EFFECT OF CHITOSAN COATING CONTAINING ACTIVE AGENTS ON MICROBIAL GROWTH, RANCIDITY AND MOISTURE LOSS OF MEATBALL DURING STORAGE

Yudi Pranoto[1], Sudip Kumar Rakshit[2]

ABSTRACT

Edible coatings based on chitosan were applied on meatball product in order to preserve quality during storages at ambient and refrigeration temperatures. To improve its efficacy, chitosan coatings were incorporated with garlic oil 0.2%, potassium sorbate 0.1 % and nisin 51,000 IU. The qualities of meatball assessed were total microbial growth, TBA value and percentage of moisture loss. All chitosan coatings suppressed microbial growth in meatball and strongly revealed when stored at refrigeration temperature. Incorporating garlic oil 0.2% into chitosan coating resulted in a greater reduction of rancidity level in meatball for both storages. Moisture loss of meatball was significantly reduced by all chitosan coatings and obviously shown when stored at refrigeration temperature.

Keywords*: Meatball, coating, chitosan, garlic oil, potassium sorbate, nisin*

INTRODUCTION

Meatball is a processed meat product, a very popular food throughout several countries. Asian people are familiar with this kind of meat product with various additional ingredients to meet consumer preferences. The meatball in Indonesia is called "bakso", that mainly made from meat and starch. It is a familiar street stall food and very popular among a broad social status of the people in the country. Meatball accommodates 40 percent of total processed meat in Indonesia and approximately 80 tons of meatball is produced in the capital city of Jakarta for a month (Voboril, 1999; Rahardiyan, 2004). Due to its high meat and moisture contents, the meatball is vulnerable to deterioration through microbial contamination, physical and chemical reaction, thereby, the quality decreases easily whenever kept at abuse temperature. The Department of Industrial and Trade of the Republic of Indonesia has made a standard for meatball product which is officially regulated in Indonesian National Standard (SNI, 1995) for microbial contamination on meatball.

Edible coating is one of the most effective methods to maintain food quality (Gennadios and Weller, 1990). It is a thin film prepared from edible material that acts as a barrier to the external elements like moisture, oil, vapors and thus protects the product and extends shelf life Application of edible coatings on meats to prevent shrinkage has been

practiced since at least the sixteenth centuries (Baker *et al*., 1994; Guilbert *et al*., 1996). For instance, when applied onto meat products, it is able to prevent moisture loss, dripping and rancidity (Gennadios *et al*., 1997). Lately, edible films or coatings have been improved of their characteristics by carrying food additives such as antioxidants, antimicrobial, colorants, flavors, fortified nutrient and spices (Han, 2000; Pena and Torres, 1991). In this case, the agents carried are slowly released into the food surface to afford some efficacies, and therefore, remaining at high concentration for extended period of time (Ouattara *et al.*, 2000; Coma *et al*., 2001).

Chitosan, β-1,4 linked glucosamine and *N*-acetyl glucosamine, is prepared by deacetylation of chitin. Chitosan has been proven to be nontoxic, biodegradable, biofunctional, biocompatible materials and it possess antimicrobial characteristics (Wang, 1992; Darmadji and Izumimoto, 1994). One of the reasons for the antimicrobial characteristic of the chitosan is its positively charged amino group which interacts with negatively charged microbial cell membranes, leading to the leakage of proteinaceous and other intracellular constituents of the microorganisms (Shahidi *et al*., 1999). Chitosan has been studied for coating precooked pizza and Emmental cheese to delay growth of some microbial contaminants (Rodríquez *et al*., 2003; Coma et al., 2003). Ouattara *et al*.

