Iraqi Journal of Agricultural Sciences –2026:57(5):1578-1591
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/t9gc6z89

EDIBLE FILM BASED ON ENZYMATICALLY PRODUCED NANOCELLULOSE: CHARACTERIZATION AND NOVEL USE IN PACKAGING BEEF CUTS

Hyder N. Alzobaidy*[1], Muhsin F. Alquraishi*[1]
*[1]Department of Food Science, College of Agriculture, Wasit University, Wasit, Iraq.

ABSTRACT

Sustainable food packaging solutions with biocompatible macromolecules are becoming increasingly popular. This study addresses the difficulty of extending the shelf life of meat through innovative packaging. It investigates nanocellulose coatings as a possible solution for microbial growth and oxidative stability issues. The goal of this study is to develop a novel nanocellulose (NC) edible film to protect and extend the shelf life of meat cuts. In this study, cellulase is used in a modified method to convert microcrystalline cellulose (MCC) into nanoscales. X-ray diffraction analysis of enzymatically treated MCC revealed a decrease in crystallinity from 53.8% to 42.8%. Atomic force microscopy (AFM) images of cellulose fibers treated with cellulase show particles in the nano-dimensions, and the surface roughness profile has Rq and Ra values of 0.082 µm and 0.068 µm, respectively. Field-emission scanning electron microscopy (FESEM) images revealed that NC films have uniform nanoparticle distribution, and the nanoparticles range in size from 35 to 46 nm. The microbial content and chemical properties of the NC coating solution were studied in three treatments (T1, T2, and T3) with a control. The study examined the effects of NC coating on the microbial growth of aerobic bacteria, coliform bacteria, Clostridium bacteria, yeast, and mold. The control group showed significant growth in all microbial populations over the 10-day period. The treatment groups exhibited growth at lower levels compared to the control group. Meat with NC coatings has a significantly lower pH than control group meat, and NC coatings at higher concentrations improve oxidative stability and reduce lipid oxidation. NC coatings in meat cuts act as antioxidants, boosting oxidative stability, decreasing PV, and lowering lipid oxidation. As a result, this study introduced an innovative NC edible film to preserve meat cuts.

Key words: Antioxidant properties, cellulase, Edible coating, Enzymatic treatment, Microcrystalline cellulose, Nanocellulose.

Received: 16/12/2024, Accepted: 12/3/2025, Published: 30/5/2026

INTRODUCTION

Common commercial food packaging materials include glass, aluminum cans, and polymers derived from petroleum. These materials are used to prevent food from becoming physically damaged, contaminated, or spoiled (Marsh & Bugusu, 2007). However, there is growing interest in developing sustainable packaging solutions to reduce environmental pollution caused by non-degradable plastics (Kavitha et al., 2018), (Fortunati et al., 2019). One promising approach, according to (Suhag et al., 2020), is the use of biocompatible macromolecules, such as proteins, polysaccharides, or lipids, which can be applied as thin edible coatings directly onto the food's surface. Edible coatings serve as primary packaging and have the advantage of being consumable along with the food, eliminating the need for additional packaging disposal. The growing demand for biodegradable packaging to preserve food was

Iraqi Journal of Agricultural Sciences –2026:57(5):1578-1591
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/t9gc6z89

investigated by (Homayounpour et al., 2020). They examine the effect of eco-friendly coatings containing nano-chitosan on the quality of sardine fish, observing improved attributes through nanoliposome encapsulation. Nanocellulose (NC) has emerged as a promising reinforcing agent to enhance the properties of edible coatings for food preservation and lower the price of fresh food packaging systems among the various reinforcing agents (Pirozzi et al., 2021). NC can be incorporated into various polymer materials to tailor the properties of edible coatings. It possesses desirable characteristics like low weight, density, high shape factor, excellent biocompatibility, hydrophilicity, and impressive mechanical properties such as high tensile strength, stiffness, and modulus of elasticity (Sharma et al., 2019). Enzymatic modification of cellulose requires the synergistic action of cellulolytic enzymes due to the numerous hydrogen bonds present in cellulose (Mansfield et al., 1999). NC can be obtained from cellulose through mechanical, acidic, or ionic liquid hydrolysis, as well as enzymatic hydrolysis, which is a newer method and the enzymes can be isolated from different resources (Al Zobaidy et al., 2016) (Zielińska et al., 2021). The impact of glycerol plasticization on nanocrystalline cellulose derived from sugar palm was examined by (Ilyas et al., 2018). The researchers discovered that this process led to an improvement in both water vapor permeability and resistance to biodegradation. Nisin, a naturally derived antimicrobial agent, presents a compelling opportunity for enhancing packaging materials. By combining cellulose nanofibrils with nisin, the resulting nanohybrid film demonstrates impressive attributes that contribute to improved food packaging and preservation (Yang et al., 2020). Controlling factors like microbial growth, oxidative deterioration, and moisture loss necessitates novel approaches to food packaging and preservation (Holman et al., 2018). The application of edible coatings to fresh beef meat cuts is an innovative technology in meat processing and presentation. This approach aims to preserve meat products without the use of synthetic chemical preservatives (Shafiei & Mostaghim, 2022). Edible coatings on meat cuts and products not only serve as carriers for antimicrobials but also help prevent moisture loss, maintain juiciness during storage in plastic trays, reduce oxidation rates, minimize flavor loss, and prevent the absorption of foreign odors (Bhagath & Manjula, 2019). Previous meat packaging approaches had limitations in terms of barrier properties, antimicrobial efficacy, environmental impact, and mechanical strength (Kerry et al., 2006). In contrast, the use of nanocellulose-based edible packaging holds promise for addressing these issues, providing improved preservation capabilities, increased sustainability, and potential additional functionalities. The reason for this research is to investigate novel techniques for enhancing food preservation and extending the shelf life of meat products. In order to contribute to the development of sustainable packaging solutions for the food industry by utilizing degradable film, which has the potential to reduce plastic waste and provide a safe and environmentally friendly alternative for packaging, the goal of this study is to develop a NC edible film and study its potential to protect and extend the shelf life of meat cuts.

MATERIALS AND METHODS

Materials: From the local market in the Kut region of Wasit province (south-east of Iraq), 5 kg of fresh beef (eye of round) were purchased. The samples were collected in sterile plastic bags under aseptic conditions. All utilized materials were of reagent-grade quality in the conducted processes. Microcrystalline cellulose (Chemnovatic Co., Lublin, Poland). acetate buffer solution, trichloroacetic acid, thiobarbituric acid and 1,1,3,3-tetramethoxypropane (Merck, Darmstadt, Germany). Cellulase (1,4, β-D-glucanase) (Megazyme, Bray, Ireland). Glycerol (Nice Chemical Pvt. Ltd., Kochi, India). Nisin (GoldBio Co., St. Louis, MO, USA). Peptone water, MacConkey agar and tryptose sulfite cycloserine (TSC) agar (HiMedia Laboratories Ltd., Mumbai, India). Nutrient agar and sabouraud dextrose agar (Oxoid Ltd., Hampshire, England).

