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A chitosan-based coating with or without clove oil extends the shelf life of cooked pork sausages in refrigerated storage

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ABSTRACT

Chitosan coatings, with and without clove oil, were investigated for effects on quality and shelf life of cooked pork sausages stored at a refrigerated temperature (4 ± 2 °C). The various treatments of cooked pork sausages were: untreated (control), coating with 2% chitosan (CS), and coating with a mixture having 2% chitosan and 1.5% clove oil (CS + CO). Various microbiological, physical, chemical and sensory properties were monitored over 25 days of storage. The total viable count, the psychrotrophic bacteria count, the L* value, peroxide value and the thiobarbituric acid reactive substances increased, while the a* value, the b* value, the pH and the sensory scores decreased with storage time, across all treatments. However, these changes were slowest with the CS + CO treatment. Based on sensory evaluation and microbiological quality, the shelf lives were 14 days for control, 20 days for CS, and 20 days for CS + CO treated samples, under refrigerated storage.

1. Introduction

Pork sausages are among the oldest processed meat products enjoyed by millions of consumers all over the world. Traditionally, cooked pork sausages are made of fresh pork meat and fat, chopped and mixed thoroughly with seasonings. Typical seasonings include pepper powder, spicy paprika, salt, rice wine, sugar, monosodium glutamate and ginger. After filling this meaty mixture in natural casings from the cleaned small intestine of pigs, the sausages are fully cooked (Qiu, Zhao, Sun, Zhou, and Cui, 2013; Sebranek, Sewalt, Robbins, and Houser, 2005). However, cooked pork sausage products typically have a short shelf life restricted by poor color retention, rancidity, and other quality losses.

Chitosan (ß-(1,4)-2-amino-2-deoxy-D-glucopyranose), mainly manufactured from crustacean shells (from crab, shrimp, crayfish, etc.), is derived by deacetylation of chitin (Knorr, 1984; Rinaudo, 2006; Sandford, 2003). This nontoxic, biodegradable and biocompatible substance has antimicrobial and antioxidant properties, wound-healing properties, as well as hemostatic activity, and it is receiving increased attention as a promising renewable polymeric material (Knorr, 1984; Yen, Yang, and Mau, 2009). Several reports indicate that chitosan has potential in food packaging, especially as edible films and coatings (Fan et al., 2009; Petrou, Tsiraki, Giatrakou, and Savvaidis, 2012; Suman et al., 2010).

The incorporation of chitosan and essential oils into food coatings is concurrently emerging as a promising technology, to inhibit the growth of microorganisms, to retard the oxidation at the surface, to improve the sensory quality, and to prolong the shelf life of the samples. Such studies demonstrating shelf life extension include the following. Chitosan with rosemary extract in pork sausages (Georgantelis, Ambrosiadis, Katikou, Blekas, & Georgakis, 2007) and in beef burgers (Georgantelis, Blekas, Katikou, Ambrosiadis, & Fletouris, 2007); chitosan and thyme oil in ready to cook poultry products (Giatrakou, Ntzimani, & Savvaidis, 2010a; Giatrakou, Ntzimani, Zwietering, & Savvaidis, 2010b); chitosan and cinnamon oil in refrigerated rainbow trout (Ojagh, Rezaei, Razavi, and Hosseini, 2010); chitosan and sunflower oil in pork meat hamburgers (Vargas, Albors, and Chiralt, 2011); chitosan and oregano oil in chicken breast meat (Petrou et al., 2012); and, recently, chitosan and *Zataria multiflora* essential oil in chicken breast meat ((Bazargani-Gilani, Aliakbarlu, and Tajik, 2015).

Clove oil (*Syzygium aromaticum*, Lin) is a natural essential oil with antimicrobial and antioxidant activities (Burt, 2004; Gülçin, Elmastaş, and Aboul-Enein, 2012; Gülçin, Güngör Şat, Beydemir, Elmastaş, and İrfan Küfrevioğlu, 2004; Lee and Shibamoto, 2001; Matan et al., 2006; Oskoueian, Maroufyan, Goh, Ramezani-Fard, and Ebrahimi, 2013), its active ingredient being eugenol (Lee and Shibamoto, 2001; Ordóñez, Llopis, and Peñalver, 2008). Eugenol has antifungal activity (Martini, Weidenbörner, Adams, and Kunz, 1996), and inhibits malonaldehyde formation from cod liver oil, as well as formation of hexanal (Lee and Shibamoto, 2001).