[1] Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Gadjah Mada University, Jl. Sosio Yustisia, Yogyakarta 55281, Indonesia. Email: pranoto@ugm.ac.id

[2] Department of Food Engineering and Bioprocess Technology, School of Environment, Resources and Development, Asian Institute of Technology, P.O. Box 4, Klong Luang, Pathumthani 12120, Thailand

(2000) coated muscle foods of bologna, ham and pastrami by chitosan incorporated with acetic acid, propionic acid and cinamaldehyde to inhibit surface spoilage bacteria. The use of chitosan coating offers a great advantage in preventing the growth of microbial surface on foods (Coma *et al*., 2002).

Antimicrobial agents such as organic acids, bacteriocins and spice extracts have been tested for their ability to control meat spoilage (Abugroun *et al*., 1993; Miller *et al*., 1993; Hotchkiss, 1995). Garlic oil is mainly composed of sulfur-containing compound such as allicin, diallyl disulfide and diallyl trisulfide, that possess better antimicrobial activity than the corresponding ground form (Nychas, 1995). Garlic oil remains active against some pathogenic bacteria when incorporated into chitosan based film (Pranoto *et al*., 2005). Potassium sorbate is active against yeast, mould and many bacteria (Meyer *et al*., 2002). It has been incorporated into starch based coating to control microbial growth on chicken breast (Baron and Summer, 1993). Nisin is a bacteriocin produced by *Lactococcus lactis* subsp. *lactis*. It has antimicrobial activity against a broad spectrum of Gram-positive bacteria. Nisin has widely been used in the food industry as a safe and natural preservative and has been studied of its suitability to be incorporated into cellulose, whey protein isolate, soy protein isolate, egg albumen, wheat gluten, hydroxyprophyl methylcellulose and zein films (Coma *et al.*, 2001; Ko *et al.*, 2001; Janes *et al*., 2002).

The development of complementary methods to inhibit the growth of microorganisms such as coating material-associated antimicrobial agents is an active area of research. This study aimed to preserve the quality of beef meatball during storages at ambient and refrigeration temperature by coating with chitosan incorporating active agents consisting of garlic oil, potassium sorbate and nisin.

MATERIALS AND METHODS

Meatball Preparation

The meatball used for this study was typical Indonesian meatball in which the major material constituted of the beef meat. Garlic and salt were also added into the recipe. All materials were obtained from the nearby market. The meatball formulation consisted of beef meat 1 kg, garlic 12 g and salt 20 g. Initially, beef meat was ground by using a meat grinder. Ground garlic and salt were then mixed with ground meat and minced again by using an extruder (TASIN, Type TS-102 AL, Tashing Food Machinery Co., Ltd.) until a fine dough was obtained. The dough was squeezed with hands and formed into a ball form having diameter of approximately 3 cm. Uncooked balls were then put into boiled water for 10 min, and partially cooked meatballs were taken out when they were floated up.

Proximate Composition of Meatball

Meatball was analyzed for moisture, protein and fat contents. The moisture content of meatballs was determined using gravimetric method (AOAC, 1990) by heating the sample in an oven 105 °C until steady weight was gained. Lipid content of meatball was determined with Soxhlet method using PE (petroleum ether) as a solvent, and then followed by gravimetric measurement of the extracted oil after kept in an air drying oven. Protein content was analyzed by using Micro Kjeldahl method, involving digestion, distillation and titration steps.

Chitosan Coating on Meatballs

Initially, 1 % of chitosan solution was prepared by dissolving 1 g of shrimp chitosan (molecular weight of 900,000 to 1,000,000 Dalton, degree of deacetylation approximately 95 %) into 100 mL of 1 % acetic acid solution. In order to obtain additional function, active agents namely garlic oil (ABBRA Co. Ltd., Bangkok, Thailand) at 0.2 % (v/v), potassium sorbate (Fluka Chemia GmbH, Buchs) at 0.1 % (w/v), and nisin (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) at 51,000 IU were incorporated into chitosan solution in accordance with the minimum effective level of their antimicrobial effect that had been previously studied. Coating of meatballs was done by dipping meatballs into chitosan solutions for 1 min. The meatballs were then taken out and hang up to allow draining of the excessive solution. After that, the coated meatballs were air dried in a laminar hood at ambient temperature for 30 min.

