# Development of CS/WPC/MO-NE bionanocomposites for coating Ras cheese based on Moringa essential oil nanoemulsion

**K. M. Adel [1,*], A. M. Youssef [2], R. A. Awad [3], and A. M. Gamal El Deen [1]**

[1] *Dairy Department, Faculty of agriculture, Al-Azhar University, Cairo, Egypt.*
[2] *Packaging Materials Department, National Research Centre, Giza, Egypt.*
[3] *Food Science Department, Faculty of agriculture, Ain Shams University, Cairo, Egypt.*

*Corresponding author E-mail: khaled@azhar.edu.eg (K. Adel)

## ABSTRACT

The purpose of the current work is to create and develop bionanocomposite materials and assess their suitability for covering Ras cheese. The Moringa nanoemulsion (MO-NE) was added in concentrations ranging from 2.5, 5.0, and 7.5% to the chitosan/whey protein concentrate (CS/WPC) aggregate that was used to create the bionanocomposite materials. Utilizing the SEM, TEM, and FT-IR, the manufactured nanoemulsions and bionanocomposites have been assessed. Additionally, the constructed bionanocomposites' mechanical tests, water vapour transmission rate (WVTR), and oxygen transmission rate (OTR) have also been assessed. In comparison to the uncoated cheese, the effect of covering Ras cheese with an ordered nanocomposite's impact on weight reduction, chemical, textural, and bacterial properties has been evaluated. Cheese coating decreased weight and moisture losses but had little impact on the typical ripening modifications to the composition and texture of Ras cheese. As for microbiological properties, no mould appears on the covered rind of Ras cheese, which is covered with a bionanocomposite film of MO-NE and CS/WPC.

**Keywords:** Bionanocomposite, Ras cheese, Chitosan, Whey protein concentrate, packaging.

## INTRODUCTION

In order to increase the expiration date of perishable food, lower post-contamination, and guarantee its protection in marketing, packaging is a crucial step. The acceptance and marketability of a product can also be significantly influenced by its packaging. Youssef *et al*. (2018); Youssef & El-Sayed (2018). Many different types of spoilage microorganisms can thrive well on cheese. Due to this, it may be necessary to prevent the passing of cheese at some point throughout the ripening process. Cheeses mature in hard and semi-hard forms for a period of time to develop their flavour and texture. Improper moisture losses also have an impact on cheese quality and weight loss. Cheese with the perfect coating is packaged with substances that have been used for a long time to keep away from those troubles and convey cheeses of regular fineness and warranted safety. A crucial need of the chosen coating is to prevent interfering with normal cheese ripening and enhancement of the cheese's specific flavour and texture (Poças & Pintado, 2010).

Plastic coating and wax substances had been used to coat various types of cheese. All these materials offer benefits and drawbacks when used for cheese coating, especially non-biodegradable plastics that cause environmental problems (Youssef, 2013). The use of food-grade and biodegradable polymers for cheese covering has recently been a growing field (Uput, Lazi, Popovi, & Hromi, 2015). A significant kind of cheese coating and packaging materials has developed using various polysaccharides, proteins, and their mixing. Due to its improved antibacterial film-forming properties and biodegradability Chitosan is among the most widely used researched and used natural polymers for safe-to-eat coating and packaging materials (Youssef, Abdel-Aziz, & El-Sayed, 2014; Rehan, El-Naggar, Mashable, & Wilken, 2018).

Chitosan can be combined using additional polymers and nanoparticles to create several packaging and coating choices with specialized qualities for application in meals or medicine (Youssef, El-Sayed, Salama, and Dufresne, 2016). To improve the functionality of the materials used to coat cheese so that they are fit for human consumption and biodegradable, preservatives and antimicrobials are frequently added. In cheese coating ingredients, natural oils, antimicrobials, crude extracts, and additional metallic nanoparticles are employed as antimicrobials. Metallic nanoparticles not only have antibacterial properties but also enhance the mechanical details of the created film (Zhang *et al*., 2017).

Ras cheese, which is similar to Greek Kefalotyri cheese, is the most common type of hard cheese manufactured in Egypt (Abou-Donia, 2002). Ras cheese is often composed of

cow's milk or a combination of buffalo and cow milk, and it is allowed to ripen for a minimum of three months at 12 to 15 degrees Celsius and 80% relative humidity. Ras cheese sometimes lacks packaging film lining or waxing, which permits weight loss and moisture loss. Additionally, the presence of molds and yeasts is frequently detected throughout the ripening process. Ras cheese was covered with wax mixtures to prevent it from losing moisture, while uncoated cheese had significant mold growth El-Sisi, Gap, and Kamaly (2015); Abdou, Abd El-Hamid, Dawood, Youssef, and Mahran (1977). determined that using 2% chitosan to cover Ras cheese lowers its weight and moisture, enhances the cheese's general quality in comparison to uncovered cheese, and decreases the amount of yeast and mildew. In the present paper, we prepare a new bionanocomposite coating material containing chitosan, whey protein concentrate, and Moringa oil nanoemulsion. Ras cheese was evaluated in terms of the chemical composition, texture, microbiology, and organoleptic properties of the coated and uncoated cheese during the ripening process.

## MATERIALS AND METHODS

### Materials

Chitosan (CS), having a moderate molecular weight and an 82.5% deacetylation level, as well as glycerol, Tween 20, and citric acid, were obtained from Sigma-Aldrich chemicals (Cairo, Egypt). Cow's milk, which has 3.2% protein and 4% fat, and buffalo milk, which contains 3.7% protein and 6% fat, are combined in a 9:1 ratio to make Ras cheese. Fresh after the dry salting process, Ras cheese was obtained from Green Valley Company. Whey protein concentrate 80% of the protein comes from New Zealand. The National Research Center's oil pressing section provided the Moringa oil that was used.

### Methods

#### Moringa oil nanoemulsion synthesis (MO-NE)

By mixing Moringa oil (10% v/v), Tween 20 (a nonionic surfactant), and distilled water, an oil-in-water nanoemulsion was created. A coarse emulsion was first created by adding water to the organic phase, which contained oil and surfactant in a 1:2 ratio, using a magnetic stirrer for 10 minutes at 500 rpm. Next, the mixture was heated at 60 °C for 6 hours while being stirred, and finally, it was applied to a Daihan Scientific Homogenizer for 10 minutes at 25,000 rpm. The resulting MO-NE was stored under typical circumstances for future usage.