Iraqi Journal of Agricultural Sciences –2026:57(5):1578-1591
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/t9gc6z89

The enzymatic method for NC preparation

Cellulase is a particular kind of enzyme used to break down cellulose into nanoscales. This transformation was achieved according to the method described in (Zielińska et al., 2021), with some modifications: 5 g of microcrystalline cellulose (MCC) were added to 200 ml of acetate buffer solution (0.05 M concentration and pH = 4.8). Subsequently, 3 ml of 2000 U mg-1 Cellulase (1,4, β-D-glucanase) was added to the mixture and mixed thoroughly for 24 h by a hot plate magnetic stirrer (L-81, LABINCO, Breda, Netherlands) adjusted to 250 rpm min-1 shaking speed at 40 °C. The transformed NC solution was stored at -10 °C for the following experiments.

Characterization of NC

1. X-ray Diffraction: X-ray diffraction was carried out according to the method described in (Latif & Mahmood, 2018) using a 1% NC solution sonicated (SONIC RUPTOR 400, OMNI Inc., Zhubei, Taiwan) at 50 W for 5 min to remove aggregates and homogenize the solution. Next, drops of the solution were taken and put on a glass slide, left at room temperature to dry, then scanned by an X-ray diffractometer (XRD-6000, Shimadzu, Tokyo, Japan) to identify the crystallinity nature of the material. The ratio between crystallin cellulose and amorphous materials determines the crystallinity index (*CrI*) of the cellulose, as shown in equation 1.

$$CrI\ \% = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \qquad (1)$$

where:

CrI is the crystallinity index.

I_{002} is the maximum diffraction intensity located around (22.8°–26.4°), corresponding to the crystallin materials.

I_{am} is the minimum diffraction intensity located around (15°–18°), corresponding to the amorphous materials.

2. **Atomic force microscopy**

The surface morphology of selected film samples was also observed using an atomic force microscope (SPM-9700, Shimadzu instrument, Tokyo, Japan). The measurements were performed in tapping mode using the Scan Assist-Air Probe at ambient temperature. A droplet of sonicated 1% NC was placed on a glass slide, allowed to air dry at ambient temperature. Measurements (3.5 × 3.5 μm^2 scan size) were taken from several zones of the film surface to capture images. Gwyddion analysis software version 2.62 was used to analyze the acquired photos and create three-dimensional images. The roughness values of the film surface can be determined using two statistical parameters: Rq (root-mean-square average of height deviations from the mean data plane) and Ra (average absolute value of height deviations from the mean surface) (Farhan & Hani, 2017).

3. **Field-Emission Scanning Electron Microscopy**

Field-emission scanning electron microscopy (FESEM) was utilised to visualise the surface properties and nanostructural analysis of the NC samples. The synthesised samples were examined using a MIRA 3 FESEM instrument (Tescan Co., Brno, Czech Republic) with the following analysis conditions: 15 kV accelerating voltage, 10.0 nA beam current, 12500x magnification, and a detector with a 20 µ gold/aluminum coating layer (Divsalar et al., 2018).

Preparation of the NC coating solution

The NC coating solution was prepared according to (Ilyas et al., 2018) with some modifications. To prepare the coating solution, 5 ml of glycerol were added to 495 ml of 2.5% NC solution as a plasticizer agent. Next, 200 IU of Nisin were added to the mixture and mixed well using a hotplate stirrer under continuous stirring at 500 rpm at 60–65 °C for one hour. Then, the mixture was diluted with different volumes of deionized water as follows: 20:80, 40:60, and 60:40 (V:V NC solution: Deionized Water) to perform three treatments (T1, T2, and T3), and the control was kept without coating. The solution was mixed well and sonicated at 150 w for 10 min for homogenization and to remove aggregates of the nanocompound. The beef samples slices (10 g portions) were dipped in the NC coating solution for 1 minute, then placed on a clean surface to dry and stiffen for around 10 minutes at room temperature, using the immersion technique to coat the meat. The samples were placed in the refrigerator until they were used.

Iraqi Journal of Agricultural Sciences –2026:57(5):1578-1591
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/t9gc6z89

Characterization of NC coating

1. Examining the microbial content: The total plate count (TPC) test was used to determine the microbiological population of the beef samples across the storage period at 5-7 °C. The method was achieved according to (Sobhan et al., 2021) with a few modifications. At first, 10 g of the meat samples were blended with 90 ml of 1% peptone water using a stomacher blender (BK-SHG04, Biobase Co. Ltd., Shandong, China). Different concentrations were used. To a culture medium, 1 ml of each dilution was added. Nutrient agar was used for the aerobic bacterial total viable count, and the cultivated media was incubated (UNE 400, Memmert, Schwabach, Germany) at 37 °C for 24 hrs. While for *E. coli* total viable count MacConkey agar was used, the prepared media were incubated for 24 hrs. at 37 °C. *Clostridium perfringens* total count was achieved by transferring 1 ml of each dilution into the center of solidified tryptose sulfite cycloserine (TSC) agar and incubating at 37 °C for 24 hrs. in anaerobic conditions, which were established by using an anaerobic jar and gas Pak (Oxiod Ltd., Hampshire, England). It reduces the oxygen in the jar space and creates anaerobic conditions. The total yeasts and molds count was achieved by spreading 1 ml of each dilution on sabouraud dextrose agar. Then the inoculated plates were incubated at 25 °C for 5 days, and the total fungi count was calculated.

2. **Chemical characterization:** To assess the pH variations in meat during storage, a method introduced by (Moosavi-Nasab et al., 2016) was employed. In this technique, 10 grams of meat slices were blended (Bomann, Kempen, Germany) with 10 volumes of deionized water for five minutes. The pH of the resulting mixture was then measured using a pH meter (HI 2211, Hanna Instruments Inc., Romania). The measurement of lipid oxidation involved determining the thiobarbituric acid reactive substances (TBARS) values, which were expressed as milligrams of malondialdehyde (MDA) per kilogram. The process included centrifuging (DM0424, DLAB Scientific Co., Ltd., Kuala Lumpur, Malaysia) a mixture of samples and trichloroacetic acid, followed by vortexing (V2H, Boeco, Hamburg, Germany) supernatant with thiobarbiturate. After homogenization, the samples were incubated for 2 hours in a water bath (WNE 14, Memmert, Schwabach, Germany) at 97 °C. Absorbances were subsequently measured at 532 nm using a spectrophotometer (UV-2505, Labomed Inc., Los Angeles, CA, USA). The calibration curve was established using 1,1,3,3-tetramethoxypropane (99%), a precursor of MDA. The final results were reported in terms of 1 M of methoxy propane equivalent per gram of veal specimens, with values representing multiples of the molecular weight of tb MDA (Eq. 2).

$$TBARS = \frac{(A \times V) \times k}{W} \quad (2)$$

TBARS= mg MDA/kg
A: Absorbance at 532 nm
V: Sample volume (ml).
k: Conversion factor
W: Sample weight (g).

