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Novel active packaging based on carboxymethyl cellulose-chitosan -ZnO NPs nanocomposite for increasing the shelf life of bread

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ABSTRACT

In this study a new nanocomposite film and coating based on chitosan-carboxymethyl cellulose-oleic acid (CMC-CH-OL) incorporated with different concentrations (0.5, 1 and 2%) of zinc oxide nano particles (ZnO NPs) have been suggested as a packaging material to increase the shelf life (microbial and staling) of sliced wheat bread. Water vapor permeability (WVP) of the CMC-CH film was significantly ($P < 0.05$) decreased after incorporation of oleic acid as well as ZnO NPs (2%) from 8.27×10^{-7} to 5.28×10^{-7} and 1.96×10^{-7} g/m.h.pa, respectively. Results of moisture content and water activity (a_w) showed better maintenance of moisture content for breads stored by active coatings compared to the control one (bread without coating). Differential scanning calorimetry (DSC) thermograms of control bread showed an endothermic peak corresponding to amylopectin retrogradation. Control exhibited the highest firmness over 15 days of storage in all other samples. The results of microbial tests revealed an increase in microbial shelf life of sliced wheat bread from 3 to 35 days for CMC-CH-OL-ZnO NPs 2% in compared to the control. All active coatings lessened the number of yeasts and molds in sliced bread during 15 days, and further improvement in antimicrobial properties obtained for coatings contains 1 and 2% ZnO NPs with no fungal growth during 15 days.

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1. Introduction

Bread is one of the oldest and main prepared food of most people all over the world. Due to increasing of the world's population as well as reducing of the food sources, food preservation management plays an important role in reducing food loss (Fazeli, Shahverdi, Sedaghat, Jamalifar, & Samadi, 2004). Mold spoilage and staling are two main factors, which restrict the quality of bread. Normally, shelf-life of bread without any preservative method is about 3–4 days (Muizniece Brasava et al., 2012). Limited shelf life of bread causes great economic losses annually around the world (Baik & Chinachoti, 2000; Maga & Ponte, 1975). Fungi is the most common spoiler in bakery products and most of the times, fungal growth specifies shelf life of bread and bakery products. Due to the presence of about 40% of water, bread has a water activity (a_w) of around 0.96, which makes it susceptible to mold growth (Cioban, Alexa, Sumalan, & Merce, 2010). *Penicillium, Aspergillus, Monilia, Mucor, Endomyces, Cladosporium, Fusarium, Alternaria* and *Rhizopus* include the most popular fungi in bread and bakery products (Alhendi & Choudhary, 2013; Cioban et al., 2010; Dal Bello et al., 2007; Luciana Gerez, Torino, Rollan, & De Valdez, 2009; Muhianldin, Hassan, & Saari, 2013; Rodriguez, Nerin, & Batlle, 2008). Recently, it was mentioned that about 60% of spoilage in bakery products caused by *Penicillium* spp. and *Aspergillus niger* (Alhendi & Choudhary, 2013). Addition of organic acids (propionic acid and its salts) (Muhianldin et al., 2013) is a common method to inhibit fungal growth in bread. However, consumers are demanding for food products without any chemical preservatives (Gutierrez, Sanchez, Batlle, & Nerin, 2009). As a solution, application of active packaging is a promising approach to increase the microbial shelf life of bread without direct addition of chemical agents. In this regards, previous studies have shown that the microbial shelf life of bread can be increased by active packaging such as cellulose acetate films incorporated with sodium propionate (Soares, Rutishauser, Melo, Cruz, & Andrade, 2002); gliadin films incorporated with cinnamaldeyde (Balaguer, Lopez-Carballo, Catala, Gavara, & Hernandez-Munoz, 2013); methylcellulose films incorporated with clove and oregano essential oils (Otoni, Pontes, Medeiros, & Soares, 2014); and Ag/TiO_2-polyethylene packaging (Mihaly Cozmuta et al., 2014). In addition, other methods such as modified atmosphere packaging

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(MAP) (Del Nobile, Matoriello, Cavella, & Giudici, Masi, 2003; Rasmussen & Hansen, 2001); combination of both active packaging and MAP (Degirmencioglu et al., 2011; Gutierrez et al., 2009; Muizniece Brasava et al., 2012; Nielsen & Rios, 2000) have been used to increase the microbial shelf life of bread.

