



# Enhancement of Egyptian soft white cheese shelf life using a novel chitosan/carboxymethyl cellulose/zinc oxide bionanocomposite film



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## ABSTRACT

A novel bionanocomposites packaging material prepared using chitosan (CH), carboxymethyl cellulose (CMC), and zinc oxide nanoparticles (ZnO-NPs), namely CH/CMC/ZnO bionanocomposites, was prepared by casting method. The CH/CMC/ZnO bionanocomposites were investigated using FT-IR, TEM, SEM, XRD, and TGA. The acquired bionanocomposites exhibited improved mechanical and thermal properties compare with the biocomposites (CH/CMC) blend. The soft white cheese were manufactured, packaged within the prepared bionanocomposites films and stored at 7 °C for 30 days. The influence of packaging material on packaged cheese (rheological properties, colour measurements, moisture, pH and titratable acidity) were assessed. Furthermore, the effect of packaging material on the total bacterial counts, mold & yeast and coliform in cheese was evaluated. The prepared bionanocomposites displayed good antibacterial activity against gram positive (*Staphylococcus aureus*), gram negative (*Pseudomonas aeruginosa*, *Escherichia coli*) bacteria and fungi (*Candida albicans*). Moreover, the packaging films assisted in increasing the shelf life of white soft cheese. Therefore, it can be used in food packaging applications.

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## 1. Introduction

Current efforts are devoted in using nanotechnology to meet the requirements of consumers in confirming food's quality with extended shelf life during storage, distribution and in using antimicrobial agents to extend the shelf life of foods under storage and distribution conditions. Food industries have to choose which packaging material is more suitable for their food product according to the advantages and disadvantages of their choice or maybe what other characteristics can be incorporated in the packaging material based on the end use properties of the food product (Duncan, 2011). Besides, the rising demand for sustainability and environmental safety, a growing number of studies have been focused to improve food packaging materials that could rapidly damage and completely mineralize in environment (Majeed et al., 2013). Biopolymers have been one of the promising substitutes to be

exploited and established into eco-friendly food packaging materials attributable to its biodegradability (Tang, Kumar, Alavi, & Sandeep, 2012).

Polysaccharides are most promising applicants because they are made or come from naturally abundant products and are readily biodegradable. Chitosan (CH) is a partly deacetylated derivative of chitin, which is the second most abundant natural biopolymer next to cellulose. Structurally, chitosan is composed of glucosamine and Na-cetyl glucosamine units linked by  $\beta$ -1-4 glucosidic bonds. Moreover, chitosan has been broadly examined for food packaging film (Youssef, Abdel-Aziz, & El-Sayed, 2014), artificial skin, bone substitutes, water engineering and so on, due to its good mechanical properties, nontoxicity, readily biocompatibility, biodegradability. Providentially, carboxymethyl cellulose (CMC) is a natural biodegradable and biocompatible anionic polymer gained from natural cellulose by chemical modification, and it is very similar to CH in structure, therefore, there is strong ionic crosslinking bond due to the difference in their structures, amino groups of (CH) vs. carboxyl groups of (CMC), which result in biocompatibility in the prepared CH/CMC blend (Qiu and Li, 2005).

Because of their antimicrobial properties, metal nanoparticles are used in the food industry, particularly for coating of food

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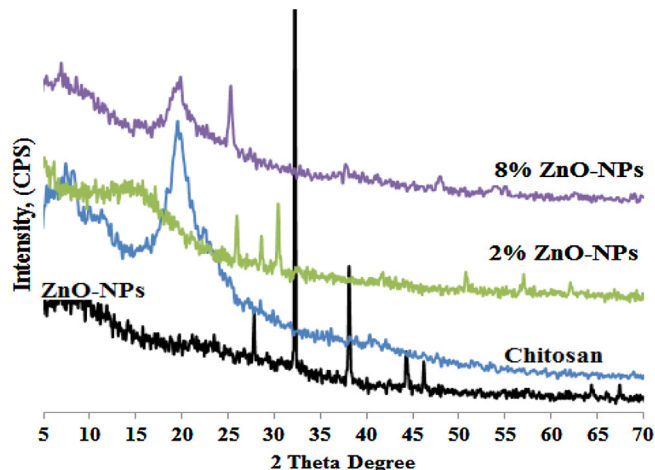
**Table 1**  
The formulations, weights, volumes, yields and thickness of the prepared bionanocomposites films.