For the evaluation of peroxide values (PV), the procedure described by (Shahamirian et al., 2019) was followed (Eq. 3). All the previous tests were achieved during the storage period (1,5, and 10 days at 5-7 °C).

$$PV = \frac{(V_1 - V_0) \times N \times 1000}{m} \quad (3)$$

PV: Peroxide value
V_1: The amount of $Na_2S_2O_3$ used for the sample (ml).
V_0: The amount of $Na_2S_2O_3$ used for the blank (ml).
N: Normality of $Na_2S_2O_3$
M: Sample wieght (g).

Statistical analysis

The data collected was analyzed using GenStat software (version 10.3, VSN International Ltd., UK) An analysis of variance (ANOVA) was conducted, and significant differences between the sample groups were determined using Duncan's test. The Duncan test was performed by ranking group means and conducting pairwise comparisons to identify significant differences among multiple groups subsequent to ANOVA analysis. The results, obtained from running samples in triplicates, were presented as means. Differences were considered statistically significant at a p-value of less than 0.05.

Iraqi Journal of Agricultural Sciences –2026:57(5):1578-1591
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/t9gc6z89

RESULTS AND DISCUSSION

Crystalline structure: The morphology and tensile properties of the enzymatically transformed NC were analyzed using X-ray diffraction, and the results are shown in **Figure (1)** For MCC **(A)** and NC **(B)**, X-ray analysis revealed the presence of cellulose in its polymorphic form at 2 Ɵ angles of 15.5° and 22.5°, with an increase in the width at half-height of the diffraction peaks. The intensity of the diffraction curve of MCC was approximately 1200, whereas in the case of NC, the intensity is approximately 700, confirming that enzymatic hydrolysis is responsible for the changes in the molecular structure of MCC. Based on the results of the calculations, the MCC has a degree of crystallinity of 53.8%. Yet, cellulase treatment decreased crystallinity to 42.8 percent. The addition of enzymes to MCC altered its crystalline phase composition, as evidenced by a dramatic shift in the relative intensities of the diffraction peaks. Previous research by (Zielińska et al., 2021) demonstrated that the mechanical properties of cellulose fibers are affected by their crystallinity index (*CrI*), which is determined by the ratio of crystalline to amorphous regions and by the orientation of the crystalline and amorphous domains within the fibers. (Ribeiro et al., 2019) found that a decrease in the degree of crystallinity is reflected by an increase in the width at half-height of the diffraction peaks and a decrease in the intensity of the diffraction peaks.

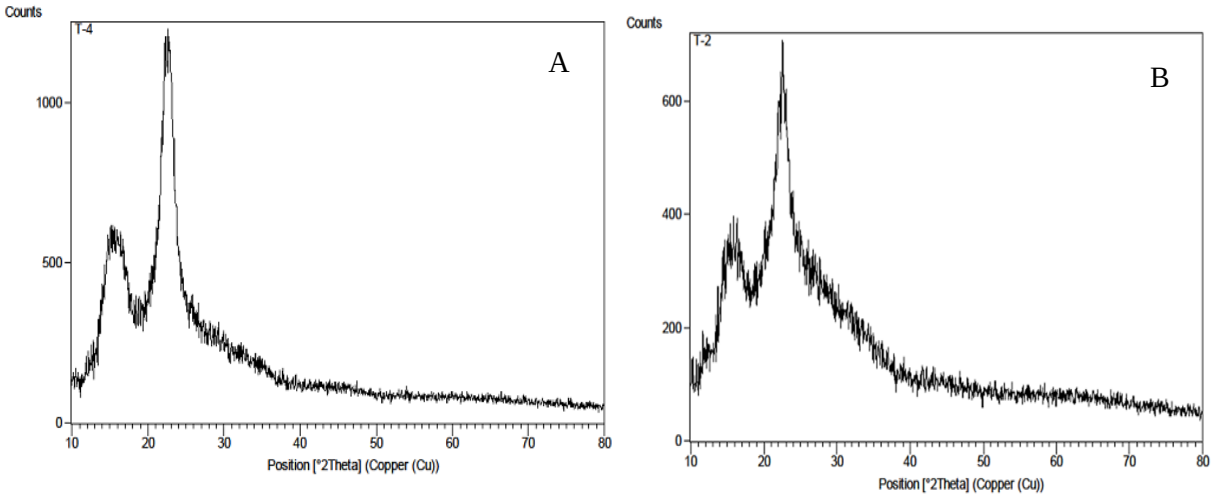

Figure 1. X-ray diffractogram of (A): microcrystalline cellulose (MCC) and (B): nanocellulose (NC).

Topographical and morphological characteristics of NC

1. Atomic force microscope AFM: Figure (2) depicts the AFM image of a diluted suspension of cellulose fibers created by treating MCC with cellulase. During scanning, two modes are used to record data: one for the height image (Figure 2. B) and one for the amplitude image (Figure 2. A). The height image displays topographical detail by following the surface with the probe, whereas the amplitude image depicts the contrast between soft and hard polymer segments (García et al., 2007). It is evident from Figure (2.B) that the cellulose gained after enzyme hydrolysis under the present experimental conditions consists primarily of particles in the nano-dimension, and the height of the cellulose fibers surface film is less than 275 nm. In the cellulose structure, the bright regions represent the crystalline regions, whereas the dark regions represent the amorphous regions along the fiber axis (Mandal & Chakrabarty, 2011). The surface features of the NC film were influenced by the enzymatic treatment. The control NC film displayed a rough surface with Rq and Ra values of 0.082 µm and 0.068 µm, respectively. The surface topography of Bacterial Nanocellulose (BNC) products was studied by (Abol-Fotouh et al., 2020) using Atomic Force Microscopy (AFM) in tapping mode, focusing on a surface area of 30x30 µm. The BNC generated using the date waste-derived medium displayed the smoothest

Iraqi Journal of Agricultural Sciences –2026:57(5):1578-1591
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/t9gc6z89

surface structure, characterized by an Rq measurement of 0.19 µm. The precise mechanisms by which cellulases act on cellulose involve the hydrolysis of glycosidic bonds in the cellulose structure, breaking it down into shorter polysaccharide chains and ultimately into individual sugar molecules(Jayasekara & Ratnayake, 2019). This enzymatic action allows for the controlled degradation and transformation of cellulose into NC. Overall, cellulases are key enzymes in the extraction of NC, enabling the conversion of cellulose fibers into nanoscale components through their hydrolytic activity (Michelin et al., 2020).