Along with mold contamination, staling is another factor which limits the shelf life of bread. Staling is a complicated phenomenon that its exact mechanism is not completely founded. However, starch retrogradation which involves physicochemical changes in bread structure, is suggested as one main mechanism for bread staling. Moreover, other mechanisms such as cross linking between starch and protein, partial drying, glassy-rubbery change, and moisture transformation from crumb to crust of bread are involved in staling (Baik & Chinachoti, 2000; Besbes, Jury, Monteau, & Bail, 2014; Gray & Bemiller, 2003). Generally, staling is divided to crust staling and crump staling. Crust staling is attributed to migration of moisture from crumb to crust, but crump staling is related to physicochemical alteration in starch (Bhatt & Nagaraju, 2009). Some studies have been carried out on the effects of different hydrocolloids (Bhattacharya, Erazo-Castejon, Doehlert, & Mc Mullen, 2002; Guarda, Rosell, Benedito, & Galotto, 2004; Shalini & Laxmi, 2007), and anti-staling agents (Purhagen, Sjoo, & Eliasson, 2011; Purhagen, Sjoo, & Eliasson, 2012) on the bread staling. In addition, Licciardello, Cipri, & Muratore, (2014) studied the usefulness of packaging material on the control of bread staling. Ahmadi, Azizi, Abbasi, Hadian, and Sareminezhad (2011) exhibited retardation in bread staling by measuring mechanical and organoleptic tests in breads were kept by edible active coatings of HPMC-corn starch and sunflower oil. In another study, Salehifar, Beladi Nejad, Alizadeh, and Azizi (2013) studied on the effects of LDPE- multi walled carbon nanotube (MWCNT) nanocomposite films on the shelf life of Lavash bread. Increase in bread shelf life have reported by improvement in water and oxygen barrier properties of LDPE-MWCNT nanocomposites films.

Application of biopolymers is a favorable approach to produce active biodegradable packaging. Chitosan (CH) is an amino polysaccharide polymer that shows superior antimicrobial properties (Mitelut, Tanase, Popa, & Popa, 2015). For reasons of biodegradability, having desirable film forming properties, high permeability to gases, and non-toxicity, chitosan is known as a promising polymer for active packaging (Kanatt, Rao, Chawla, & Sharma, 2012). Carboxymethyl cellulose (CMC), a derivative of cellulose, shows good barrier properties against oxygen and lipids, and it forms transparent films that is a desirable feature to consumer acceptance (Ebrahimzadeh, Ghanbarzadeh, & Hamishehkar, 2016; Ghanbarzadeh & Almasi, 2011). Since CMC is an ionic polymer, and CH is a cationic polymer, they are biocompatible by formation of strong ionic cross-linking bonds (Youssef, El-Sayed, El-Sayed, Salama, & Dufresne, 2016). Thus, in this study, to produce antimicrobial films with transparent views, chitosan and CMC are mixed and CMC-CH blend film is produced. On the other hand, decrease in water vapor permeability (WVP) of films is an important parameter in food packaging application. We hypothesize that reduction of water vapor transmission from packaging film may lead to a reduction in the rate of bread staling. Previous studies (Preda, Amica & Marcovich, 2012; Vargas, Albors, Chiralt, & Gonzalez-Martinez, 2009; Wang et al., 2014), have shown the effects of lipid compounds on WVP and hydrophobicity of films. Therefore, in this study oleic acid (OL) was added to the CMC-CH blend and an emulsified film was produced.