Total Microbial Growth

Meatball sample (50 g) was stomached (Stomacher, BagMixer®400, Interscience, France) with 100 ml of sterile 0.1 % peptone solution. A series of decimal dilution was carried out with sterile 0.1 % peptone solution in the test tubes, and 0.1 ml of diluted solution was directly spread onto Total Plate Count (Hi Media Laboratories Limited, Mumbai 400-086, India) agar plates. The plates were then incubated at 37 °C for 24 h. Total colony growth was finally counted using a colony counter and expressed in CFU/g of meatball.

Rancidity of Meatball

Rancidity occurrence in the meatballs was evaluated using thiobarbituric acid (TBA) value through distillation method, according to Tarlagis *et al*. (1960) as cited in Egan, Kirk, & Sawyer (1981). Ten g of meatball sample was blended with 50 mL of distilled water for 2 min. The mixture was transferred into Kjeldahl flask, and the jar was rinsed with 47.5 mL of distilled water. The sample was then treated with 2.5 mL of 4 mol l^{-1} HCl to bring pH to 1.5. A boiling chip and 1

drop of antifoam solution were added to control bubbling and foaming during distillation process. About 50 mL of distillate was collected. Five milliliter of 0.02 mol l^{-1} TBA reagent in 90 % acetic acid (Fluka Chemia GmbH, Buch, Steinheim, Germany) was added into 5 mL of collected distillate. The solution was mixed, stoppered and placed in a boiling water bath for 35 min and then immediately cooled in an ice bath for 10 min. The amount of colored compound formed was measured of absorbance at 538 nm with a spectrophotometer (Unicam Model UV-200). The TBA value was expressed as milligrams of malonaldehyde per kilogram of meatball.

Weight Loss of Meatball

Weight loss of the meatballs was determined as a percentage of reduced weight during storages. Therefore, the weight of initial meatballs and the final weight taken during sampling were measured accordingly. The value was expressed in percentage (%).

Statistical Analysis

Experimental data were analyzed by using Excel Release 2002 (Microsoft Inc.) and SPSS Release 11.0.0 (SPSS Inc.) software. Replication was conducted in a triplicate. Experimental design of randomized complete block was used to tabulate data followed by least significant difference (LSD) test to determine the significant difference ($p<0.05$) between treatment means.

RESULTS AND DISCUSSION

Analysis of proximate composition revealed that meatball made from our experiment had contents of lipid 5 %, protein 30 % and moisture 60 %. This composition makes this product susceptible to deterioration that consequently leads to decrease in quality. This effort, therefore, is to apply a novel method of edible coating based on chitosan in order to extend the shelf life of meatball. This study presents the beneficial effect of chitosan coatings, in terms of antimicrobial and antioxidative activity, and also moisture loss reduction during storages. The meatball was processed according to the typical Indonesian meatball, which is mainly made from ground beef meat.

Chitosan Coatings on Microbial Growth of Meatballs

Application of edible coatings on meatball was con-ducted to follow up the previous study on the antimicrobial or bioactive edible films based on chitosan as reported earlier (Pranoto *et al*., 2005). Figure 1 shows the effect of chitosan coatings on the growth of microorganisms in meatballs stored at ambient temperature. Meatballs had initial microbial count

