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Nanocomposite coating based on chitosan and ZnO nanoparticles to maintain the storage quality of meatball

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Abstract

The effect of nanocomposite coating from chitosan incorporated with ZnO nanoparticles (ZnO NPs) at various concentrations (0, 0.05, and 0.1%) on meatball characteristics during storage was investigated. Meatball quality including microbial growth, pH, a_w, and sensory attributes was evaluated after immersed into nanocomposite solution for 1 min and drained. The combination of chitosan and ZnO NPs exhibited a synergistic effect in suppressing gram-negative bacterial growth. Chitosan-ZnO NPs 0.05% could prolong the shelf life of meatball beyond 24 hrs indicated by aerobic microbes of 4.30 log CFU/g which was lower than the threshold for consumption (5 log CFU/g). Coating solution also significantly influenced the pH of meatballs up to 24 hrs of storage allowing lower sensory acceptability. Moreover, the low organoleptic score for taste and overall acceptability attributes were found as a consequence of the addition of ZnO NPs. Finally, the results of the present study suggested that chitosan-based coating containing ZnO NPs could be used as an alternative to maintaining meatball quality, particularly in microbial decay context.

1. Introduction

Meatball is a processed meat product, round-shaped, with the addition of some ingredients such as tapioca flour, white onion, pepper, egg and salt. The meatball in Indonesia is commonly known as "bakso" that mainly manufactured from meat and starch (Pranoto and Rakshit. 2008). Due to its ingredient which is mostly based on the meat, consequently, meatball is suitable for microbial growth and classified a highly perishable food. National Standardization Agency of Indonesia (SNI-01-3818-2014) has regulated that the maximum level requirement of meatball microbial counts is 5 log CFU/g.

An alternative method to maintain the quality of perishable food products is edible coating application which could be produced from different materials such as lipids, proteins, and polysaccharides or blended of those components. Previous studies recommended chitosan, an amino polysaccharide polymer derived from chitin, as a beneficial matrix due to its antimicrobial and barrier properties on both gas and water vapor (Butler *et al.,* 1996; Romanazzi *et al.,* 2013; Chaparro-Hernández *et al.,* 2015). Furthermore, coatings performance could be improved by supplementing antimicrobial substances to delay deterioration and keep the quality of food products (Gennadios and Weller, 1990; Jeon *et al.,* 2002; Iturriaga *et al.,* 2012). Zinc oxide nanoparticles (ZnO NPs) is an inorganic filler as well as an antimicrobial agent that could be supplemented into the coating matrix polymer (Meindrawan *et al.,* 2018; Yuliani *et al.,* 2018; Lavinia *et al.,* 2020).

Due to the high consumption of meatball in Indonesia, this study has concerned about extending the shelf life of Indonesian meatball product by retarding microbial activities. Hence, the aim of the present research was to evaluate the effect of nanocomposite edible coating from chitosan and ZnO NPs on the characteristics of meatball.

2. Materials and methods

2.1 Preparation of meatball and coating solution

Meatball was prepared by blending of ground meat 250 g and spices including salt and pepper respectively 5 and 3 g, a clove of minced garlic, and an egg white. Subsequently, 50 g tapioca flour and 50 g chopped ice cubes were added and mixed. The mixed dough was shaped and boiled for 15 mins, air drained and cooled to ambient temperature before they were coated with nanocomposite.

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Nanocomposite coatings were prepared by dispersing ZnO NPs (0, 0.05 and 0.1%), 20±5 nm (Wako Pure Chemical Industries Ltd, Japan) in acetic acid solution 1% (Merck (Germany). Subsequently, 3% chitosan was added slowly and blended with constant stirring at temperature 45°C until completely dissolved. Later, meatball samples were immersed for one min in this solution and drained. Finally, after being air-dried, the sample was placed into polyethylene terephthalate plastic trays (11 x 8 cm) and stored at 25°C for 48 hrs.

2.2 Antibacterial activity of nanocomposite

Agar well diffusion method was selected to evaluate the antibacterial activity of coating solutions as described by Wolf and Gibbon (1996) and Balouri *et al.* (2016) with slight modification. Bacterial inoculum, *Escherichia coli* and *Staphylococcus aureus* (IPB laboratory collection), were set up to 0.5 Mac Farland (10^6 bacterial cells/mL) before transferred into petri dishes containing Nutrient Agar (NA). Then, around 6 mm wells were punched into the inoculated NA plates, followed by filling of coating solution±50 μL. Subsequently, the presence of inhibition zones was measured.