#### Manufacturing of CS/WPC/MO-NE bionanocomposite film

The procedure was used to create the CS/WPC/MO-NE biofilms. First, 100 mL of distilled water and 3 g of CS were put into a 250 mL flask, to produce a homogenous mixture (3 weight %), the temperature was kept at 70 °C while being stirred. WPC (3wt %) was dissolved in 60 °C hot distilled water in another beaker. The two polymer solutions (60:40 % wt/wt) were combined to create a mixture of CS and WPC. In a separate step, the polymer solution was continuously mechanically stirred while being cross-linked with citric acid (10% wt/wt based on the polymer blend). The cross-linked blend was then produced by curing the mixture at 150 °C for 10 min. Then, glycerol (3 wt %) and MO-NE were added to a polymer blend in varying amounts (i.e., 2.5%, 5%, and 7.5%) after the solution had been allowed to cool to room temperature. To allow the solvent to evaporate and the creation of a film, the prepared suspension was put into clear, round Petri dishes made of glass. The biofilms under investigation were coded as CS/WPC/XMO-NE, where x reflects the percentage of MO-NE in the nanocomposite.

#### Ras cheese was coated with the prepared bionanocomposite film.

The cheese wheel was purchased immediately from Green Valley Company after pressing and salting (one week after manufacture). For each treatment, the cheese wheel was weighed (around 3.5 kg). Uncoated control was the first. The second was CS/WPC coated. The third, fourth, and fifth treatment were coated with suspensions that, respectively, contained 2.5, 5, and 7.5% of MO-NE. The cheese wheel was layering fluid onto its several layers by brushing, which were then required to dry and form a film for two hours at room temperature. To ripen, the covered cheeses were kept for three months at 12±2 °C and around 80% relative humidity. For analysis, representative samples were collected every month.

#### Analysis of cheese's physicochemical composition

The surface layer of the cheese was removed down to a depth of 2 mm. A representative sample (about 25 g) was obtained from the inside of the cheese,

crushed, and stored for examination in a closed glass container. Dry matter (DM), titratable acidity (TA), fat, total nitrogen (TN), and soluble nitrogen (SN) were measured in cheese samples as stated in AOAC, 2002. With the use of an electronic pH meter that has a combination electrode, the pH levels of the cheese were determined **(Hanna, Germany).**

**Loss of weight**

Each block of cheese was accurately weighed both before and after the storage period. The formula below was used to calculate the mass (W):

$$W\% = \frac{m_i - m_t}{m_t} \times 100$$

Where mt is the weight at time t and mi is the starting weight.

**Analysis of the textural profile**

Using the double compression tester, TPA, or texture profile analysis, was done on a sample of Ras cheese from Food Technology Corporation, Slinfold, West Sussex, UK (Multiple test 1d memesin). The TPA examination was carried out at five distinct locations on the sample surface using a cylinder probe with a 25-mm diameter. It was recorded how much force (N) changed with time (s) on the created plot. The following variables were chosen in accordance with the International Dairy Federation's (1991) definition: Springiness (mm) = length 2nd compression/length 1st compression (L2/L1), Cohesiveness (N) = maximum force of first compression, Hardness (N) = maximum force of second compression. Gumminess (N) is equal to hardness and cohesiveness, while chewiness (mJ) is equal to gumminess and springiness.

**Microbiological evaluation of Ras cheese**

The number of yeasts and moulds was counted using sterile potato dextrose agar and a 10% solution of lactic acid to acidify the mixture to pH 3.5. (APHA, 1994). For four days, the plates were incubated aerobically at 25°C. Using violet bile agar media, the number of coliform bacteria was counted (Mossel, 1985). For 18 hours, the plates were incubated at 37°C. Plate count agar media was used to count the total number of microorganisms (Oxoid). The plates were incubated for 48 hours at 37 °C. (APHA, 1992).

**Characteristics of bionanocomposite films.**

***Analysis of IR spectral data.***

On a Shimadzu 8400S FT-IR Spectrophotometer, the prepared sample were recorded in the 400–4000 $cm^{-1}$ range.

***Scanning electron microscopy (SEM).***

The morphology of the generated nanocomposite was determined using scanning electron microscopy (SEM). (High Resolution Quanta FEG 250-SEM, Czech Republic) Without coating and under a low vacuum.

***Transmission electron microscopy (TEM).***

A transmission electron microscope (TEM) model JEOL JEM-1230 with an approximately 80 kV acceleration voltage was used to investigate the structure and morphology of the manufactured MO-NE. A small drop was placed on a copper grid covered in a Lacey carbon layer, and it was left to dry at first in the open air before being placed under a strong vacuum.

***Water vapour transmission rate of the bionanocomposites film.***

WVTR was measured using a GBI W303 (B) Water Vapor Permeability Analyzer (China) utilizing the cup method. The amount of water vapour that permeates the tested film was used to calculate its water vapour permeability. Additionally, WVTR was calculated using the following standards: the amount of water vapour transferred over a unit of space in a controlled environment of 38 °C and 4% humidity for a unit of time. (E96D1653). The following formula was used to determine the WVTR:

**WVTR** $= \Delta m \times h / S \times T \times \Delta p$

where $\Delta m$ is the weight change, h is the film samples' thickness (mm), S is the area where water vapour is transferred ($m^2$), T is the time (s), and $\Delta p$ is the partial pressure of water vapour on the film samples' two sides.

***Oxygen Transmission Rate (OTR) of the bionanocomposites film***

Following ASTM (American Society for Testing and Materials) Standard F1927 at 23 °C and regulated relative humidity, the OTR of the films was measured using a Mocon Ox-Tran 2/21 (ST or SH module, Minneapolis, MN, USA) (RH). Using an oxygen-free carrier gas, the diffusion cell was cleansed of any remaining oxygen before clamping in flat sheet samples. The oxygen molecules then passed through the film to the interior chamber (90% RH), where they were carried to the sensor by the carrier gas. Next, pure oxygen (99.9%) was

supplied into the exterior chamber of the diffusion cell (50% RH). The oxygen transmission rate (OTR) was determined by accounting for the oxygen pressure gradient in the steady-state flow rate and expressed in [cc/m2 •day •atm]. Each film was measured three times. Using the same tools and following ASTM Standard F1307 at 23 °C with 50% RH outside and 90% RH inside the package, the OTR of the thermoformed trays was measured.

***Procedure cytotoxicity at various concentrations MO-NE***

The following operations were all carried out in a sterile setting while employing a Class II A2 Laminar Flow Biosafety Cabinet (manufactured by Labconco). In a $CO_2$ incubator, cells were suspended in DMEM medium with a 1% antibiotic-antimycotic mixture (10,000 U/ml Potassium Penicillin, 10,000 g/ml Streptomycin Sulfate, and 25 g/ml Amphotericin B), 1% L-glutamine, and 5% fetal bovine serum (Sartorius stedium, biotech).