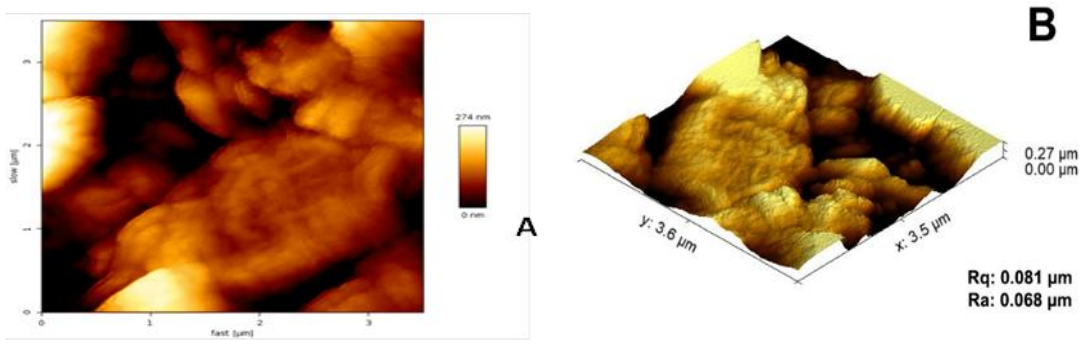

Figure 2. Image of enzymatically hydrolyzed (NC) by atomic force microscope, A: nanoscale; B: height of nanoparticles. Rq: root-mean-square average of height deviations from the mean data plane, Ra: average absolute value of height deviations from the mean surface

2. Field-Emission Scanning Electron Microscopy: In order to evaluate the surface properties and morphology, NC was subjected to FESEM analysis. Scanning electron micrographs of NC film are shown in Figure (3). As demonstrated in Figure (3A), the morphology of the surface of NC films is homogeneous and slightly rough. Moreover, the nanocrystals seem to be uniformly distributed into the matrix without any notable aggregation clusters. From Figure (3B), it can be observed that the NC exhibited small nanoparticles with sizes ranging from 35 to 46 nm. This suggests that the enzyme hydrolysis effectively converted the cellulose microfibers to nanoscales. (Mariño et al., 2015) found that enzymatic treatment separated fiber bundles, resulting in 600 nm-diameter nanofibers. FESEM showed numerous cellulose nanofibers from effective enzymatic hydrolysis. Scanning electron microscopy was utilized by (Boiko et al., 2023) to examine cellulose fiber degradation. Cellulosic fibers were shown to become severely fragmented after treatment with cellulases; the rough fibers became thinner, and the surface obtained a smooth structure.

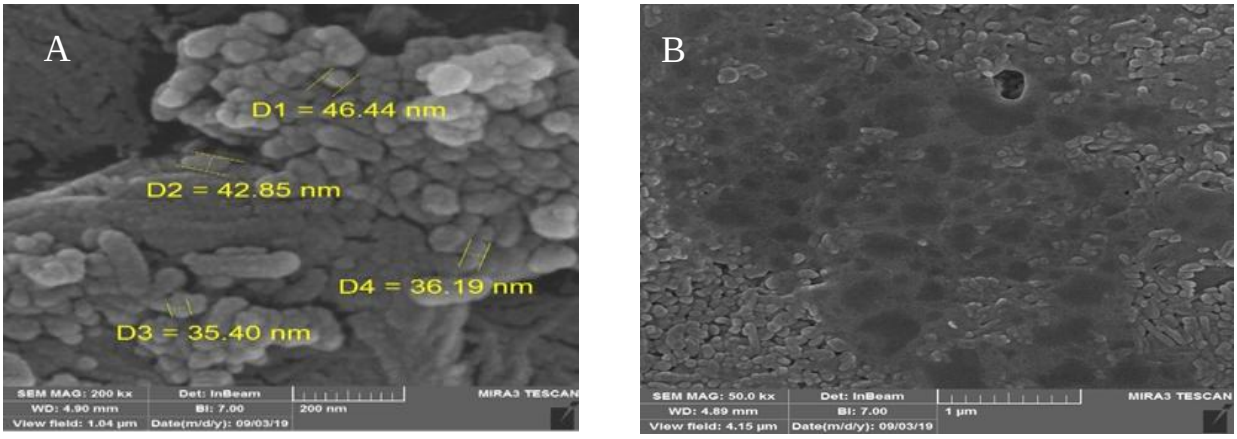

Figure 3. field emission scanning electron micrograph NC, (A): Morphology and (B): size of nanoparticles

Nanoparticles, such as NC particles, can provide various contributions to coatings: Nanoparticles can enhance the strength, hardness, and durability of coatings, leading to

Iraqi Journal of Agricultural Sciences –2026:57(5):1578-1591
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/t9gc6z89

improved mechanical performance (Zhou et al., 2013). Nanoparticles can create a dense and uniform barrier layer in coatings, reducing permeability to moisture, gases, and UV radiation, thereby improving the protective qualities of the coating (Ma et al., 2017), (Ilyas et al., 2018), (Costa et al., 2021). Controlled release of active ingredients, nanoparticles can be used as carrier for active ingredients, such as antimicrobial agents or flavor compounds, allowing for controlled release over time and providing desired functionalities to the coating. The nanofiber mat has a high surface area and porous morphology to enable higher loading of active compounds (Almasi et al., 2021). Certain nanoparticles can modify the optical properties of coatings, such as transparency, color, or reflectivity, offering aesthetic and functional enhancements (Ma et al., 2017) (Farhan & Hani, 2017).

Microbial content: The results shown in **Table (1)** indicate the total plate count (CFU) of different microbial populations over a span of 10 days. The experiment examined the effects of NC coating on microbial growth. For aerobic bacteria, the control group showed a significant increase in CFU from 2.7×10^3 on Day 1 to 3.0×10^6 on Day 10. Treatment groups (T1, T2, T3) also exhibited growth, although at lower levels compared to the control. Regarding coliform bacteria, the control group displayed an increase in CFU from 0.53×10^2 on Day 1 to 2.0×10^5 on Day 10. The treatment groups (T1, T2, T3) showed varying levels of CFU, with T3 exhibiting the lowest counts. The results for Clostridium bacteria indicate a negative result for all groups throughout the experiment. A negative result for Clostridium means that the bacteria either did not grow or were not present in the samples analyzed or competed with other antimicrobial organisms. While it is not possible to draw any conclusions about the effect of the NC coating on Clostridium based on these results, it is important to note that the absence of growth or detection of Clostridium bacteria can still provide useful information. For yeasts and molds, the control group demonstrated an increase in CFU from 2.3×10^3 on Day 1 to 17×10^3 on Day 10. The treatment groups (T1, T2, T3) showed significantly lower CFU counts compared to the control, with T3 having the lowest counts. Overall, the bacterial count was within the acceptable limits for cold meat cuts 10^6-10^7 according to FDA guidelines.