of 1.78 Log CFU/g. It increased steadily when stored until 2 days, reaching 6.85 Log CFU/g. Over 2 days of storage, the increase was slow down, about 7.53 Log CFU/g at the end of storage. All coating treatments significantly ($p<0.05$) reduced microbial growth during storage up to 3 days. Incorporation of active agents showed a greater inhibitory effect when the meatballs were kept longer. According to Indonesian National Standard (SNI, 1995) for meatball product, the maximum total plate count is 10^5 CFU/g of meatball. After 1 day of storage, the control meatball already passed this allowable line, whereas coated ones passed the line after 2 days of storage. Coating meatballs based on chitosan greatly extended the shelf life by suppressing microbial growth. Similar work was also obtained by Coma *et al*. (2003), who found that chitosan coating increased the lag phase of microbial contaminations on cheese product.
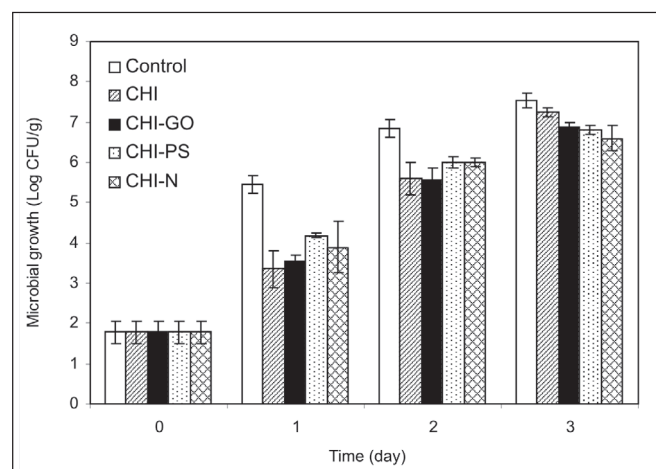

Figure 1. Total microbial growth of meatballs during storage at ambient temperature; control, coated with chitosan (CHI), coated with chitosan incorporating garlic oil 0.2 % (CHI-GO), coated with chitosan incorporating potassium sorbate 0.1% (CHI-PS) and coated with chitosan incorporating nisin 51,000 IU (CHI-N)

When meatballs stored at refrigeration temperature, they did not reach the maximum permissible level until 20 days of storage (Figure 2) with an initial load of 1.78 log (CFU/g), whether for control or coated ones. The pattern of microbial growth (in control) that increased at the initial storage, then remained constant or even decreased was also observed by Cagri *et al*. (2002) and Gill and Holley (2000) in bologna, ham and summer sausage stored at refrigeration temperature. In this study, chitosan coating significantly ($p<0.05$) sup-pressed microbial growth during storage, therefore, they showed much lower microbial growth compared to control for all the days assessed. In addition, incorporating active agents significantly ($p<0.05$) enhanced the inhibitory effect

as compared to unincorporated ones. It was consistent with the phenomenon investigated by Pranoto *et al.* (2005) in improving antimicrobial edible films based on chitosan. Obvious inhibitory effect of chitosan coating is conceivable as chitosan itself has innate antimicrobial activity (Wang, 1992; Darmadji and Izumimoto, 1994; Shahidi *et al.*, 1999). Study on the combined effects of incorporating spice oils of tyme oil and *trans*-cinnamalydehyde into protein based coating, and gamma irradiation has also been carried out by Ouattara *et al.* (2001). In term of aerobic plate counts, these antimicrobial coatings were able to extend the shelf life of precooked shrimp for 4 to 5 days when stored at 4 °C. From this study, it was also shown that the refrigeration storage is the appropriate way to reduce microbial growth of meatballs.
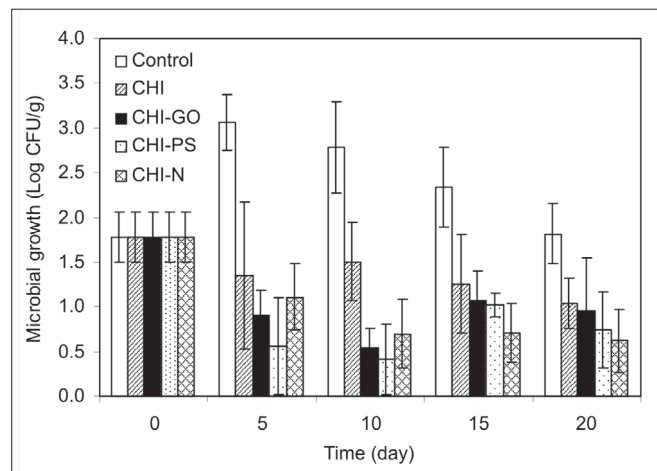