Cells were batch cultured for 10 days, then seeded at a density of 10 x 103 cells/well in fresh complete growth medium in 96-well plastic plates at 37°C for 24 h under 5% $CO_2$ either alone (negative control) or with various Moringa, concentrations to give a final concentration of (600, 300, 150, 75, 37.5 ug/ml). The medium was removed from the wells after 48 hours of incubation, 20 ul of MTT salt (2.5 g/ml) was added, and the wells were then incubated for an additional four hours at 37°C with 5% $CO_2$. 200 µL of 10% sodium dodecyl sulphate (SDS) in 0.01 m HCL was added to each well and allowed to sit overnight at 37oC to stop the reaction and dissolve the crystals that had formed.

A known cytotoxic natural substance at a concentration of 100 g/ml was chosen as a positive control since it causes 100% fatality under identical conditions (Thabrew, *et al*., 1997). The absorbance was then measured at 595 nm with a reference wavelength of 620 nm using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA). Viability = Drug Absorbance / Control Absorbance x 100 Viability - 100 times cytotoxicity.

***Mechanical properties of the prepared bionanocomposites film.***

Using a universal testing machine (Hants, UK) equipped with a 5 KN load cell and operating at a rate of 5 mm/min on the samples, the mechanical characteristics of the produced CS/WPC/MO-NE bionanocomposite were measured following the ASTM D638-918 standard.

***Size of the particles***

A dynamic light scattering technique was used to measure the nanoemulsion's particle size (Zetasizer Nano-Zs, Malvern Instruments, and Worcestershire, UK). The measurement was done using backscattering light, and the average particle diameters were given as Z-average diameter in nm.

## RESULTS AND DISCUSSION

**Evaluation of the prepared Moringa oil nanoemulsion's morphology (MO-NE)**

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) techniques were used to analyse the morphologies. The surface morphology of the created MO-NE was examined using TEM investigations, which revealed that the particles' diameters are less than 100 nm, as shown in Fig. 1 A. It shows a uniform, good dispersion of MO-NE that contains poorly regarded particles and has gathered tiny crystals.

**Size of the Particle Moringa oil Nano Emulsion**

The DLS method is used to analyse particle size. The particle size of the emulsion, which had the following composition: water: Moringa oil: tween80 (80:10:10), was found to be 202.9 nm. **Fig. 1B**'s depiction of particle size (Jasmina *et al*. 2017)

**Structure evaluation of the prepared bionanocomposites.**

The function of the bending vibrations of -OH and -NH and the characteristic FT-IR spectrum of chitosan/whey protein concentrate (Fig. 2a) showed the band of absorption at wavelength 3280 $cm^{-1}$. The C=O and C-O stretching were also indicated by the absorption bands between 1720 and 1380 $cm^{-1}$. The absorption band at 1570 $cm^{-1}$ was also caused by the N-H bending of amino groups, whereas the absorption bands at 1190 and 1060 $cm^{-1}$, respectively, were caused by the C-O-C skeleton is stretched symmetrically, and the C-O stretching vibrating (Tang, Qian, & Shi, 2007).

Accordingly, Figure 2a shows the FT-IR spectra of CS/WPC, which are provided with all of the main characteristic bands of CS and WPC. The intra-hydrogen bond that was established between the OH of chitosan and

the NH2 of WPC may be to blame for the change in the absorption bands between 3600 and 2960 $cm^{-1}$. CS/WPC/2.5% MO-NE FT-IR spectra are shown in Figure 2b, c. Water MO-OH bending modes are represented by the band seen around 1630 $cm^{-1}$, whereas MO-O bending modes are represented by the peak at 1385 $cm^{-1}$.

**Morphological characteristics of the synthesised bionanocomposites**

The morphological structure and dispersion of the created CS/WPC/MO-NE bionanocomposites are visible using scanning electron microscopy. The SEM images of CS/WPC blends and CS/WPC/MO-NE bionanocomposites loaded with various MO-NE content are shown in Figure 3. The SEM results from Fig. 3a demonstrated that the chitosan and whey protein concentrate in the CS/WPC mixture were homogeneous, and had a good correlation. Additionally, the SEM showed good dispersion of the obtained MO-NE up to 7.5% and indicated that little of the CS/WPC matrix experiences agglomeration, as demonstrated by enhancing the MO-generated NE's concentration in the CS/WPC blend from 2.5 to 7.5%. (Fig. 3b, c).

**Mechanical characteristics of the CS/WPC/MO-NE prepared bionanocomposites**

The tensile study can be used to determine important mechanical parameters such as the elastic modulus, yield strength, ultimate tensile strength, ductility, and toughness. The synthesised CS/WPC/MO-NE bionanocomposites films with various MO-NE ratios' mechanical characteristics are displayed in Table 1. It shows that by increasing the loaded quantities by the corresponding percentages (2.5, 5.0, and 7.5%) based on the CS/WPC blend, the mechanical characteristics of the manufactured bionanocomposites based on MO-NE were lowered. When the amount of MO-NE in the matrix was increased from (2.5, 5.0, and 7.5%), the tensile strength reduced from 45.36 mm for the blank CS/WPC blend to (8.16, 8.72, and 12.27 mm), respectively. This result can be explained by changing the loaded amounts by the following percentages, depending on the CS/WPC blend: 2.5, 5.0, and 7.5%. That good interfacial adhesion is attained at low MO-NE loadings. This enables a component of the tensile strength while allowing the interfacial arrangement of the created bionanocomposite. Elongation was reduced to 35.07% by including 7.5% MO-NE in the films, compared to 151.19% for a pure CS/WPC blend, by increasing the amount of MO-NE in the bionanocomposites matrix. Although MO-NE has a higher contact surface area with the polymer matrix than other inorganic fillers, the tensile strength and elongation have decreased as a result of the good interaction between the CS/WPC blends and the filler. Other research studies have proven that the zone of connection between the MO-NE and CS/WPC blends is one of the important factors linked to the development of bionanocomposites with better characteristics (Archanaa *et al*., 2013; Hayeemasae *et al*., 2015; Pinto *et al*., 2015; Archanaa *et al*., 2013).

