH-CGH and NaOH-CGH treatments, as the peroxide formation rates did not differ significantly between them ($p \geq 0.05$).

For Kashar cheese, shelf life was further evaluated using the rate constant of peroxide value formation, which results from oxidation processes in the cheese. In these calculations, the initial peroxide level was assumed to be 0 mmol O_2/kg fat, and deterioration was defined as the formation of 2 mmol O_2/kg fat peroxide values. Based on these assumptions, the shelf life was estimated to be 155 days for uncoated cheese and 555 days for cheese coated with hydrogels.

This approach aligns with standard methods in food science, where kinetic models are applied to predict microbial and chemical changes over time. The reliance on zero-order kinetics in this study suggests that the rate of deterioration remained constant over the observed period, fitting the linear relationship between the measured parameters and time. The use of peroxide levels as an indicator of oxidative stability is widely recognized, particularly for fatty foods like cheese, where lipid oxidation plays a critical role in shelf life. The significant extension of shelf life with hydrogel coatings underscores the potential of innovative packaging solutions to improve food preservation and reduce waste.

CONCLUSIONS

In this study, chitin-glucan, extracted from biological waste, was successfully converted into a hydrogel and applied as a coating material for Kashar cheese. The application of hydrogel coatings

demonstrated significant benefits in extending the shelf life of cheese and maintaining its quality during storage. Using zero-order kinetics for shelf-life estimation, it was determined that the uncoated cheese had a shelf life of 155 days, while cheese coated with either KOH-CGH or NaOH-CGH showed a remarkable extension of shelf life to 555 days. This extension highlights the effectiveness of hydrogel coating in reducing oxidation, as indicated by the peroxide value formation. The KOH-CGH and NaOH-CGH coatings effectively reduced oxygen permeability and delayed fat hydrolysis, contributing to better preservation of cheese quality. Among the tested hydrogels, KOH-CGH demonstrated superior inhibitory effects on LAB, which are responsible for souring defects in cheese. This suggests that the choice of solvent system plays a critical role in enhancing the antimicrobial properties of the hydrogel matrix. Although chitin-glucan is known for its antifungal properties, this effect was not prominent in its hydrogel form, potentially due to the low concentration of chitin-glucan or structural changes during hydrogel synthesis. Additionally, hydrogel-coated samples experienced greater water loss than uncoated samples, leading to increased hardness, adhesiveness, and gumminess, as well as reduced resilience. This study highlights the potential of chitin-glucan hydrogels as sustainable and functional coatings for extending the shelf life of high-moisture food products. These findings establish a foundation for advancements in biodegradable food packaging systems, addressing global challenges in reducing food waste and improving product quality through innovative materials. Given its biodegradability and low cost, chitin-glucan hydrogel presents a sustainable option for extending the shelf life of high-moisture foods. Future work should optimize formulations and assess their performance under industrial-scale processing.

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CONFLICT OF INTEREST

A. Çağrı Mehmetoğlu and Ö. Aslan declare that there are no conflicts of interest.

SUPPLEMENTARY MATERIAL

Supplementary material is available at www.ftb.com.hr.

AUTHORS' CONTRIBUTIONS

A. Cagri-Mehmetoğlu designed the experiments, analyzed the data, and reviewed the manuscript. Ö. Aslan collected the data and wrote the manuscript. Both authors completed and authorized the final version of the manuscript.