The effects of chitosan-based coatings, with or without clove oil, on the shelf life of a meat product under refrigerated storage are poorly understood. Therefore, the objective of this study was to evaluate such effects on the quality and shelf life of pork sausage. If the shelf life of sausages in retail stores could be extended, this would reduce losses

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from having to dispose potentially spoiled product. This provides the economic motivation of the current study.

2. Materials and methods

2.1. Chemicals and media

Chitosan powder from shrimp shells (food grade), with a 200 mesh particle size, 8.97×10^5 molecular weight, and 80% degree of deacetylation, was purchased from Sinudom Agriculture Products Co., Ltd. (Surat Thani, Thailand). Pure clove oil of the genus *S. aromaticum*, Lin was purchased from Thai China Flavors and Fragrances Industry Co., Ltd. (Bangkok, Thailand). The major component of this oil was eugenol at 70–80% (manufacturer's data). The chemicals used were analytical grade, and included chloroform, methanol, trichloroacetic acid, potassium iodide, anhydrous sodium sulfate, hydrochloric acid, sodium hydroxide, sodium thiosulfate, thiobarbituric acid, potassium dihydrogen phosphate (Merck, Darmstadt, Germany), acetic acid (Lab-Scan, Bangkok, Thailand), and 1,1,3,3-tetramethoxypropane (Sigma-Aldrich, St. Louis., MO, USA). The medium for microbiological analyses was analytical grade plate count agar (Merck, Darmstadt, Germany).

2.2. Preparation of chitosan and clove oil solutions

A stock solution of chitosan was prepared by dissolving 2 g in 100 ml of 1% (*w*/*v*) glacial acetic acid, and stirring overnight at room temperature (final chitosan concentration = 2% *w*/*v*). To prepare chitosan solution incorporating clove oil, 0.5 ml of glycerol/g chitosan and 0.1% *w*/*v* of Tween 60 were added in the chitosan solution, then stirring for 30 min. The glycerol concentration gave suitable viscosity to the chitosan solution for use in manual dip coating. After that, 1.5 g clove oil was added to 100 ml of the chitosan mixture with glycerol and Tween 60, and stirred for 6 h at room temperature (final clove oil concentration = 1.5% *w*/*v*). All stirring was done with a magnetic stirrer in a glass beaker at room temperature.

2.3. Sample preparation

Cooked pork sausages, approximately 500 ± 10 g for each treatment, were purchased from Charoen Pokphand Foods PCL (Bangkok, Thailand). The samples were given a 3 min dip treatment in the 2% chitosan solution (CS); or the 2% chitosan solution incorporating 1.5% clove oil (CS + CO). Then the samples were dried for 60 min in aseptic laminar airflow conditions, and packed into PE ziplock bags holding 35 g per bag. The control samples (without coatings) were treated following the same procedure. The packed samples were kept in a refrigerator at 4 ± 2 °C for up to 25 days. Intermediate samples were taken randomly every 5 days to evaluate physical, chemical, microbiological and sensory properties. The optimal concentration of clove oil used was 1.5% by volume, based on preliminary experiments including microbiological analyses, physical analyses and sensory evaluations (Songsaeng, 2014). The sensory evaluations of the cooked pork sausage samples were investigated for effects of chitosan-based coating at 2.0% *v*/*v* incorporated with clove oil at 0.5, 1.0 and 1.5% *v*/*v*. The 1.5% *v*/*v* concentration of clove oil was the best among these treatments, giving the highest overall acceptability.