**The prepared CS/WPC/MO-NE bionanocomposites were analyzed for both water vapour transmission rate and oxygen transmission rate.**

The water vapour transmission rate (WVTR) is an important consideration when determining if a material is suitable for use in food packaging. Therefore, the stability and quality of the food throughout its shelf life are significantly impacted by the movement of water vapour from the environment in the area; either moisture is added to the food or moisture is lost from the food to the environment. It is revealed in WVTR for the created CS/WPC/MO-NE bionanocomposite films (Table 2). The information on the WVTR device showed that CS/WPC/MO-NE films had better WVTR than CS/WPC films. The WVTR values in the manufactured CS/WPC/MO-NE bionanocomposites dramatically increased as the MO-NE loadings were increased. The film's WVTR value increased to 1250.33, 1432.48, and 1614.89 $g/m^2$.day and 2063.98 $g/m^2$.day), respectively, with the addition of 2.5, 5.0%, and 7.5% MO-NE.

The water vapour transmission mechanism can be broken down into several stages, with the first stage involving the evaporation and liquefaction of water vapour, the bionanocomposites film that has been created. The next stage involves the transmission of liquid water through the film, and the last stage involves the evaporation of water vapour from the opposite side of the film (Saxena & Ragauskas, 2009). The OTR of each of the studied bionanocomposites is shown in Table 2. The films had improved oxygen barrier qualities; depending on the individual film thickness, the OTR of the blank and treatments varied between 7.54, 9.36, 13.70, and 7.5 $g/m^2$/day. These findings are in accordance with permeability information compiled by Vandewijngaarden *et a*l. (2014).

**Cytotoxicity of different concentration of MO-NE.**

A human hepatocellular carcinoma cell line HePG 2, was used to examine the material. Using MTT tests, the sample concentration ranges from 500 to 31.25 g/ml. The two concentrations of Moringa oil nanoemulsion, 2.5 and 7.5%, had toxicological results of 163.949 and 259 ug/mL, respectively.

**Gross composition of cheese**

According to Table 3, packaging and the elongation of the storage period had a substantial (P<0.05) impact on the moisture content of Ras cheese. When compared to cheese coated with varying amounts of MO-NE, the cheese coated without MO-NE in the coat (control) sample showed a more pronounced decrease in moisture content. After 90 days of storage, 2.5% MO-NE film-coated cheese had the highest moisture content (31.0%) and control cheese had the lowest (27.11%). The fact that the coated cheese's moisture content increased as it ripened shows that the nanocomposite used enhanced the cheese's barrier qualities. Film against water vapour permeability utilising chitosan and whey protein concentrate films in agreement with previous research (El-Sisi *et al*, 2015).

There were significant differences between the treatments and the Ras cheese's fat composition dramatically increased (P<0.05) when being stored; Table 3. The rise in the TSs during storage was the primary cause of the increase in the fat percentage. Ras cheese's changes in fat content during preservation were agreed with by Abd Elsalam *et al*, (2011). The lipolysis that often takes place in Ras cheese during maturation is to blame for the departure resulting from the strong bond between the contents of fat and moisture during the final phases of cheese maturation (AbouDonia, 2002)

. Low-fat content is caused by the free fatty acids that are released from fat during hydrolysis, evading the Gerber determination method utilized in the current study. Additionally, in accordance with Table 3, the total nitrogen (TN) component of Ras cheese from all treatments and the control increased (P<0.05) over the course of the storage time, reaching the maximum levels after 90 days of storage, which may be mostly attributable to moisture losses**.** Contrary to what El-Sisi *et al*. found, there were no appreciable variations in TN content among the various treatments (2015). The amount of proteolysis that has taken place while the Ras cheese has been stored is indicated by its soluble nitrogen (SN) level.

The findings in Table 3 show that the SN content of the control and other treatments increased significantly (P<0.05) during storage, which was a contributing factor in the major variations in cheese ripening between the various treatments.

This shows that Ras cheese ripened normally without interference from the covering. During ripening, the proteins in Ras cheese are subjected to proteolysis, which results in a complex medley of protein degradation products (Abd ElSalam *et al.,* 2011). During storage, Ras cheese's titratable acidity significantly rose (P<0.05), while cheese from the other treatments and the control cheese saw a significant pH reduction (P<0.05). Table 3. No significant differences (P<0.05) in the percent acidity and pH between the control and coated Ras cheese from the various treatments were observed, indicating that the addition of MO-NE in the coating material did not block the natural acid formation.

The weight of the cheese was calculated each month, and weight decreases were expressed as a percentage of the starting cheese weight. In comparison to coated cheese without the addition of MO-NE in the coat, the weight losses from the various treatments were significantly (P<0.05) lower for coated cheese Table 3. After 90 days of storage, cheese covered with 2.5% MO-NE film lost the least amount of weight (25.16%). Weight loss and storage time were shown to be significantly correlated (P<0.05), which is mostly due to moisture losses. The increased barrier qualities of the employed coating to water vapour can be used to explain why reduced-fat cheese that has been MO-NE-coated loses weight. Similar results were obtained for cheeses covered by chitosan and an edible film made of whey protein concentrate (El-Sisi *et al*., 2015). Kavas, Kavas, and Saygili (2015) found that cheese samples with film on them avoided rind formation and reduced economic losses.

**Texture profile analysis of Ras cheese**

Results in Table 4 show that throughout ripening, Ras cheese's hardness was considerably (P>0.05) higher in all treatments. These increases were mostly brought on by moisture loss during storage. The hardness of cheese covered with bionanocomposite films without additional MO-NE or with films having various concentrations of MO-NE did not differ significantly (P > 0.05). On the cohesion of Ras cheese, both the storage time

and coating had significant (P>0.05) effects. As a result, cohesiveness was reduced with longer storage, and this drop was more obvious in cheese covered with films that had a high percentage of MO-NE. On the other hand, the Ras cheese's other material characteristics were unaffected by coating and storage time (P > 0.05).

**Organoleptic properties of Ras cheese coated and uncoated CS/WPC/MO-NE bionanocomposites**

Odor and taste have been sensory used instinctively from the beginning of humans to improve in eating selection and improve the edibility of foods (Maarse, 1991). Table 5 displays the overall results of the sensorial assessment of Ras cheeses. It was found that Ras cheeses containing MO-NE did not significantly ($p \leq 0.05$) better control cheese in taste, body and texture, and appearance. As indicated in Table 5, the addition of MO-NE improved the flavour of Ras cheese treatments when compared to control cheese. It might be because MO-NE contains volatile chemicals, which essentially impact cheese flavour and make it more edible. Arimboor *et al*., 2015; Fadavi & Beglaryan, 2015; Ibrahim *et al*., 2019; Solhi *et al*., 2020b. Additionally, Table 5 showed that there were no significant ($p \leq 0.05$) variations between the body and texture of the experimental cheeses containing MO-NE and the control cheeses. Rheological characteristics of these findings supported them. Table 5 shows that every treated cheese scored considerably ($p \leq 0.05$) higher than control cheese in terms of appearance of Ras cheese. Ras cheese's better appearance may be attributable to characteristics MO- NE.