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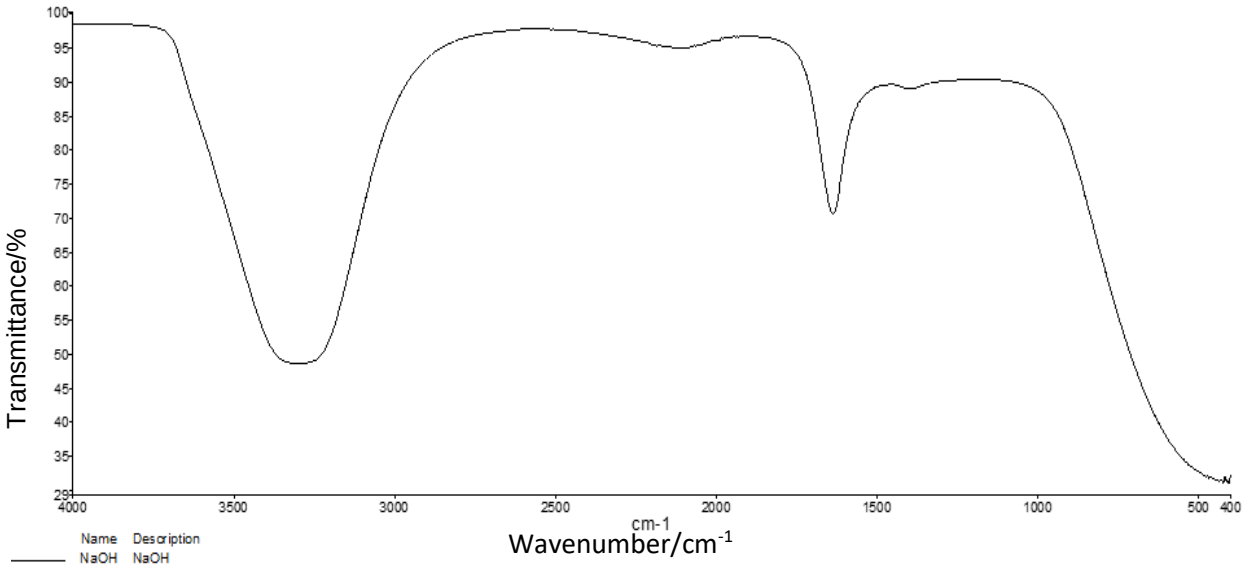
a)



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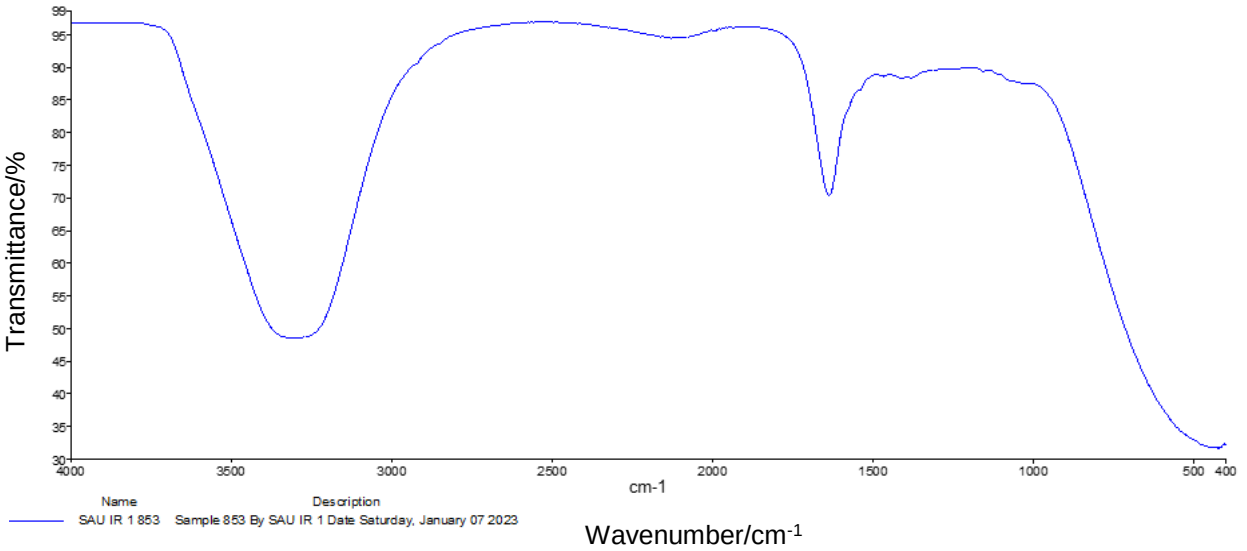

Fig. 1. FT-IR analysis result of chitin-glucan hydrogel created in: a) NaOH-based solvent system and b) KOH-based solvent system

a) b)