Zinc oxide is an interesting agent in food and pharmacy fields due to its antimicrobial properties, high stability, non-toxicity, and good UV absorbance properties. Additionally, it is generally recognized as safe (GRAS) from FDA (182.8991 code) (Shahmohammadi Jebel & Almasi, 2016). The antimicrobial efficiency of ZnO NPs in active films, have reported by several studies (Arfat, Benjakul, Prodpran, Sumpavapol, & Songtipya, 2014; Pantani, Gorrasi, Vigliotta, Murariu, & Dubois, 2013; Sanuja, Agalya & Umapathym, 2015). It seems that, addition of ZnO NPs in active packaging is a promising method to inhibit fungal growth in bread. Additionally, according to the previous studies (Arfat et al., 2014; Mohammadi Nafchi, Alias, Mahmud, & Robal, 2012; Shahabi-Ghahfarrokhi, Khodaiyan, Mousavi, & Yousefi, 2015) ZnO NPs has positive effects on the mechanical and barrier properties of the films which is useful property in food packaging application.

Due to our knowledge, there are scarce reports on the CMC-CH film formulation. One is reported very recently by Youssef et al. (2016); that showed reduction of microbial growth in cheese by CMC-CH-ZnO NPs films. Also, in another work a CMC-CH film was prepared using layer by layer method and used as a coating for preserving of citrus fruits (Arnon, Granit, Porat, & Poverenov, 2015). The third one is reported by Hu, Wang, and Wang (2016); for CMC and quaternized chitosan. As far as we know, there is no study with CMC-CH-OL-ZnO NPs formulation. Additionally, a few studies have been carried out on the application of active packaging on bread staling. Thus, the objective of current work is production of active emulsified packaging based on CMC-CH-OL incorporated by ZnO NPs at different concentrations by solution casting method and investigation of the effects of this type of packaging on the microbial and physicochemical properties of sliced wheat bread.

2. Material and methods

2.1. Materials

Chitosan (low molecular weight, 20–300 cP 1, 50–190 kDa, deacetylation degree 75–85%) and carboxymethyl cellulose sodium salt (CMC) (high viscosity, 1500–3000 cP 1% in H_2O 25 °C) were bought from Sigma Aldrich. Analytical grade glycerol, oleic acid and tween 80 were obtained from Merck (Darmstadt, Germany), potato dextrose agar (PDA) were purchased from Biokar Diagnostics (Beauvais, France). ZnO NPs with average size of <25 nm was purchased from Sigma Aldrich. All other chemicals were of analytical grade.

2.2. Preparation of films

Films were elaborated as explained by Vargas et al. (2009) with some modifications. The ratio of Chitosan and CMC was 1:2; briefly, chitosan (0.2 g) was dispersed in 50.0 mL acidic water (0.5% v/v acetic acid) and stirred overnight; the pH of dispersion was adjusted to 6.8 with NaOH solution (3.0 M). ZnO NPs powder at different concentrations (0.5, 1 and 2% based on solid matter) was dispersed in water (50.0 mL) and sonicated for 30 min in an ultrasonic bath (Sonorex-super, Bandelin, Germany, 40 KHz). Then, 0.4 g CMC was dissolved in ZnO NPs suspension and the solution was mixed by stirrer for 1 h. Later, chitosan and CMC solutions were mixed together. Then, tween 80 (0.2 mL) was added as an emulsifier. After stirring for 15 min, OL (0.3 mL) was added, and the mixture, which has become turbid and milky white at the end, was homogenized by ultrasound probe (Bandelin Sonopuls, Germany, 31 KJ) for 15 min. Glycerol (0.3 mL) was added to plasticizing the solution and the mixture was stirred again for 15 min. Finally, 50.0 mL of each solution were poured into the petri dishes (9.0 cm diameter) and were dried at 25 °C and relative humidity (RH) of 50% for 72 h.

2.3. Preparation of coating on the surface of bread slices

In order to have a maximum contact between the active compounds and bread slices, active coatings were prepared as a model system to better estimation of staling rate and microbial

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