**Microbiological analysis of Ras cheese**

During ripening, Ras cheese's microbial cell population changes Table 6. Because the cheese used in this study was prepared from raw milk, it had high levels of bacteria and mould Table 6, 7. (Without coating). Throughout the control and every other treatment in the coated cheese, there was a substantial (P<0.05) drop in the total bacterial count (TBC), mould count, and coliform count over the storage time. However, yeast counts considerably (P<0.05) rose throughout the control and every other treatment in the coated cheese during the storage period. An earlier study (Youssef *et al*., 2018) found that the coating material utilized had poor oxygen permeability, which would reduce the oxygen content in the cheese matrix and enhance lactobacillus development. The current findings concur with those published by Kong, Chen, Xing, and Park (2010), who discovered that lactic acid bacteria were more viable in cheeses coated with chitosan than in cheeses that weren't.

With the exception of the coliform populations, where its count was significantly (P<0.05) reduced at the end of the storage period by raising the concentration of MO-NE, these differences were observed in the bacterial, mould, and yeast counts between the coated and uncoated cheese throughout the storage period. As a result, the Ras cheese coating significantly impacted the documented natural changes in Ras cheese microflora during ripening (Abdou *et al.*, 1977; Abd El-Salam *et al*., 2011).

Table 7 displays the surface mould growth of Ras cheese that has been coated and uncoated, with or without the addition of MO-NE during storage.

The surface of the control cheese had significant mould development, while the surfaces of cheese that had been including films that contain 2.5, 5.5%, and 7.5% MO-NE mould were not affected. These results indicate that chitosan and MO-NE had an inhibitory effect on surface mold growth.

## CONCLUSIONS

In the investigation, Ras cheese was covered with materials that had an appropriate tensile elasticity and penetration to water vapour. These materials were made from chitosan, whey protein concentrate, glycerol, and different amounts of MO-NE. The coating material with 2.5% MO-NE lost the least amount of weight while the cheese was ripening without changing its natural ripening procedures. It also inhibited mould growth on the cheese surface.

**Conflict of interest:**

The authors declare that there is no conflict of interest in this study

## REFERENCES

Abd El-Salam, M.H., Hippen, A.R., Assem, F.M., El-Shafei, Kawther, Tawfik, N. F., ElAassar, M. 2011: Preparation and properties of probiotic cheese high in conjugated linoleic acid content. International Journal of Dairy Technology, 64(1), 64–74.

Abdou, S.M., Abd El-Hamid, L.B., Dawood, A.H.M., Youssef, A.M., Mahran, G.A. 1977: Studies on Cephalotyre “Ras” cheese

coating.II. EffECT on ripening. Egyptian Journal of Dairy Science, 5, 191–200.

Abou-Donia, S.A. 2002: Recent development Ras cheese research: A review. Egyptian Journal of Dairy Science, 30, 155–166.

American Public Health Association, (APHA) 1992: Compendium of Methods for the Microbiological Examination of Food. 3rd Edition. American Public Health Association, Washington D.C., USA.

American Public Health Association, (APHA) 1994: Standard Methods for Examination of Dairy Products. 16th Ed. Washington, USA.

AOAC 2002: Official Methods of Analysis. 25th Edition. Association of the Official Analytical Chemists, Arlington VA, USA.

Archanaa, D., Singh, B.K., Dutta, J., Duttaa, P.K. 2013: In vivo evaluation of chitosan–PVP–titanium dioxide nanocomposite as wound dressing material.Carbohydrate Polymers, 95, 530–539.

Arimboor, R., Natarajan, R.B., Menon, K.R., Chandrasekhar, L.P., Moorkoth, V. 2015: Red pepper (*Capsicum annuum*) carotenoids as a source of natural food colors: Analysis and stability—A review. *Journal of Food Science and Technology*, *52*(3), 1258–1271. https://doi. org/10.1007/s1319 7-014-1260-7.

El-Sisi, A.S., Gapr, A.M., Kamaly, K.M. 2015: Use of chitosan as an edible coating in Ras cheese. Biolife, 3, 564–570. https://doi.org/10.17812/blj2015.32.32.

Fadavi, A., Beglaryan, R. 2015: Optimization of UF-Feta cheese preparation, enriched by peppermint extract. *Journal of Food Science and Technology*, *52*, 952–959.

Hayeemasae, N., Rathnayake, W., Ismail, H. 2015: Nanosized $TiO_2$-reinforced natural rubber composites prepared by latex compounding method. Journal of Vinyl and Additive Technology. https://doi.org/10.1002/vnl.21497.

Ibrahim, O.A., Mohamed, A.G., Bahgaat, W.K. 2019: Natural peppermint- flavored cheese. *Acta Scientiarum Polonorum Technologia Alimentaria*, *18*(1), 75–85. https://doi.org/10.17306 /J.AFS.0607.

Jasmina, H., Džana, O., Alisa, E., Edina, V., Ognjenka, R. 2017: Preparation of nanoemulsions by high-energy and lowenergy emulsification methods. In *CMBEBIH 2017* (pp. 317-322). Springer, Singapore.

Kavas, G., Kavas, N., Saygili, D. 2015: The effects of thyme and clove essential oil fortified edible films on the physical, chemical and microbiological characteristics of kashar cheese. Journal of Food Quality, 38, 405–412.

Kong, M., Chen, X.G., Xing, K., Park, H.J. 2010: Antimicrobial properties of chitosan and mode of action: A state of the art review. International Journal of Food Microbiolology, 144, 51–63. https://doi.org/10.1016/j.ijfoodmicro.2010.09.012.

Maarse, H. 1991: *Volatile compounds in foods and beverages*. New York,NY: Marcel Dekker Inc. *767* p.

Mossel, D.A.A. 1985: Media for Enterobacteriaceae. International Journal of Food Microbiology, 2, 27–32.

Pinto, D., Bernardo, L., Amaro, A., Lopes, S. 2015: Mechanical properties of epoxy nanocomposites using titanium dioxide as reinforcement-a review. Construction and Building Materials, 95, 506–524.

Poças, M.F., Pintado, M. 2010: Packaging and the shelf life of cheese. In G. L. Robertson (Ed.). Food packaging and shelf life: A practical guide (pp. 103–126). CRC Press.

Rehan, M., El-Naggar, M.E., Mashaly, H.M., Wilken, R. 2018: Nanocomposites based on chitosan/silver/clay for durable multi-functional properties of cotton fabrics.Carbohydrate Polymers, 182, 29–41.