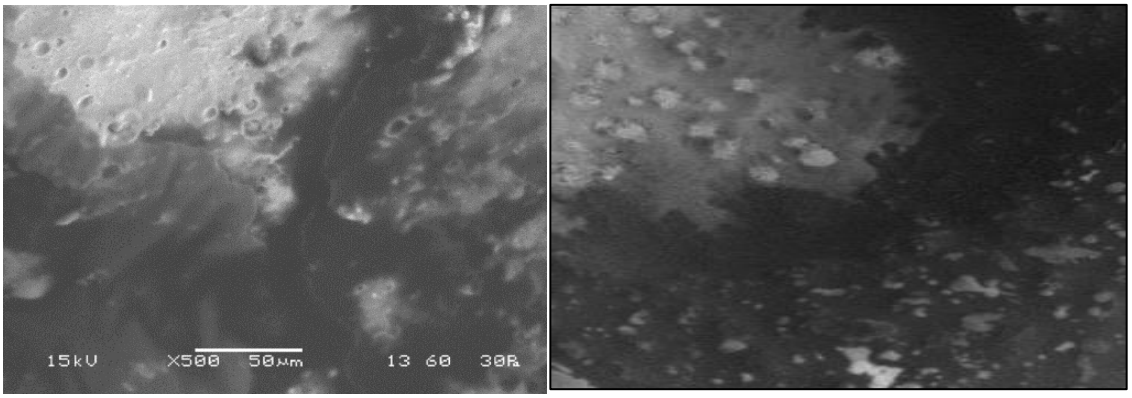

Fig. 2. Scanning electron microscope (SEM) images of hydrogel regions of Kashar cheese sample coated with: a) KOH-CGH and b) NaOH-CGH observed under 500×magnification

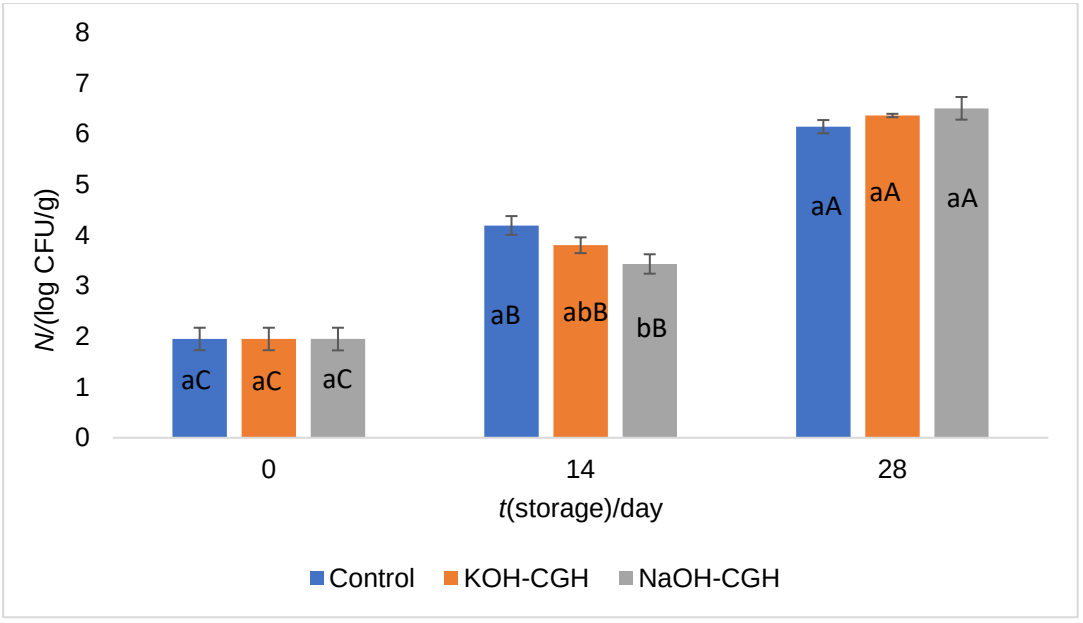

Fig. 3. The growth of yeast-mold in Kashar cheese samples coated with chitin-glucan hydrogel prepared with KOH (KOH-CGH) and NaOH (NaOH-CGH) at 4 °C for 28 days storage (log CFU/g). Control: distilled water. The difference between means denoted by the same uppercase letter (A, B, C) (the effect of storage time) and the same lowercase letter (a, b, c) (the effect of coating treatments) is insignificant ($p \leq 0.05$). Results are presented as mean value±standard deviation (N=3)