Table 1. Microbial growth on meat cuts treated with NC coating

Microbe	Treatment	Total count Log (CFU)		
		Day 1	Day 5	Day 10
Aerobic bacteria	Control	3.44^{g}	4.89^{d}	6.48^{a1}
	T1	3.37^{h}	4.82^{e}	6.07^{b}
	T2	3.37^{h}	4.72^{f}	6.05^{b}
	T3	3.31^{h}	4.72^{f}	5.89^{c}
Coliform	Control	1.73^{g}	3.04^{c}	5.31^{a2}
	T1	2.19^{f}	2.59^{d}	5.10^{b}
	T2	2.27^{ef}	2.44^{de}	5.03^{b}
	T3	2.23^{ef}	2.37^{def}	4.96^{b}
Clostridium	Control	-	-	-
	T1	-	-	-
	T2	-	-	-
	T3	-	-	-
Yeasts and molds	Control	3.36^{de}	3.55^{bc}	4.23^{a3}
	T1	3.10^{fg}	3.20^{ef}	3.72^{b}
	T2	3.00^{g}	3.36^{de}	3.62^{bc}
	T3	3.00^{g}	3.30^{e}	3.52^{cd}

$^{a-h}$ Lettered values in the table are significantly different ($p < 0.05$), ^{1}L.S.D. = 0.05713, ^{2}L.S.D. = 0.2512, ^{3}L.S.D. = 0.1862. Each value is the mean of n (n=3). T1, T2 and T3 represent NC: DW (20:80, 40:60 and 60:40 respectively).

Iraqi Journal of Agricultural Sciences –2026:57(5):1578-1591
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/t9gc6z89

Based on these results, it can be observed that the NC coating had varying effects on different microbial populations. By comparing the CFU counts of molds and yeasts in the control group to those in the treatment groups, it is possible to assess the efficacy of combining nisin and NC as a protective measure against mold and yeast contamination in meat cuts (Aloui & Khwaldia, 2016). However, the effects on aerobic bacteria and coliform bacteria were less pronounced, with some treatment groups showing similar or slightly reduced CFU counts compared to the control. (Ortiz et al., 2018) revealed that the incorporation of clove essential oil and microfibrillated cellulose in soybean protein films enhances their antimicrobial properties, particularly against foodborne pathogens. The presence of microfibrillated cellulose facilitates the release of active compounds from clove essential oil, leading to improved antimicrobial activity. The assessment of microbial degradation in beef, using polylactic acid/nanocellulose-chitosan mixture (PLA/NCM) film, over a 5-day period, as observed in (Sobhan et al., 2021), highlights critical implications for fresh meat quality. The initial total viable count of 1.95 log (CFU/g) gradually increased to 6.9 log CFU/g after 5 days at refrigerant conditions.

Chemical properties of NC coating

1. Ph: The results in **Figure (4)**. Present the pH values of meat cuts coated with NC using three different treatments over a period of 10 days, compared to a control group. In summary, based on the pH results, it appears that NC coating influenced the pH of the meat cuts differently compared to the control group. Treatments 1, 2, and 3 showed a pH of 6.5, 6, and 5.7, respectively. The lower pH observed in treatment T3, which had the highest NC concentration in the coating solution, can be explained by factors such as the acidic nature of NC, pH buffering capacity, potential chemical reactions with meat constituents, and altered ion diffusion (Abitbol et al., 2016) (Silva et al., 2020).

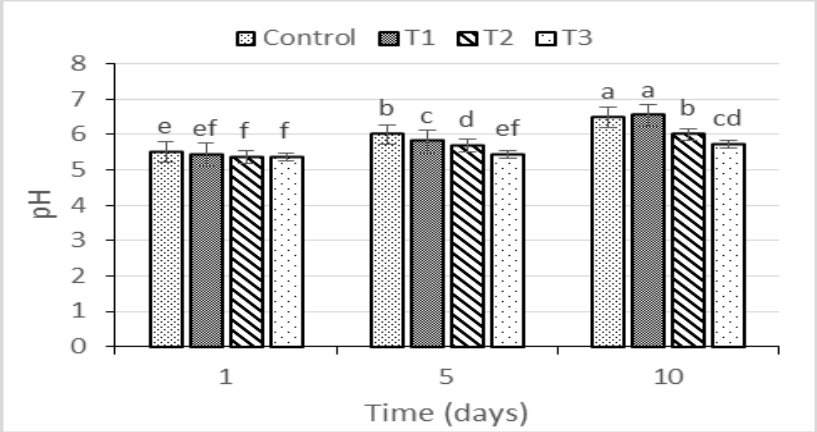

Figure 4. values of pH over time for the various treatments. Letters indicate significant differences ($p < 0.05$).

In accordance with (Dehnad et al., 2014), pH analyses demonstrated that chitosan–nanocellulose film effectively maintained lower pH levels in ground meat samples stored at 25°C for 4 and 6 days, with reductions of 0.12 and 0.2, respectively, compared to control samples. However, for meat stored for 2 days, edible films showed no significant pH alterations compared to control samples. On the other hand, it was reported by (Alexandre et al., 2021), the incorporation of basil extract as an edible coating in meat did not result in any significant differences in pH values among treatments and display conditions.

2. Antioxidant properties of NC coating

Figure 5(A) displays measurements of TBARS, an indicator of lipid oxidation that can provide insight into the meat's quality and oxidative stability. According to the provided data, the application of NC coating to meat cuts can help reduce lipid oxidation and improve the meat's oxidative stability. The significantly lower TBARS values of higher concentrations of NC (T2 and T3) compared to the control group and T1 indicate a more

Iraqi Journal of Agricultural Sciences –2026:57(5):1578-1591
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/t9gc6z89

pronounced protective effect. NC coating in cuts of meat can inhibit lipid oxidation and improve oxidative stability. The PV values represent the level of oxidative rancidity in the meat. Lower PV values typically indicate reduced lipid oxidation and enhanced oxidative stability. Figure 5(B) demonstrates that NC coating effectively inhibits lipid oxidation and enhance the oxidative stability of meat cuts. Compared to the control group and T1, higher concentrations of NC (T2 and T3) provided significantly greater protection against lipid oxidation. The decreasing PV values from Day 1 to Day 10 demonstrate an improvement in oxidative stability over time. (Salimiraad et al., 2022). NC coating effectively reduces lipid oxidation in meat cuts by acting as antioxidants, creating a barrier against oxygen diffusion, chelating metal ions, modulating moisture content, and interacting with lipid constituents. These mechanisms contribute to improved oxidative stability and lower PV. (M'barek et al., 2022) conducted a study to evaluate the antioxidant and antimicrobial activities of NC and its coated forms with nanoparticles (NPs). They observed that the NC and its coated forms exhibited significant scavenging abilities against the DPPH radical, with a maximum of 82.94% at a concentration of 200 mg/L.