Saxena, A., Ragauskas, A.J. 2009: Water transmission barrier properties of biodegradable films based on cellulosic whiskers and xylan. Carbohydrate Polymers, 78, 357–360.

Solhi, P., Azadmard-Damirchi, S., Hesari, J., Hamishehkar, H. 2020b: Production of the processed cheese containing tomato powder and evaluation of its rheological, chemical and sensory characteristics. *Journal of Food Science and Technology*, 1–8.

Šuput, D.Z., Lazić, V.L., Popović, S.Z., Hromiš, N.M. 2015: Edible films and coatings – Sources, properties and applications. Food and Feed Research, 42, 11–22.

Tang, Z., Qian, J., Shi, L. 2007: Preparation of chitosan nanoparticles as carrier for immobilized enzyme. Applied Biochemistry & Biotechnology, 136, 77–96.

Thabrew, M.I., Hughes, R.D., McFarlane, I.G. 1997: Screening of hepatoprotective plant components using a HepG2 cell cytotoxicity assay. *J Pharm Pharmacol*.49:1132-5.

Vandewijngaarden, J., Murariu, M., Dubois, P., Carleer, R., Yperman, J., Adriaensens, P., Schreurs, S., Lepot, N., Peeters, R., Buntinx, M. (2014) Gas permeability properties of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate). J. Polym. Environ. 2014, 22, 17.

Youssef, A.M. 2013: Polymer nanocomposites as a new trend for packaging applications. Polymer Plastics Technology & Engineering, 52, 635–660.

Youssef, A.M., El-Sayed, S.M. 2018: Bionanocomposites materials for food

packaging applications: Concepts and future Outlook. Carbohydrate Polymers, 193, 19–27.

Youssef, A.M., Abdel-Aziz, M.S., El-Sayed, M.S. 2014: Chitosan nanocomposite films based on Ag-NP and Au-NP biosynthesis by Bacillus subtilis as packaging material.International Journal of Biological Macromolecules, 69, 185–191.

Youssef, A.M., El-Sayed, S.M., El-Sayed, H.S., Salama, H.H., Assem, F.M., Abd ElSalam, M.H. 2018: Novel bionanocomposite materials used for packaging skimmed milk acid coagulated cheese (Karish). International Journal of Biological Macromolecules, 115, 1002–1011.

Youssef, A.M., El-Sayed, S.M., El-Sayed, H.S., Salama, H.H., Dufresne, A. 2016: Enhancement of Egyptian soft white cheese shelf life using a novel chitosan/carboxymethyl cellulose/zinc oxide bionanocomposite film. Carbohydrate Polymers, 151, 9–19.

Zhang, X.D., Xiao, G., Wang, Y., Zhao, Y., Su, H., Tan, T. 2017: Preparation of chitosan-$TiO_2$ composite film with efficient antimicrobial activities under visible light for food packaging applications. Carbohydrate Polymers, 169, 101–107.

**Table 1:** Mechanical properties of CS/WPC/MO-NE bionanocomposites loadings with different concentrations of MO-NE.

| Treatments | Mo-NE (%) | Tensile strength (mm) | Elongation (%) | Tensile stress (MPa) |
|---|---|---|---|---|
| CS/WPC blend | 0.0 | 45.36 | 151.19 | 1.24 |
| CS/WPC/MO-NE | 2.5 | 8.16 | 23.31 | 0.25 |
| CS/WPC/MO-NE | 5.0 | 8.72 | 26.23 | 0.18 |
| CS/WPC/MO-NE | 7.5 | 12.27 | 35.07 | 0.01 |

**Table 2:** Water vapor transmission rate (WVTR) and Oxygen transmission rate (OTR)

| Treatments | Mo-NE (%) | OTR, $g/m^2.day$ | WVTR, $g/m^2.day$ |
|---|---|---|---|
| CS/WPC (Blend) | 0.0 | 7.54 | 1250.33 |
| CS/WPC/Mo-NE bionanocomposites | 2.5 | 9.36 | 1432.48 |
| CS/WPC/Mo-NE bionanocomposites | 5.0 | 13.70 | 1614.89 |
| CS/WPC/Mo-NE bionanocomposites | 7.5 | 16.27 | 2063.98 |

**Table 3:** Changes in the chemical composition of Ras cheese during ripening as affected by coating

| Property | Ripening period (days) | Uncoated | Coated 0% MO-NE | Coated 2.5% MO-NE | Coated 5.0% MO-NE | Coated 7.5% MO-NE |
|---|---|---|---|---|---|---|
| Dry matter (DM) (%) | Fresh | 62.46 | 65.10 | 61.20 | 65.50 | 62.70 |
| | 30 | 67.24 | 68.50 | 65.10 | 68.96 | 67.97 |
| | 60 | 70.60 | 72.17 | 67.31 | 72.31 | 69.64 |
| | 90 | 72.89 | 74.65 | 69.00 | 74.12 | 71.43 |
| Fat % | Fresh | 32 | 34 | 32 | 33 | 33 |
| | 30 | 34 | 38 | 36 | 36 | 37 |
| | 60 | 35 | 39 | 37 | 38 | 38 |
| | 90 | 36 | 40 | 38 | 39 | 39 |
| Total nitrogen | Fresh | 3.97 | 4.18 | 3.91 | 4.21 | 4.03 |
| | 30 | 4.28 | 4.39 | 4.16 | 4.43 | 4.35 |
| | 60 | 4.46 | 4.63 | 4.29 | 4.58 | 4.44 |
| | 90 | 4.59 | 4.76 | 4.42 | 4.66 | 4.48 |
| Total protein | Fresh | 24.82 | 26.13 | 24.42 | 26.32 | 25.20 |
| | 30 | 26.93 | 27.43 | 26.03 | 27.66 | 27.18 |
| | 60 | 28.24 | 28.96 | 26.79 | 28.65 | 27.77 |
| | 90 | 28.94 | 29.73 | 27.60 | 29.13 | 28.00 |
| Soluble Nitrogen | Fresh | 3.06 | 3.39 | 3.12 | 3.30 | 3.13 |
| | 30 | 3.21 | 3.21 | 3.32 | 3.38 | 3.41 |
| | 60 | 3.51 | 3.55 | 3.39 | 3.56 | 3.38 |
| | 90 | 3.52 | 3.69 | 3.53 | 3.61 | 3.46 |
| Salt | Fresh | 3.27 | 3.11 | 3.17 | 3.34 | 3.16 |
| | 30 | 4.22 | 3.7 | 3.84 | 4.42 | 4.16 |
| | 60 | 4.98 | 5.54 | 5.14 | 4.88 | 4.58 |
| | 90 | 5.24 | 5.78 | 5.61 | 5.61 | 5.45 |
| Ash (%) | Fresh | 4.37 | 4.64 | 4.18 | 4.45 | 4.26 |
| | 30 | 4.54 | 4.76 | 4.43 | 4.69 | 4.49 |
| | 60 | 4.73 | 5.04 | 4.70 | 4.97 | 4.71 |
| | 90 | 5.11 | 5.67 | 4.79 | 5.08 | 4.98 |
| Acidity % | Fresh | 0.45 | 0.49 | 0.46 | 0.41 | 0.40 |
| | 30 | 0.71 | 0.79 | 0.81 | 0.79 | 0.83 |
| | 60 | 0.97 | 1.01 | 1.11 | 1.17 | 1.20 |
| | 90 | 1.29 | 1.31 | 1.43 | 1.41 | 1.54 |
| pH | Fresh | 6.21 | 6.30 | 6.18 | 6.22 | 6.31 |
| | 30 | 4.72 | 4.69 | 4.61 | 4.71 | 4.53 |
| | 60 | 4.31 | 4.40 | 4.21 | 4.03 | 4.14 |
| | 90 | 3.60 | 3.69 | 3.41 | 3.33 | 3.32 |
| weight loss % | | 38.47 | 37.67 | 25.16 | 33.30 | 30.55 |