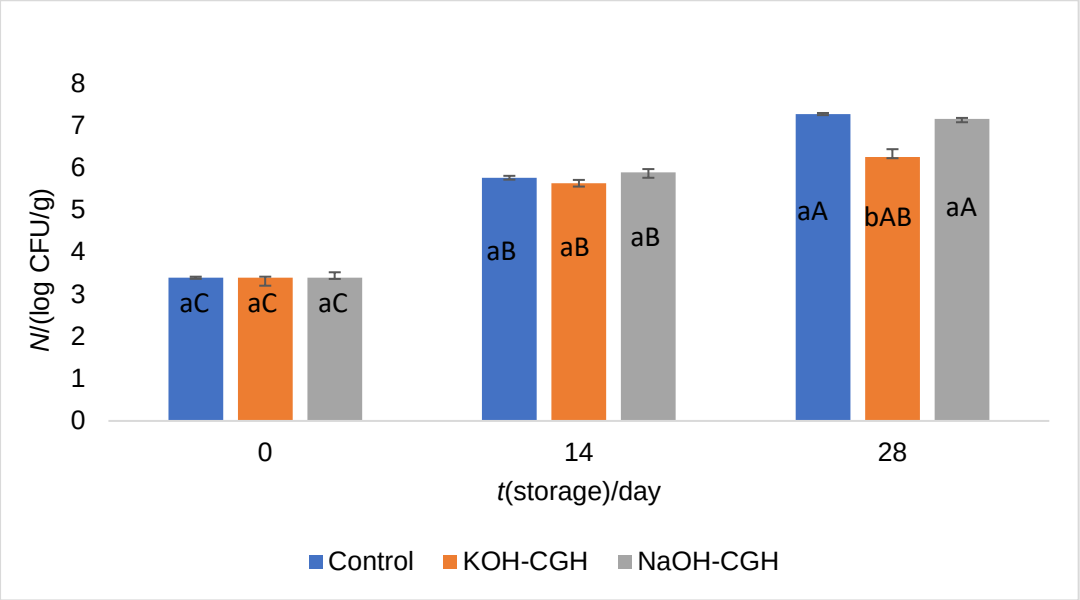

Fig. 4. The growth of LAB in Kashar cheese samples coated with chitin-glucan hydrogel prepared with KOH (KOH-CGH) and NaOH (NaOH-CGH) at 4 °C for 28 days of storage (log CFU/g). Control: distilled water. The difference between means denoted by the same uppercase letter (A, B, C) (the effect of storage time) and the same lowercase letter (a, b, c) (the effect of coating treatments) is insignificant ($p \leq 0.05$). Results are presented as mean value±standard deviation (N=3)