Sample name	CH (g)	CMC (g)	ZnO-NPs (g)	Volume (ml)	Film Weight (g)	Yield%	Film Thickness ( $\mu\text{m}$ )
Blank (CH/CMC)	2	2	–	100	2.00	100	0.94
CH/CMC/ZnO (2%)	2	2	0.04	100	2.02	100	0.95
CH/CMC/ZnO (4%)	2	2	0.16	100	2.08	100	0.95
CH/CMC/ZnO (8%)	2	2	0.32	100	2.16	100	0.95

processing equipment and packaging material to diminish food spoilage and foodborne diseases by pathogenic bacteria (Youssef, Kamel, & El-Samahy, 2013; Morris, 2011). ZnO-NPs are an eco-friendly and have attracted extensive attention owing to its good antibacterial properties, high stability, photocatalytic activity and non-toxicity (Wang, 2004). Furthermore, ZnO-NPs have several significant applications in biomedical field (Riggio, Raffa, & Cuschieri, 2010), as food additive, and in catalysis (Liu, Morishima, Yatsui, Kawazoe, & Ohtsu, 2011). ZnO-NPs have revealed effective antibacterial activity on a broad spectrum of bacteria (gram positive and gram-negative bacteria) (Huang et al., 2008). It works on biocidal effects on bacterial, fungal, and viral species (Adams, Lyon, & Alvarez, 2006). The successful preparation of chitosan/ZnO nanocomposites has been described and enhancement in the antibacterial properties of chitosan with the inclusion of ZnO was observed (Patale & Patravale, 2011). The incorporation of ZnO nanoparticles on biopolymers matrix as nanofillers could improve not only the fabricated bionanocomposites, mechanical and barrier properties but also create other functions and applications in food packaging such as antimicrobial agent (Youssef, Abou-Yousef, El-Sayed, & Kamel, 2015). The bionanocomposites can be as a smart food packaging whereby it can observe property of the packaged food such as microbial contamination or expiration date and uses some mechanism to record and convey information about the quality or safety of the packaged food (Azeredo, 2009).

Soft white cheese is the main kind of cheese available to the public in large quantities on the markets of Egypt. Packaging of cheese is one of the most significant steps in the long trip from the producer to the consumer, since most of the cheese factories are far away from the consumption. Packaging of cheese must provide common protection of the product from mechanical damage and poor environmental conditions through handling and distribution. Optimum packaging solutions could prevent or minimize quality changes, resulting in increased shelf life as well as quality preservation. Various types of cheeses have to be packaged within different packaging designs (Youssef, EL-Sayed, Salama, EL-Sayed, & Dufresne, 2015). Most fresh cheeses are packaged in air atmosphere owing to their short shelf life. A number of experiments confirmed that the chemical composition and sensory characteristics, colour and body of soft white cheese made from pasteurized cow milk during a storage period of 45 days in vacuum packaging did not significantly change (Osman, Abdalla, & Mohamed, 2009).

Based on the above statements, the current work is a step towards using bionanocomposites as packaging materials. Bionanocomposite films fabricated from chitosan, CMC and ZnO-NPs were carefully evaluated regarding their physical, chemical and mechanical properties. To the best of our knowledge, this is the first work where such a system (CH/CMC/ZnO bionanocomposite) was manufactured and characterized in terms of morphological, mechanical and thermal properties with different ZnO-NP contents. The prepared bionanocomposite films play an important role for increasing the shelf life of Egyptian soft white cheese and keeping the chemical and physical properties of packaged cheese after the storage period. This new CH/CMC/ZnO bionanocomposite films can be used as eco-friendly and sustainable material for food packaging with improved antimicrobial activity



**Fig. 1.** XRD patterns for chitosan, ZnO-NPs and CH/CMC/ZnO bionanocomposites loaded with 2% and 8 wt% ZnO-NPs.

## 2. Materials and methods

### 2.1. Materials

Chitosan (CH) powder (Aldrich Chemical), medium molecular weight  $MW = 161,000 \text{ g/mol}$ , degree of deacetylation,  $DD = 75.6\%$ , and viscosity  $1406 \text{ mPas}$  (1% in 1% acetic) were used without further purification. Glacial acetic acid (HAc) obtained from Aldrich Chemicals was used as the solvent for CH. CMC ( $M_w 4.2 \times 10^8$ , degree of substitution of 0.7) was purchased from Kelong Chemical Agent Factory, Chengdu, China. Zinc-acetate (puriss, Reanal, Hungary) ( $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ ) was used to prepare ZnO nanoparticles. Buffalo's milk containing (5% fat and 8.57% (solid nonfat, SNF)) was used for manufacturing soft white cheese. Chymocin rennet powder was obtained from Chr. Hansen's Lab., Denmark, and was used for manufacturing cheese. NaCl and  $\text{CaCl}_2$  were used for salting soft white cheese and were obtained from Rankem Chemical Company. All materials were used without further purification.

#### 2.1.1. Pathogenic strains

*Staph. aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus cereus* B-3711 and *Bacillus subtilis* (ATCC 6633) were provided by the Northern Regional Research Laboratory Illinois, USA. *Listeria monocytogenes* 598 was provided by the Department of Food Science, University of Massachusetts, Amherst MA, USA. *Escherichia coli* O175: H7 were isolated and serologically identified by dairy microbiological Lab., National Research Center. *Aspergillus niger* and *candidia albicans* (ATCC 36232) were provided by the Institute of Applied Microbiology, University of Tokyo, Japan.

#### 2.2. Manufacture of ZnO nanoparticles

In order to prepare ZnO-NPs, stock solutions of  $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$  (0.2 M) were prepared in 50 mL methanol under stirring. To this stock solution 50 mL of NaOH (0.3 M) in methanol was added under continuous stirring in order to reach the pH (10) value of reactants. These solutions were transferred

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