**Table 4:** Textural properties of Ras cheese as affected by packaging and ripening period.

| Parameters | Ripening period (days) | Uncoated | Coated 0% MO-NE | Coated 2.5% MO-NE | Coated 5.0% MO-NE | Coated 7.5% MO-NE |
|---|---|---|---|---|---|---|
| Hardness (g) | Fresh | 52.25 | 67.58 | 68.69 | 72.36 | 75.62 |
| | 90 | 98.63 | 112.58 | 115.69 | 116.36 | 118.87 |
| Cohesiveness ratio | Fresh | 0.74 | 0.74 | 0.72 | 0.73 | 0.71 |
| | 90 | 0.41 | 0.42 | 0.39 | 0.39 | 0.37 |
| Springiness Mm | Fresh | 2.79 | 2.62 | 2.31 | 2.60 | 2.59 |
| | 90 | 2.81 | 2.61 | 2.30 | 2.61 | 2.60 |
| Gumminess (N) | Fresh | 38.83 | 50.05 | 52.23 | 55.88 | 57.45 |
| | 90 | 53.67 | 64.13 | 66.45 | 68.96 | 70.02 |
| Chewiness (N.mm) | Fresh | 108.50 | 131.27 | 132.55 | 135.68 | 139.64 |
| | 90 | 117.61 | 143.17 | 145.22 | 146.12 | 149.88 |
| Adhesiveness g/sec | Fresh | 0.0211 | 0.0323 | 0.0481 | 0.0497 | 0.0499 |
| | 90 | 0.5111 | 0.5323 | 0.5581 | 0.5597 | 0.5611 |

**Table 5:** Scoring of organoleptic properties resultant Ras cheese coated CS/WPC/MO-NE bionanocomposites after 3 months ripening.

| | Uncoated | Coated 0% MO-NE | Coated 2.5% MO-NE | Coated 5.0% MO-NE | Coated 7.5% MO-NE |
|---|---|---|---|---|---|
| Flavor (50) | 43 | 45 | 44 | 47 | 45 |
| Body &texture (40) | 35 | 35 | 34 | 34 | 37 |
| Appearance (10) | 8 | 9 | 8 | 9 | 9 |
| Total (100) | 86 | 89 | 86 | 90 | 91 |

**Table 6:** Changes in the microbiological quality of coated and uncoated in middle Ras cheese whale during ripening.

| | Storage period (3months) | | | |
|---|---|---|---|---|
| | fresh | 30 day | 60 day | 90 day |
| Total bacterial Count (log cfu ml-1) | | | | |
| Uncoated | 12.81 | 12.14 | 11.13 | 10.75 |
| Coated 0% MO-NE | 12.2 | 11.05 | 10.88 | 9.96 |
| Coated 2.5% MO-NE | 11.9 | 11.02 | 10.64 | 9.74 |
| Coated 5% MO-NE | 11.7 | 11.0 | 10.30 | 9.53 |
| Coated 7.5% MO-NE | 10.6 | 10.2 | 9.50 | 7.99 |
| Yeast (log cfu ml-1) | | | | |
| Uncoated | 4.07 | 4.39 | 5.02 | 6 |
| Coated 0% MO-NE | 3.90 | 4.02 | 4.44 | 5.2 |
| Coated 2.5% MO-NE | 3.80 | 3.96 | 4.22 | 4.95 |
| Coated 5% MO-NE | 3.55 | 3.93 | 4.47 | 4.58 |
| Coated 7.5% MO-NE | 3.04 | 3.52 | 4.12 | 4.52 |
| Coliform (log cfu ml-1) | | | | |
| Uncoated | 3.68 | 2.10 | 1.02 | ND |
| Coated 0% MO-NE | 3.23 | 2.05 | 1.25 | ND |
| Coated 2.5% MO-NE | 3.45 | 1.95 | 1.04 | ND |
| Coated 5% MO-NE | 3.09 | 1.84 | 1.20 | ND |
| Coated 7.5% MO-NE | 3.69 | 2.22 | 1.12 | ND |

ND Not detected

**Table 7:** Changes in the microbiological quality of coated and uncoated on surface Ras cheese whale during ripening.

| | Storage period (3months) | | | |
|---|---|---|---|---|
| | fresh | 30 day | 60 day | 90 day |
| | mold (log cfu ml-1) | | | |
| Uncoated | ND | 5.6 | 6.3 | 6.47 |
| Coated 0% MO-NE | ND | ND | ND | ND |
| Coated 2.5% MO-NE | ND | ND | ND | ND |
| Coated 5% MO-NE | ND | ND | ND | ND |
| Coated 7.5% MO-NE | ND | ND | ND | ND |