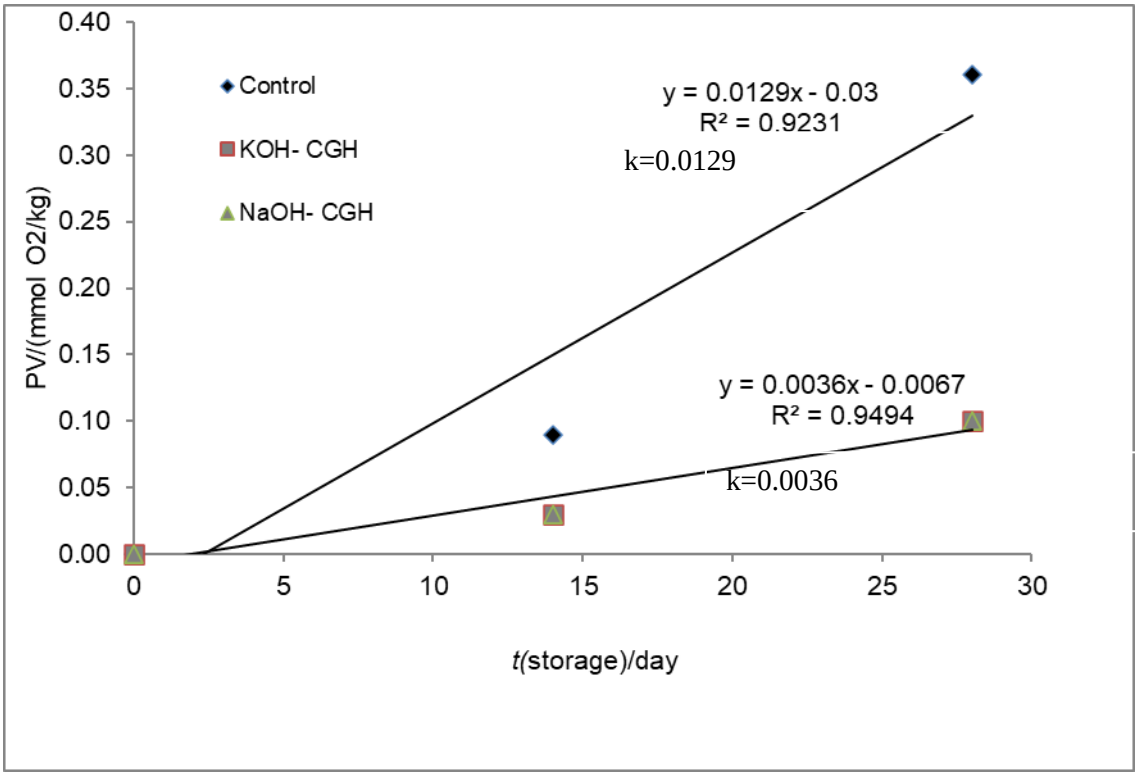

Fig. 5. Peroxide value of Kashar cheese coated with chitin-glucan hydrogel prepared with KOH (KOH-CGH) or NaOH (NaOH-CGH) during 28 days of storage at 4 °C. The same regression curve was used for KOH-CGH and NaOH-CGH due to statistically similar peroxide formation rates ($p \geq 0.05$). Control: samples prepared with distilled water

Table 1. Effect of different coatings on pH, mass loss, moisture content, and peroxide value (PV) of Kashar cheese during storage at 4 °C

Parameter	*t*(storage)/day	Coating		
		Control	KOH-CGH	NaOH-CGH
pH	0	(5.96±0.1) ^{Ac}	(6.11±0.4)^{Bb}	(6.18±0.9)^{Aa}
	14	(5.91±0.5)^{Bb}	(6.14±0.8)^{Aa}	(6.15±0.7)^{Aa}
	28	(5.81±0.3)^{Cc}	(6.13±0.5)^{ABa}	(5.97±0.8)^{Bb}
Mass loss/(%)	14	(0)	(0.06±0.01)^{Ba}	(0)
	28	(0.06±0.01)^{c}	(0.09±0.04^{Ab}	(0.15±0.04)^{a}
w(moisture)/(%)	0	(40.85±5.3)^{Aab}	(42.1 ±6.8)^{Aa}	(40.33±7.4)^{Ab}
	14	(33.17±5.4)^{Ba}	(32.57±3.6)^{Ba}	(30.92±4.6)^{Bb}
	28	(32.98±7.1)^{Ba}	(24.11±1.4)^{Cc}	(26.03±1.9)^{Cb}
PV/(mmol O_2/kg)	0	(0.00±0.00)^{Ca}	(0.00±0.00)^{Ca}	(0.00±0.00)^{Ca}
	14	(0.09±0.01)^{Ba}	(0.03±0.00)^{Bb}	(0.03±0.00)^{Bb}
	28	(0.36±0.09)^{Aa}	(0.10±0.01)^{Ab}	(0.10±0.01)^{Ab}

Control: samples prepared with distilled water, KOH-CGH: chitin-glucan hydrogel prepared with potassium hydroxide, NaOH-CGH: chitin-glucan hydrogel prepared with sodium hydroxide. The difference between means denoted by the same uppercase letter (A, B, C) in the same column (the effect of storage time) and the same lowercase letter (a, b, c) in the same row (the effect of coating treatments) is insignificant ($p \leq 0.05$). Results are presented as mean value±standard deviation (*N*=3)

Table 2. The effect of coatings on the texture properties of fresh Kashar cheese at the last day of storage

Coating	Hardness/ mJ	Adhesiveness/ mJ	Cohesiveness/ (g/s)	Resilience/ mm	Gumminess/ g
Control	$(93.50\pm0.07)^{a}$	$(-42.17\pm0.50)^{a}$	$(0.63\pm0.14)^{b}$	$(0.33\pm0.07)^{c}$	$(58.91\pm0.07)^{a}$
KOH-CGH	$(151.25\pm0.05)^{b}$	$(-40.77\pm0.26)^{ab}$	$(0.59\pm0.09)^{a}$	$(0.25\pm0.08)^{b}$	$(88.48\pm0.09)^{b}$
NaOH-CGH	$(126.50\pm0.05)^{c}$	$(-40.11\pm0.27)^{b}$	$(0.68\pm0.11)^{c}$	$(0.21\pm0.04)^{a}$	$(85.39\pm0.09)^{c}$