Figure 2. Total microbial growth of meatballs during storage at refrigeration temperature 4°C; control, coated with chitosan (CHI), coated with chitosan incorporating garlic oil 0.2 % (CHI-GO), coated with chitosan incorporating potassium sorbate 0.1% (CHI-PS) and coated with chitosan incorporating nisin 51,000 IU (CHI-N)

Chitosan Coatings on Rancidity Level of Meatballs

Meat products contain a high lipid content that leads to oxidation and consequently causing decrease in quality. In precooked meat products, it remains of a great concern to the meat industry because of the increase demand for convenient foods (Ahn *et al.*, 2002; Dzudie *et al.*, 2004). Oxidation reaction, in some cases, forms toxic products which are harmful to human health (Ferrari and Torres, 2002). This study, therefore, was subjected to protect or limit oxidation reaction in meatball by using edible coating based on chitosan. The extent of lipid oxidation can be evaluated by measurement of malonaldialdehyde (MDA) reacting with thiobarbituric acid (TBA) that had been conducted to assess oxidation in various meat products such as precooked beef patties, cooked ground beef, sausage, beef loins and turkey breast meat (Antony *et*

al., 2000; Dzudie *et al.*, 2004; Ferrari and Torres, 2002; Ahn *et al.*, 2002; Wu *et al.*, 2000). Application of edible coatings on meatball as a model of meat product is expected to reduce the occurrence of lipid oxidation reaction during storages. The effect of edible coatings on rancidity of meatballs during storage at ambient temperature is shown in Figure 3. Initial TBA value of meatball was 0.33, which is about the same level with beef patties (Dzudie *et al.*, 2004) and lower than cooked ground beef (Wu *et al.*, 2001; Ahn *et al.*, 2002). In general, TBA value increased with the increase of storage time. After 3 days of storage at ambient temperature, meatball (control) had TBA value of 1.50. It shows that all coatings gave lower thiobarbituric acid (TBA) value compared to uncoated one (control) during storage. However, only chitosan coatings incorporated with garlic oil (CHI-GO) and potassium sorbate (CHI-PS) significantly ($p<0.05$) revealed in reduction of rancidity within all days observed.
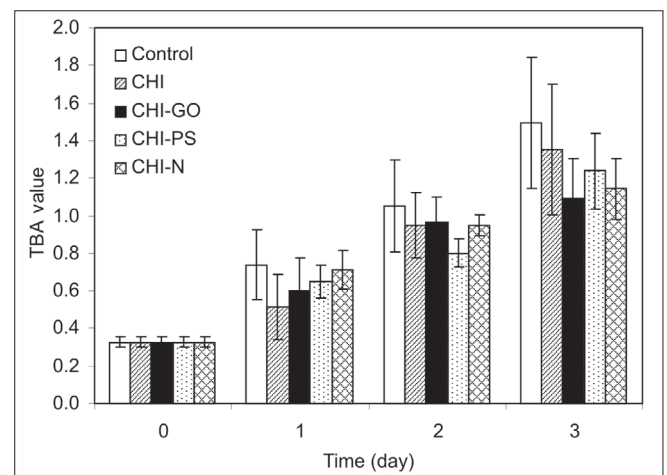

Figure 3. Rancidity level of meatballs during storage at ambient temperature; control, coated with chitosan (CHI), coated with chitosan incorporating garlic oil 0.2 % (CHI-GO), coated with chitosan incorporating potassium sorbate 0.1 % (CHI-PS) and coated with chitosan incorporating nisin 51,000 IU (CHI-N)