2.3 Meatball characteristics during storage

The microbial count was determined by using the pour plate method. Plate count agar used as growth medium was purchased from Merck (Germany). As much as 1 g of 5 g crushed meatball were mixed in 9 mL 0.1% peptone water as 10^{-1} dilution. Afterwards, those of samples were poured on the media and stored at 37°C for 48 hrs referring to the FDA Bacteriological Analytical Manual (1998). Colony were counted into log colony forming units per g of sample (log CFU/g). *pH* and water activity (a_w) analysis were also conducted by using pH meter and a_w meter respectively.

Sensory evaluation was conducted by 32 untrained panelists to judge taste, aroma, visual appearance, texture, and overall acceptability of the sample at 0 hrs. A 9-point hedonic scale was used (1-dislike extremely, 2-dislike very much, 3-dislike moderately, 4-dislike slightly, 5-neither like nor dislike, 6-like slightly, 7-like moderately, 8-like very much, and 9-like extremely).

2.4 Statistical analysis

Statistical Package for Social Scientists (SPSS, version 16.0) was selected for statistical analysis. The results were evaluated by analysis of variance (ANOVA) and compared by Duncan's Multiple Range Test (DMRT) when needed at a significance degree of $p < 0.05$.

3. Results and discussion

3.1 Antibacterial activity

The antibacterial activity of ZnO NPs was tested following agar well diffusion method (Table 1) which exhibited a synergistic effect of ZnO NPs and chitosan in the terms of suppressing especially gram-negative bacteria. This was probably associated with the thick peptidoglycan layer that could inhibit the nanoparticles to penetrate into the cell owned gram-positive bacteria. A similar result was reported by Li *et al*. (2009) that polyurethane film loaded with ZnO NPs showed a stronger antibacterial ability for E. coli in comparisons with gram-positive type bacteria.

Table 1. Inhibition zone of *S. aureus* and *E. coli*

Coating component	*S. aureus* (mm)	*E. coli* (mm)
Control	0^a	0^a
Chitosan-ZnO NPs 0%	3.28 ± 0.33^b	3.75 ± 0.65^b
Chitosan-ZnO NPs 0.05%	4.45 ± 0.45^b	6.05 ± 1.15^{bc}
Chitosan-ZnO NPs 0.1%	4.40 ± 0.20^b	8.05 ± 0.05^c

Different superscript letters in the same column show significant differences.

3.2 Meatball characteristics during storage

Total aerobic microbes increased over the storage period for all samples as exhibited in Figure 1. Coated meatballs exhibited lower microbial amount than uncoated one for all storage times. Furthermore, at 24 hrs, only chitosan-ZnO NPs 0.05% coating could suppress aerobic microbes in an amount of 4.30 log CFU/g which did not exceed the threshold for consumption referring to SNI-01-3818-2014 (5 log CFU/g) in comparisons with other samples. Chitosan matrix, which might electrostatically interact with negatively charged of microbial membranes, combined with 0.05% ZnO NPs filler might be synergized as antimicrobial agent. The penetration ability of ZnO NPs into cells led to disrupt the membrane, replicating process, enzyme functions (Arabi *et al.,* 2012; Yousef and Danial 2012). However, the addition of ZnO NPs 0.1% into chitosan showed lower antimicrobial activity in comparison with ZnO NPs 0.05%. The higher concentration of ZnO powders might facilitate agglomeration.

The a_w values of uncoated and nanocomposite coated meatballs after 0, 24, and 48 hrs were not statistically different, ranging from 0.98 -0.97, as shown in Figure 2a. This study found that both chitosan alone and loaded with ZnO NPs coating at various concentrations did not affect the a_w of meatballs which were relatively high allowing microbes to grow. Changes in pH of samples during storage are shown in Figure 2b. At 0 and 24 hrs storage, the pH of uncoated and coated samples was significantly different. Nevertheless, at the end of

storage, they were not statistically different. A plausible explanation was that 3% chitosan coating solution, which was relatively an acid condition, could affect the pH of samples. This was in accordance with Hermandz-Munoz *et al.* (2008) that chitosan ranging from 1-1.5% was not able to influence significantly the pH of samples.
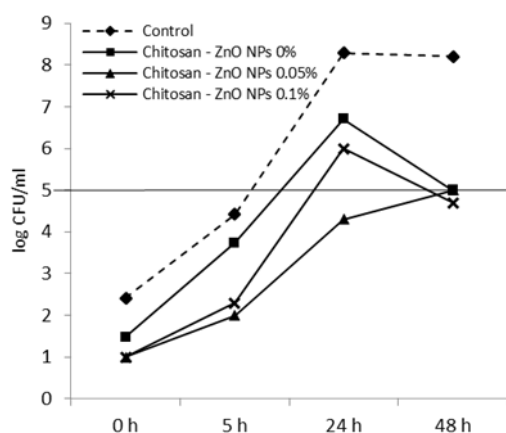