ND Not detected

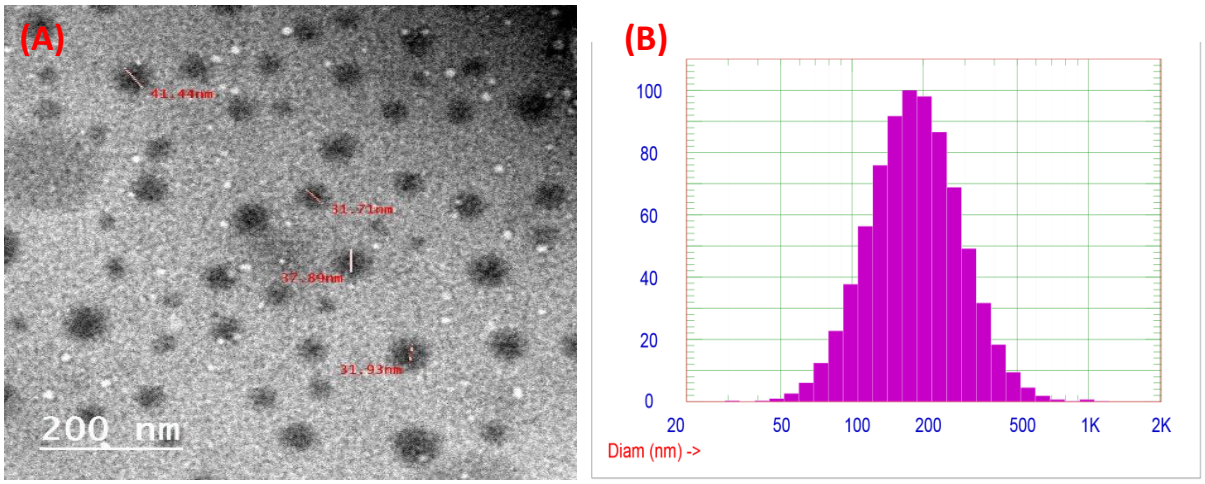


**Figure 1**: A) TEM and B) particle size distribution images of MO-NE

**Figure 2**: FT-IR. , a) CS/WPC blend, b) CS/WPC/2.5 %MO-NE, and c) CS/WPC/7.5 %MO-NE bionanocomposites.

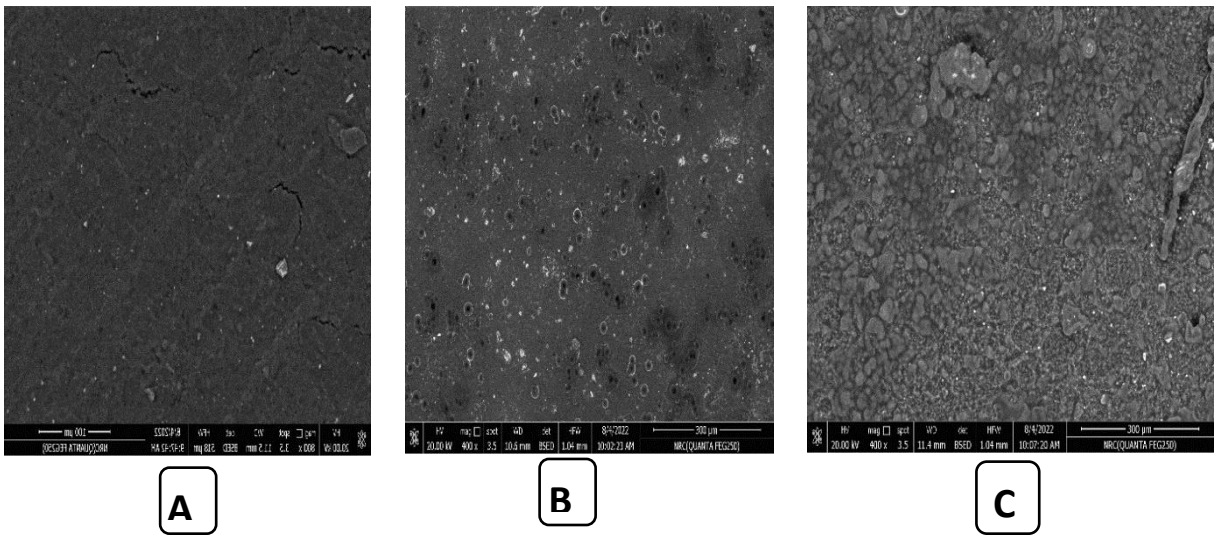


**Figure 3:** The SEM images of a) the prepared CS/WPC blend as well as CS/WPC/MO-NE bionanocomposites containing different ratios of MO-NE, b) 2.5% MO-NE and c) 7.5% MO-NE).

## تطوير المركبات الحيوية CS / WPC / MO-NE لتغليف جبن الراس بمستحلب نانوى من زيت المورينجا الأساسي

**خالد محمد عادل [1,*]، أحمد محمد عبدالوهاب يوسف [2*]، رزق عزب عواد [3*]، عبد الحكم محمد جمال الدين [1].**

*[1] قسم الألبان, كلية الزراعة، جامعة الأزهر، القاهرة، مصر.*
*[2] قسم التعبئة والتغليف، المركز القومى للبحوث، الدقى الجيزة، مصر.*
*[3] قسم علوم الأغذية، كلية الزراعة، جامعة عين شمس، القاهرة، مصر.*

[*] البريد الاليكتروني للباحث الرئيسي:khaled@azhar.edu.eg

### الملخص العـربي

الغرض من البحث هو إنشاء وتطوير مواد المركبات الحيوية وتقييم مدى ملاءمتها لتغطية جبن الراس. تمت إضافة مستحلب Moringa Nanoemulsion (MO-NE)بتركيزات تتراوح من 2.5 و 5.0 و 7.5٪ إلى الشيتوزان وبروتين اللبن المركز (CS / WPC) الذي تم استخدامهم لإنشاء مواد المركبات الحيوية. باستخدام SEM و TEM و FT-IR ، تم تقييم المستحلبات النانوية المصنعة والمركبات الحيوية. بالإضافة إلى ذلك ، تم أيضًا تقييم الاختبارات الميكانيكية للمركبات الحيوية المركبة ، ومعدل انتقال بخار الماء (WVTR) ، ومعدل انتقال الأكسجين .(OTR) بالمقارنة مع الجبن غير المطلي ، تم تقييم تأثير تغطية جبن الراس بمركب نانوي مرتب على إنقاص الوزن والخصائص الكيميائية والتركيبية والبكتيرية. قلل طلاء الجبن من فقدان الوزن والرطوبة ولكن كان له تأثير ضئيل على تعديلات النضج النموذجية لتكوين وقوام جبن الراس. أما بالنسبة للخصائص الميكروبيولوجية فلم يظهر العفن على القشرة المغطاة لجبن الراس. وهي مغطاة بغشاء من مستحلب المورينجا النانوى MO-NE مع الشيتوزان وبروتين اللبن المركز.(CS/ WPC)

**الكلمات الاسترشادية:** مركبات النانو الحيوية, جبن الراس, الشيتوزان,مركز بروتينات الشرش, التعبئة.