Figure 4 shows the effect of edible coatings on rancidity of meatball stored at 4 °C of refrigeration temperature. It was observed that high increase in TBA value occurred within 5 days of storage for all meatballs. It was noted that TBA value remained constant after 10 days of storage, and finally reached 10.73 for the control after 20 days. This value is close to the Turkey breast meat after stored for 24 h at 4 °C (Antony *et al.*, 2000). Incorporating active agents into chitosan coating significantly ($p<0.05$) resulted in lowering TBA value. Meanwhile, coating with chitosan (CHI) showed no significant difference ($p>0.05$) in TBA value compared to control. Incorporating garlic oil into chitosan revealed the

highest rancidity inhibition. It was conceivable because garlic oil contains compounds having antioxidative characteristic (Meyer *et al*., 2002). Study on the incorporation of antioxidant into edible coating has also been conducted by Wu *et al*. (2001), who incorporated tocopherol into starch-alginate based coating onto precooked ground beef patties in order to reduce lipid oxidation.
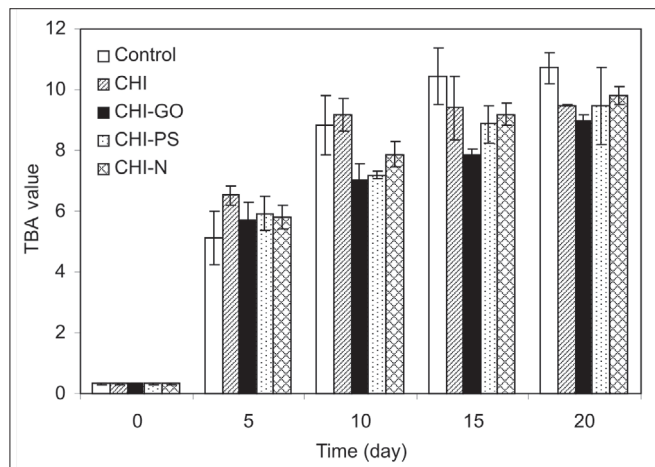

Figure 4. Rancidity level of meatballs during storage at refrigeration temperature 4 ℃; control, coated with chitosan (CHI), coated with chitosan incorporating garlic oil 0.2 % (CHI-GO), coated with chitosan incorporating potassium sorbate 0.1% (CHI-PS) and coated with chitosan incorporating nisin 51,000 IU (CHI-N)

Chitosan Coating on Moisture Loss Protection of Meatballs

Moisture loss protection of chitosan coatings on meatball could be estimated from their weight loss during storages. Figure 5 shows the effect of edible coatings on the weight loss of meatball during 3 days of storage at ambient temperature. Meatball lost its weigh steadily during storage, and reached 15.96 % after 3 days. In general, coating treatments showed less moisture loss compared to the control. This study also showed that incorporating garlic oil into chitosan coating improved its capability to reduce moisture loss. Chitosan is known to have hydrophilic groups. Incorporating such hydrophobic material would help the coating to act as moisture barrier, thereby, reduced moisture loss of coated meatballs (Wu *et al.*, 2001; Ross *et al*., 2001).

Effect of coatings on the retained moisture content was clearly observed in the meatball stored at refrigeration temperature (4 ℃) as shown in Figure 6. The percentage of moisture loss after 20 days of storage was 0.90 %, which is much lower than that of exposed at ambient temperature for 3 days. The control showed the highest moisture loss compared to coated meatballs. In this experiment, however,
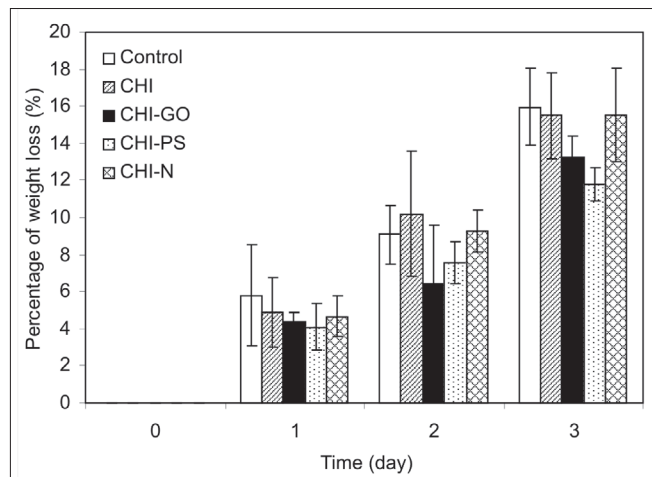