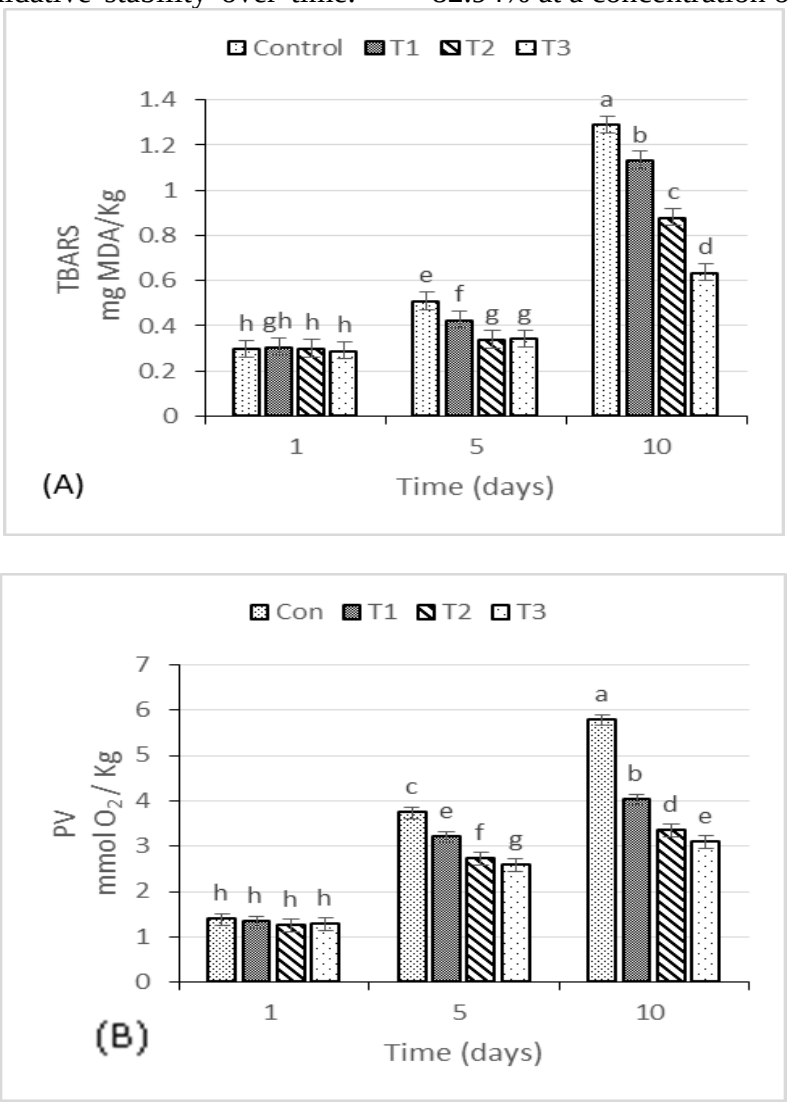

Figure 5. Values of (A) TBARS, and (B) PV over time for the various treatments. Letters indicate significant differences ($p < 0.05$).

CONCLUSION

This study introduced an innovative NC edible film to protect meat cuts and extend their shelf life. MCC was enzymatically transformed into nanoscale cellulose using a modified cellulase method. XRD diffractograms confirm that enzymatic hydrolysis is responsible for the changes in the molecular structure of MCC.

Iraqi Journal of Agricultural Sciences –2026:57(5):1578-1591
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/t9gc6z89

AFM and FESEM images confirm the uniform distribution of nanocrystals on the NC film surface. The NC coating treatment shows potential for reducing microbial growth, particularly for yeast and mold. Additionally, the NC coating contributes to improved pH levels, oxidative stability, and reduced lipid oxidation in meat cuts. These findings highlight the potential of NC-based coatings for extending the shelf life and preserving the quality of meat products while offering sustainable and biocompatible packaging solutions, but other factors that influence meat quality and safety, such as sensory attributes (flavor, texture, and appearance), and the potential allergenicity of the NC, were not extensively addressed. Further research is needed to explore the long-term effects and scalability of this technology in practical applications.

ACKNOWLEDGEMENT

We would like to acknowledge the assistance of Professor Nabil Raheem Lahmod in statistical analysis of this study.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR/S DECLARATION

We confirm that all Figures and Tables in the manuscript are original to us. Additionally, any Figures and images that do not belong to us have been incorporated with the required permissions for re-publication, which are included with the manuscript.

Author/s signature on Ethical Approval Statement.

Ethical Clearance and Animal welfare

Funds: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

AUTHOR'S CONTRIBUTION STATEMENT

Hyder Najy Alzobaaidy contributed to the conceptualization of the study, conducted the experimental work, performed field sampling, carried out data analysis and interpretation, and prepared the initial draft of the manuscript. Muhsin F. Alquraishi participated in laboratory experiments, assisted in sample collection, contributed to data analysis and interpretation, supported literature review and manuscript writing, and contributed to the critical revision of the manuscript. Both authors have read and approved the final version of the manuscript.

REFERENCES

Abitbol, T., A. Rivkin, Y. Cao, Y. Nevo, E. Abraham, T. Ben-Shalom, S. Lapidot, & O. Shoseyov. 2016. Nanocellulose, a tiny fiber with huge applications. Current Opinion in Biotechnology. 39: 76–88. https://doi.org/10.1016/j.copbio.2016.01.002

Abol-Fotouh, D., M.A. Hassan, H. Shokry, A. Roig, M.S. Azab, & A.E.-H.B. Kashyout. 2020. Bacterial nanocellulose from agro-industrial wastes: low-cost and enhanced production by *Komagataeibacter saccharivorans* MD1. Scientific Reports. 10(1): 3491. https://doi.org/10.1038/s41598-020-60315-9

Al Zobaidy, H.N., K.A. Shakir, & G. M. Strasburg. 2016. Characterization of L-asparaginase purified from pole beans. Iraqi Journal of Agricultural Sciences. 47(1): 129-137. https://www.iraqoaj.net/iasj/download/bda5ac2d999c4868

Alexandre, S., A.C.P. Vital, C. Mottin, R.M. do Prado, M.G. Ornaghi, T.R. Ramos, A. Guerrero , E.J. Pilau, & I.N. do Prado. 2021. Use of alginate edible coating and basil (Ocimum spp) extracts on beef characteristics during storage. Journal of Food Science and Technology. 58: 3835–43. https://doi.org/10.1007/s13197-020-04844-1

Almasi, H., M. Jahanbakhsh Oskouie, & A. Saleh. 2021. A review on techniques utilized for design of controlled release food active packaging. Critical Reviews in Food Science and Nutrition. 61(15): 2601–21. https://doi.org/10.1080/10408398.2020.1783199

Aloui, H., & K. Khwaldia. 2016. Natural antimicrobial edible coatings for microbial safety and food quality enhancement. Comprehensive Reviews in Food Science and Food Safety. 15(6): 1080–1103. https://doi.org/10.1111/1541-4337.12226

Bhagath, Y. B., & K. Manjula. 2019. Influence of composite edible coating systems on preservation of fresh meat cuts and products: a brief review on their trends and applications.

Iraqi Journal of Agricultural Sciences –2026:57(5):1578-1591
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/t9gc6z89

International Food Research Journal. 26(2): 377-392.