Control: samples prepared with distilled water, KOH-CGH: chitin-glucan hydrogel prepared with potassium hydroxide, NaOH-CGH: chitin-glucan hydrogel prepared with sodium hydroxide. The difference between mean values indicated with the same letter in the same column is insignificant ($p\geq0.05$). Results are presented as mean values±standard deviation (N=3)

Table 3. Effect of different coatings on *L*, a** and *b** values of Kashar cheese during storage at 4 °C

Colour parameter	*t*(storage)/day	Coating		
		Control	KOH-CGH	NaOH-CGH
*L **	0	$(87.37\pm0.90)^{ABb}$	$(78.38\pm0.42)^{Aab}$	$(79.19\pm0.15)^{Aa}$
	14	$(86.20\pm0.80)^{Bb}$	$(76.05\pm0.52)^{Aa}$	$(76.95\pm0.28)^{Aa}$
	28	$(83.70\pm0.33)^{Aab}$	$(72.01\pm0.09)^{Ab}$	$(73.03\pm0.32)^{Ba}$
*a **	0	$(2.57\pm0.18)^{Cc}$	$(3.06\pm0.16)^{Ba}$	$(3.52\pm0.26)^{Cb}$
	14	$(1.07\pm0.03)^{Ab}$	$(1.91\pm0.02)^{Aa}$	$(2.66\pm0.29)^{Aa}$
	28	$(0.44\pm0.16)^{Bc}$	$(1.74\pm0.06)^{Bb}$	$(1.34\pm0.03)^{Ba}$
*b **	0	$(17.04\pm0.99)^{Aa}$	$(16.86\pm0.99)^{Aa}$	$(16.05\pm0.86)^{Aa}$
	14	$(15.63\pm0.80)^{Bb}$	$(16.31\pm0.10)^{Aa}$	$(15.27\pm0.07)^{Ab}$
	28	$(16.07\pm0.33)^{ABa}$	$(16.11\pm0.10)^{Aa}$	(15.44(0.15)^{Ab}

Control: samples prepared with distilled water, KOH-CGH: chitin-glucan hydrogel prepared with potassium hydroxide, NaOH-CGH: chitin-glucan hydrogel prepared with sodium hydroxide. The difference between mean values denoted by the same uppercase (A, B, C) letter in the same column (the effect of storage time) and the same lowercase letter (a, b, c) in the same row (the effect of coating treatments) is insignificant ($p\leq0.05$). Results are presented as mean value±standard deviation (N=3)

Supplementary material

Table S1. The content of biological wastes in the medium composition

Fruit and vegetable waste	Zucchini, carrots, cabbage (raw and pickled), tomatoes (raw and canned), peppers (capia, charleston), eggplant, onions, potatoes, cauliflower, parsley, lettuce, dill, arugula, mint, leek, rosemary, celery, garlic, tangerine, orange, lemon, watermelon, banana, pineapple, sea buckthorn, apple, quince, persimmon, pear, grapefruit, kiwi
Protein waste	Mushrooms, eggs (raw and boiled), cheese (white cheese, cheddar cheese), red meat, beans with meat, chickpeas with meat
Polysaccharide waste	Bread (wholegrain, rye, white, whole wheat), cake, pancakes, bagel, pastry (with dill, cheddar, tomato), bagel, raisin bun, croissant (chocolate and plain), sugar bun, moon cake

Table S2. The chemical composition of biological wastes in the medium composition

w/%	PR	PS	FV*
Total dry matter	$(37.93\pm0.02)^{b}$	$(76.41\pm0.46)^{a}$	$(13.05\pm0.04)^{c}$
Protein	$(18.97\pm0.30)^{a}$	$(12.97\pm0.47)^{b}$	$(1.73\pm0.12)^{c}$
Fat	$(14.65\pm0.07)^{a}$	$(9.15\pm0.64)^{b}$	$(1.35\pm0.0)^{c}$
Ash	$(0.66\pm0.01)^{a}$	$(0.64\pm0.04)^{a}$	$(0.31\pm0.06)^{b}$
Polysaccharide	$(2.54\pm0.02)^{b}$	$(3.48\pm0.03)^{a}$	$(2.11\pm0.03)^{c}$
Total sugar	$(4.92\pm0.01)^{c}$	$(8.6\pm0.02)^{a}$	$(6.57\pm0.01)^{b}$