Figure 5. Weight loss of meatballs during storage at ambient temperature; control, coated with chitosan (CHI), coated with chitosan incorporating garlic oil 0.2 % (CHI-GO), coated with chitosan incorporating potassium sorbate 0.1 % (CHI-PS) and coated with chitosan incorporating nisin 51,000 IU (CHI-N)

coating with chitosan (CHI) significantly ($p<0.05$) resulted in a lower moisture loss (0.33 %) compared to chitosan coatings incorporated with active agents.

The protection of moisture loss is closely related to water vapor permeability value of the film formed on the surface of meatball. As observed by Pranoto *et al*. (2005), incorporating such antimicrobial agents tend to increase water permeability value of chitosan films. The value is getting higher when the level of agents incorporated increases. The agents incorporated contribute to extent molecular interaction
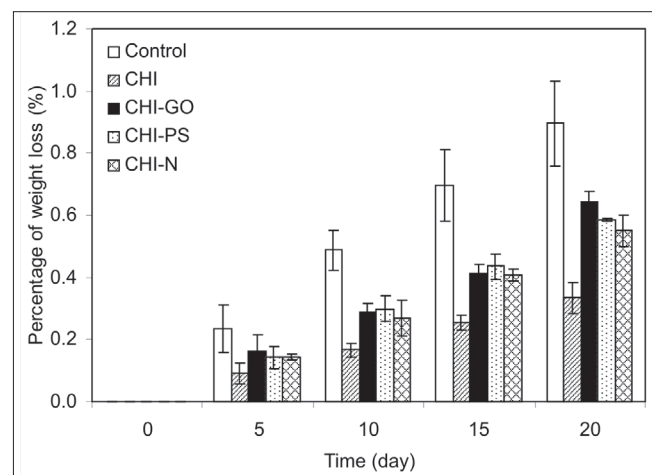

Figure 6. Weight loss of meatballs during storage at refrigeration temperature 4 ℃; control, coated with chitosan (CHI), coated with chitosan incorporating garlic oil 0.2 % (CHI-GO), coated with chitosan incorporating potassium sorbate 0.1 % (CHI-PS) and coated with chitosan incorporating nisin 51,000 IU (CHI-N)

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in the chitosan films, therefore, loosening compactness of the film structure. Furthermore, it caused the moisture easily passing through chitosan coatings. In this case, the role of hydrophobic characteristic of garlic oil to hinder moisture transport was inferior when the meatball was stored at longer period under refrigeration environment. Similar result was also studied by Wu *et al*. (2000), in which chitosan coating did reduce moisture loss after 3 days of storage at 4 °C when applied onto precooked beef patties.

CONCLUSIONS

The final outcomes of the use of the chitosan coating on meatball showed some positive results. Chitosan coating significantly reduced microbial growth during storages. Incorporating active agents enhanced the efficacy of antimicrobial. Garlic oil clearly revealed the additional function of antioxidative activity when incorporated into chitosan coating. Adding any agents into coating material, however, led to increase in permeability value of film formed, and as a result it made easy for moisture contained in the meatball to pass through. The concentration of chitosan and the thickness of film formed on the meatball surface need to be investigated further in order to optimize the coating.

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