Boiko, S., M. Netsvetov, & V. Radchenko. 2023. Cellulose biosaccharification by Irpex lacteus wood decay fungus. Maderas-Cienc Tecnol. 25. doi.org/10.4067/S0718-221X2023000100435

Costa, S.M., D.P. Ferreira, P. Teixeira, L.F. Ballesteros, J.A. Teixeira, & R. Fangueiro. 2021. Active natural-based films for food packaging applications: The combined effect of chitosan and nanocellulose. International Journal of Biological Macromolecules. 177: 241–51. https://doi.org/10.1016/j.ijbiomac.2021.02.105

Dehnad, D., H. Mirzaei, Z. Emam-Djomeh, S.-M. Jafari, & S. Dadashi. 2014. Thermal and antimicrobial properties of chitosan–nanocellulose films for extending shelf life of ground meat. Carbohydrate Polymers. 109: 148–54. https://doi.org/10.1016/j.carbpol.2014.03.063

Divsalar, E., H. Tajik, M. Moradi, M. Forough, M. Lotfi, & B. Kuswandi. 2018. Characterization of cellulosic paper coated with chitosan-zinc oxide nanocomposite containing nisin and its application in packaging of UF cheese. International Journal of Biological Macromolecules. 109: 1311–18. https://doi.org/10.1016/j.ijbiomac.2017.11.145

Farhan, A., & N. M. Hani. 2017. Characterization of edible packaging films based on semi-refined kappa-carrageenan plasticized with glycerol and sorbitol. Food Hydrocolloids. 64: 48–58. https://doi.org/10.1016/j.foodhyd.2016.10.034

Fortunati, E., A. Mazzaglia, & G.M. Balestra. 2019. Sustainable control strategies for plant protection and food packaging sectors by natural substances and novel nanotechnological approaches. Journal of the Science of Food and Agriculture. 99(3): 986–1000. https://doi.org/10.1002/jsfa.9341

García, R., R. Magerle, & R. Perez. 2007. Nanoscale compositional mapping with gentle forces. Nature Materials. 6(6): 405–11. https://doi.org/10.1038/nmat1925

Holman, B.W.B., J.P. Kerry, & D.L. Hopkins. 2018. Meat packaging solutions to current industry challenges: A review. Meat Science. 144: 159–68. https://doi.org/10.1016/j.meatsci.2018.04.026

Homayounpour, P., H. Jalali, N. Shariatifar, M. Amanlou, & A. Khanjari .2020. Protective effect of nanochitosan incorporated with free/nanoliposome Cumin (*Cuminum cyminum* L.) aqueous extract on sardine fish. Journal of Aquatic Food Product Technology. 29(9): 949–61. https://doi.org/10.1080/10498850.2020.1819497

Ilyas, R.A., S.M. Sapuan, M. R. Ishak, & E.S. Zainudin. 2018. Sugar palm nanocrystalline cellulose reinforced sugar palm starch composite: Degradation and water-barrier properties. in: *IOP* Conference Series: Materials Science and Engineering. (Vol. 368, p. 12006) (IOP Publishing). https://doi.org/10.1016/j.carbpol.2018.09.002

Jayasekara, S., & R. Ratnayake. 2019. Microbial cellulases: an overview and applications. Cellulose. 22: 92. https://doi.org/10.5772/intechopen.84531

Kavitha, G., R. Rengasamy, & D. Inbakandan. 2018. Polyhydroxybutyrate production from marine source and its application. International Journal of Biological Macromolecules. 111: 102–8. https://doi.org/10.1016/j.ijbiomac.2017.12.155

Kerry, J.P., M.N. O'grady, & S.A. Hogan .2006. Past, current and potential utilisation of active and intelligent packaging systems for meat and muscle-based products: A review. Meat Science. 74(1): 113–30. https://doi.org/10.1016/j.meatsci.2006.04.024

Latif, M.H.A., & Y. F. Mahmood. 2018. Isolation and characterization of microcrystalline cellulose and preparation of nano-crystalline cellulose from tropical water hyacinth. Ibn AL-Haitham Journal For Pure and Applied Science. 31(1): 180–88. https://doi.org/10.1016/j.ijbiomac.2018.01.098

M'barek, I., Z. Isik, Y. Ozay, S. Özdemir, G. Tollu, Y. Moussaoui, & N. Dizge. 2022. Nanocellulose synthesis from *Tamarix aphylla* and preparation of hybrid nanocellulose composites membranes with investigation of antioxidant and antibacterial effects. Separation and Purification Technology. 292: 120815. https://doi.org/10.1016/j.seppur.2022.120815

Iraqi Journal of Agricultural Sciences –2026:57(5):1578-1591
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/t9gc6z89

Ma, I.A.W., A. Shafaamri, R. Kasi, F.N. Zaini, V. Balakrishnan, R. Subramaniam, & A.K. Arof. 2017. Anticorrosion properties of epoxy/nanocellulose nanocomposite coating. Bioresources. 12(2): 2912–29. https://doi.org/10.1016/j.seppur.2022.120815

Mandal, A., & D. Chakrabarty. 2011. Isolation of nanocellulose from waste sugarcane bagasse (SCB) and its characterization. Carbohydrate Polymers. 86(3): 1291–99. https://doi.org/10.1016/j.carbpol.2011.06.030

Mansfield, S. D., C. Mooney, & J. N. Saddler .1999. Substrate and enzyme characteristics that limit cellulose hydrolysis. Biotechnology Progress. 15(5): 804–16. https://doi.org/10.1021/bp9900864

Mariño, M., L. Lopes da Silva, N. Durán, & L. Tasic. 2015. Enhanced materials from nature: nanocellulose from citrus waste. Molecules. 20(4): 5908–23. https://doi.org/10.3390/molecules20045908

Marsh, K., & B. Bugusu. 2007. Food packaging—roles, materials, and environmental issues. Journal of Food Science. 72(3): R39–55. https://doi.org/10.1111/j.1750-3841.2007.00301.x

Michelin, M., D.G. Gomes, A. Romaní, M. de L.T.M. Polizeli, & J. A. Teixeira. 2020. Nanocellulose production: exploring the enzymatic route and residues of pulp and paper industry. Molecules. 25(15): 3411. https://doi.org/10.3390/molecules25153411

Moosavi-Nasab, M., E. Shad, E. Ziaee, S.H.A. Yousefabad, M.T. Golmakani, & M. Azizinia. 2016. Biodegradable Chitosan Coating Incorporated with Black Pepper Essential Oil for Shelf Life Extension of Common Carp (*Cyprinus carpio*) during Refrigerated Storage. Journal of Food Protection. 79(6): 986–93. https://doi.org/10.4315/0362-028X.JFP-15-246

Ortiz, C. M., P. R. Salgado, A. Dufresne, & A. N. Mauri. 2018. Microfibrillated cellulose addition improved the physicochemical and bioactive properties of biodegradable films based on soy protein and clove essential oil. Food Hydrocolloids. 79: 416–27. https://doi.org/10.1016/j.foodhyd.2018.01.011

Pirozzi, A., G. Ferrari, & F. Donsì. 2021. The use of nanocellulose in edible coatings for the preservation of perishable fruits and vegetables. Coatings. 11(8): 990. https://doi.org/10.3390/coatings11080990