2.4. Microbiological analysis

Total viable counts (TVC) and psychrotrophic bacteria counts were determined as follows. Twenty-five grams of a sausage sample was blended for 2 min with 225 ml of sterile Butterfield's phosphate-buffered water using a sterile blender jar (Waring, Torrington, CT), to obtain sample concentration 0.1 g/ml. By sequential 10-fold dilutions, sample concentrations from 10^{-2} to 10^{-8} g/ml were obtained in sterile Butterfield's phosphate diluent. The TVC and psychrotrophic counts were determined by the pour plate method, using plate count agar. The diluted samples were incubated at 35 °C for 48 h, or at 7 °C for 10 days (BAM, 2001). The counts are expressed as log CFU/g.

2.5. Physical and chemical analyses

2.5.1. Surface color measurement

The surface color of each type of sample was measured for the L*, a* and b* values, using a Hunter Lab color analyzer (Color Flex, USA) calibrated with a standard white plate. The L* value represents lightness (L* = 0 for black and L* = 100 for white), while the a* value represents the red/green scale, with positive values for red and negative for green. The b* value represents the yellow/blue scale, with positive for yellow and negative for blue. The illuminant used was *C (D65), and the standard observer angle was 10°. The measured area was 1.25 in. in diameter.

2.5.2. Determination of pH

Ten grams of a sausage sample was homogenized thoroughly with 20 ml distilled water. A high-shear homogenizer (IKA homogenizer, Model T25 digital ULTRA-TURRAX, Germany) was applied at 12,000 rpm for 1 min. The pH of the homogenate was measured using a pH meter (Mettler Toledo, SevenEasy, USA) (Songsaeng, Sophanodora, Kaewsrithong, and Ohshima, 2010).

2.5.3. Lipid extraction

Lipid was extracted following Bligh and Dyer (1959). The sample (30 g) was homogenized in 210 ml of a chloroform:methanol:distilled water (60:120:30) mixture, at a speed of 10,000 rpm for 1 min, at 4 °C (IKA homogenizer, Model T25 digital ULTRA-TURRAX, Germany). The homogenate was diluted with 60 ml of chloroform, and homogenized at 10,000 rpm for 30 s. Then, 60 ml distilled water was added, and the mixture was homogenized again for 30 s. This homogenate was centrifuged at 5000 *g* for 10 min, at 4 °C (Sorvall centrifuge, Model RC 55 Plus, USA), and the supernatant was transferred into a separating flask. The chloroform phase was drained off into a 250 ml Erlenmeyer flask containing about 2–5 g of anhydrous sodium sulfate, shaken well, and decanted into a round-bottom flask through Whatman No. 4 filter paper. The solvent was evaporated at 40 °C using a rotary evaporator (Eyela, Model N-100, Tokyo, Japan), and the residual solvent was removed by flushing with nitrogen. The extracted lipid was subjected to an analysis of PV.

2.5.4. Determination of peroxide value (PV)

The PV was determined according to the method of Low and Ng (1978). The lipid sample (1.0 g) was treated with 30 ml of an organic solvent mixture (chloroform:acetic acid, 2:3). The mixture was shaken vigorously, followed by the addition of 0.5 ml of saturated potassium iodide solution. This mixture was kept in the dark for 1 min, and then 30 ml of distilled water were added and the mixture was shaken. To the mixture, 0.5 ml of starch solution (1% *w*/*v*) was added as an indicator. The PV was determined by titrating the iodine liberated from potassium iodide with standardized 0.01 N sodium thiosulfate solution. The PV is expressed as milliequivalents of free iodine per kg of lipid.

2.5.5. Determination of thiobarbituric acid reactive substances (TBARS)

The TBARS assay was performed as described by Buege and Aust (1978). A ground sample (0.5 g) was homogenized with 2.5 ml of a solution containing 0.375% (*w*/*v*) thiobarbituric acid, 15% (*w*/*v*) trichloroacetic acid and 0.25 N HCl. The mixture was heated in a boiling water bath (95–100 °C) for 10 min to develop a pink color, then cooled with running tap water and centrifuged at 3600 *g* for 20 min, at 25 °C. The absorbance of the supernatant was measured at 532 nm. A standard curve was prepared using 1,1,3,3-tetramethoxypropane at