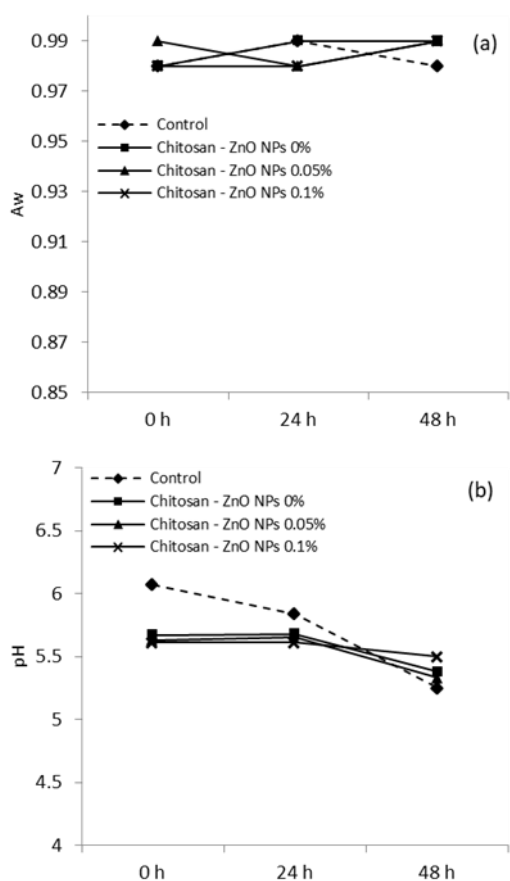Figure 1. Total aerobic microbial count during storage.

Figure 2. Characteristics of the meatball; (a) a_w and (b) pH during storage.

Sensory evaluation of samples, shown in Table 2, showed that less acceptability score for odor, taste, and overall attributes tested was found in all the coated samples especially loaded with ZnO NPs. This might be associated with a sour odor and taste of chitosan solution. Also, the addition of ZnO NP might contribute to the less acceptability of the meatball taste. Those attributes contributed to the lower score for overall acceptability. Hence, chitosan loaded with ZnO NPs could serve as an alternative method to maintain the microbial decay of meatball in which improvement was necessary, especially odor and taste attributes.

4. Conclusion

Incorporation of ZnO NPs into chitosan solution could greater suppress bacterial growth, particularly gram-negative bacteria. Chitosan-ZnO NPs 0.05% has been demonstrated as a potential coating in delaying the deterioration of meatball during storage caused by microbial decay. Nanocomposite coating treatment did not affect meatball's a_w throughout storage times. However, it influenced the pH of meatballs from 0 h to 24 hrs of storage allowing negative effect for odor and overall acceptability. Also, lower acceptability of taste and overall were contributed by ZnO NPs addition. Thus, chitosan-ZnO NPs coating could be an alternative method to maintain quality of meatball which was necessary for further improvement.

Conflict of interest

The authors declare no conflict of interest.

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Table 2. Sensory attributes of meatball

Coating component	Texture	Odor	Appearance	Taste	Overall
Control	4.59±1.55[a]	5.72±1.35[b]	6.06±1.46[a]	5.38±1.74[b]	5.25±1.57[b]
Chitosan-ZnO NPs 0%	4.91±1.41[a]	4.81±1.33[a]	6.25±1.32[a]	5.13±1.48[b]	4.97±1.43[ab]
Chitosan-ZnO NPs 0.05%	4.16±1.83[a]	4.97±1.62[a]	6.03±1.23[a]	4.25±1.80[a]	4.41±1.62[a]
Chitosan-ZnO NPs 0.1%	4.53±1.46[a]	4.91±1.45[a]	6.13 ±1.41[a]	4.22±1.9[a]	4.25±1.27[a]

Different superscript letters in the same column show significant differences.

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