Ribeiro, R.S.A., B.C. Pohlmann, V. Calado, N. Bojorge, & N. Pereira Jr .2019. Production of nanocellulose by enzymatic hydrolysis: Trends and challenges. Engineering in Life Sciences. 19(4): 279–91. https://doi.org/10.1002/elsc.201800158

Salimiraad, S., S. Safaeian, A. A. Basti, A. Khanjari, & R.M. Nadoushan. 2022. Characterization of novel probiotic nanocomposite films based on nano chitosan/nano cellulose/gelatin for the preservation of fresh chicken fillets. Lwt. 162: 113429. https://doi.org/10.1016/j.lwt.2022.113429

Shafiei, R., & T. Mostaghim. 2022. Improving shelf life of calf fillet in refrigerated storage using edible coating based on chitosan/natamycin containing Spirulina platensis and Chlorella vulgaris microalgae. Journal of Food Measurement and Characterization. 16(1): 145–61. https://doi.org/10.1007/s11694-021-01153-9

Shahamirian, M., M.H. Eskandari , M. Niakousari, S. Esteghlal, H. Hashemi Gahruie, & A. Mousavi Khaneghah. 2019. Incorporation of pomegranate rind powder extract and pomegranate juice into frozen burgers: Oxidative stability, sensorial and microbiological characteristics. Journal of Food Science and Technology. 56: 1174–83. https://doi.org/10.1007/s13197-019-03580-5

Sharma, A., M. Thakur, M. Bhattacharya, T. Mandal, & S. Goswami. 2019. Commercial application of cellulose nano-composites – A review. Biotechnology Reports. 21: e00316. https://doi.org/10.1016/j.btre.2019.e00316

Silva, E.L.P., T.C. Carvalho, & R.A. Ayub. 2020. Blackberry extend shelf life by nanocellulose and vegetable oil coating. Horticult Int J., 4(2): 54–60. https://doi.org/10.15406/hij.2020.04.00158

Sobhan, A., K. Muthukumarappan, L. Wei, R. Zhou, & H. Tummala. 2021. Development of a polylactic acid-coated nanocellulose/ chitosan-based film indicator for real-time monitoring of beef spoilage. Analytical Methods. 13(23): 2612–23. https://doi.org/10.1039/d1ay00365h

Suhag, R., N. Kumar, A.T. Petkoska, and A. Upadhyay. 2020. Film formation and

Iraqi Journal of Agricultural Sciences –2026:57(5):1578-1591
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/t9gc6z89

deposition methods of edible coating on food products: A review. Food Research International. 136: 109582. https://doi.org/10.1016/j.foodres.2020.109582

Yang, Y., Liu, H., Wu, M., Ma, J., & Lu, P. 2020. Bio-based antimicrobial packaging from sugarcane bagasse nanocellulose/nisin hybrid films. International Journal of Biological Macromolecules, 161, 627–635. https://doi.org/10.1016/j.ijbiomac.2020.06.081 https://doi.org/https://doi.org/10.1016/j.ijbiomac.2020.06.081

Zhou, S., Ding, X., & Wu, L. 2013. Fabrication of ambient-curable superhydrophobic fluoropolysiloxane/TiO2 nanocomposite coatings with good mechanical properties and durability. Progress in Organic Coatings, 76(4), 563–570. https://doi.org/10.1016/j.porgcoat.2012.11.013

Zielińska, D., K. Szentner, A. Waśkiewicz, & S. Borysiak. 2021. Production of nanocellulose by enzymatic treatment for application in polymer composites. Materials., 14(9): 2124. https://doi.org/10.3390/ma14092124

Iraqi Journal of Agricultural Sciences –2026:57(5):1578-1591
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/t9gc6z89

التغليف الصالح للأكل اعتمادا على نانوسليلوز منتج إنزيميًا: توصيف واستخدام طريقة مبتكرة في تغليف قطع لحوم الابقار

حيدر ناجي رسن[1]، محسن فالح عبد الله [2]

قسم علوم الاغذية، كلية الزراعة، جامعة واسط، العراق.

المستخلص

ازداد الاهتمام بالبحث عن حلول مستدامة لتغليف الأغذية باستخدام بوليمرات كبيرة متوافقة حيوياً مع الطبيعة. تتناول هذه الدراسة امكانية إطالة العمر الخزني للحوم من خلال التغليف المبتكر، والذي تحقق من خلال استخدام الاغشية النانوسليلوزية كحل لمشكلة النمو الميكروبي والثبات التأكسدي. تهدف هذه الدراسة إلى تطوير غشاء صالح للأكل من النانوسليلوز لحماية اللحوم واطالة عمرها الافتراضي. في هذه الدراسة، تم استخدام إنزيم السليليز بطريقة محورة لتحويل السليلوز المايكروي البلوري إلى أبعاد نانوية. كشفت تحاليل حيود الأشعة السينية للسليلوز المعالج إنزيمياً عن انخفاض في التبلور من 53.8% إلى 42.8%. أظهرت صور مجهر القوة الذرية AFM للألياف السليلوزية المعالجة بالسليلوز جسيمات بأبعاد نانوية، وبلغت قيم خشونة السطح Rq وRa المقدرة 0.082 ميكرومتر و0.068 ميكرومتر على التوالي. أظهرت صور مجهر المسح الإلكتروني ذي الانبعاث الميداني FESEM أن اغشية النانوسيللوز تحتوي على توزيع موحد للجسيمات النانوية، ويتراوح حجم الجسيمات بين 35 و46 نانومتر. تمت عملية التغليف بواسطة الطلاء النانوسليلوزي ودرس المحتوى الميكروبي والخصائص الكيميائية لمحلول طلاء النانوسليلوز في ثلاث معاملات T1و T2 و T3 مع معاملة سيطرة. بينت الدراسة تأثير الطلاء بالنانوسليلوز على النمو الميكروبي للبكتيريا الهوائية وبكتيريا القولون وبكتيريا الكلوستريديوم والخمائر والاعفان. أظهرت معاملة السيطرة نمواً كبيراً في جميع الميكروبات خلال فترة عشرة أيام، بينما أظهرت المعاملات نمواً أقل بالمقارنة مع معاملة السيطرة. أظهرت اللحوم المغلفة بالنانوسليلوز انخفاضاً ملحوظاً في درجة الحموضة مقارنة بلحوم معاملة السيطرة، كما حسنت الطلاءات بتركيزات أعلى من NC الثبات التأكسدي وقللت من أكسدة الدهون. تعمل الطلاءات النانوسليلوزية على قطع اللحوم كمضادات أكسدة، مما يعزز الثبات التأكسدي، ويقلل من قيمة البيروكسيد (PV)، ويخفض أكسدة الدهون. ونتيجة لذلك، قدمت هذه الدراسة غشاء صالحاً للأكل ومبتكراً من النانوسليلوز لحفظ قطع لحوم الابقار.

الكلمات المفتاحية: خصائص مضادات الاكسدة، السيليلوز، التغليف الصالح للأكل،المعاملة الانزيمية ، السيليلوز المايكروي البلوري، السيليلوز النانوي.