Effect of an Active Film from Chitosan and Pomegranate Rind Powder Extract on Shelf-life Extension of Pork Meat Patties

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Abstract: Chitosan (CH) film is widely used for the shelf life extension of food stuff. In order to improve its antioxidant activity, chitosan film containing pomegranate rind powder extract (PRP) was used as an active packaging material for pork meat patties stored at 4±1 ℃ for 20 days. The physical, chemical, microbiological, and sensory qualities of pork meat patties wrapped with CH-PRP film were compared with those wrapped with pure chitosan film and control group without chitosan film wrapping. A microbiological shelf-life extension of 8 days was achieved for CH and CH-PRP treatment groups when compared to the control group. Wrapping with CH-PRP film retarded the increases in thiobarbituric acid-reactive substances values and metmyoglobin content. The samples wrapped with CH-PRP film could maintain acceptable sensory quality throughout the storage. A gradual release of phenolic compound was observed from CH-PRP film during storage. The results indicated that pomegranate rind powder extract incorporated into chitosan film enhanced the antioxidative and antimicrobial activities of the film and thus maintained quality and shelf life of pork meat patties.

Key words: chitosan; pomegranate rind powder; pork; antimicrobial; antioxidant; shelf life

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壳聚糖/石榴皮提取物复合膜在猪肉饼保鲜中的应用

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摘要：壳聚糖（CH）膜被广泛的应用于食品保鲜领域中，本研究通过向壳聚糖膜中添加石榴皮提取物（PRP）制备具有抗氧化和抗菌活性的复合膜，并研究其对猪肉的保鲜效果。在4±1 ℃的条件下，采用CH-PRP膜或CH膜包裹猪肉饼，进行为期20 d的储藏保鲜，测定猪肉饼的pH值、细菌总数、硫代巴比妥酸值(TBARS)、高铁肌红蛋白(MetMb)和感官品质等指标的变化。对照组未使用膜包装。对于微生物学指标，与对照组相比，CH和CH-PRP组的保质期均延长了8 d。此外，CH-PRP膜能有效抑制硫代巴比妥酸活性物和肌红蛋白含量的增加。在整个储存期，CH-PRP组猪肉饼的感官品质一直维持在可接受的范围内。通过观察发现，CH-PRP膜在储藏过程中缓慢释放抗氧化酚类化合物。因此，壳聚糖/石榴皮提取物复合膜是一种兼具抗菌和抗氧化效果的保鲜膜，能有效延长猪肉饼的保质期。

关键词：壳聚糖；石榴皮提取物；猪肉；抗菌；抗氧化；保质期

Active packaging, the new generation of food packaging, is gaining interest from researchers and industry fields. In response to the consumers to use food with minimal chemical additives and environmental problems, current studies in active packaging have focused on the addition of natural antioxidants and/or antimicrobial agents into packaging materials[1]. The film could serve as a barrier for contaminating microorganisms on the surface of food and reduce lipid oxidation for the subsequent improvement of quality, shelf life, and safety of coated products[2].

Chitosan (CH) is generally recognized as a safe material (GRAS) by the FDA (USA, 2001). It is a valuable component for producing biodegradable packaging films due to its film-forming capability as well as its broad-spectrum antimicrobial activity against both Gram-positive, Gram-negative bacteria and fungi[3]. Previous studies have indicated that chitosan could be used effectively to inhibit the oxidation of various meat products in combination with natural active compounds[4~8]. In one of these studies, Moradi et al.

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developed novel antioxidant chitosan based on edible films incorporated with Zataria multiflora Boiss essential oil (ZEO) (5 and 10 g/L) and grape seed extract (GSE) (10 g/L) individually or in combination, for the preservation of ready-to-eat mortadella-type sausages[2,9]. Chamanara et al. studied the effect of chitosan with thyme essential oil on nutritional, textural and sensorial characteristics of rainbow trout[8].

Pomegranate (Punica granatum) is native from Iran and now also cultivated in several provinces in China. Pomegranate rind is the by-product during processing of pomegranate juice. Polyphenolic compounds in pomegranate rind extract has been reported to be acted as free radical scavengers to terminate the radical chain reactions and thus possess significant antioxidant activity[10,11]. Recently, use of pomegranate rind extract as natural antioxidant in chicken and goat meat products have been investigated[12,13].

To the best of our knowledge, the application of chitosan in combined with pomegranate rind powder extract (PRP), has not been studied to date. Thus, the objective of the present work was to determine the effectiveness of chitosan and pomegranate rind powder extract, applied individually or in combination, in pork meat patties as observed by pH, microbiological analysis, thiobarbituric acid reactive substances (TBARS), metmyoglobin (MetMb), and sensory evaluation during refrigerated storage. Release behavior of phenolic content from chitosan-based film into pork meat patties was also studied.

1 Materials and methods

1.1 Preparation of chitosan-based films

Chitosan in powder from crab shells with a deacetylation degree of 95% was purchased from Qingdao Allforlong Bio-Tech Co., Ltd. (Shandong Province, China). Fresh pomegranate was obtained from retail fruit market (Yunnan province, China). Mature and healthy pomegranate fruits were washed, cut manually, and peeled off. Pomegranate rind was dried in an air circulatory tray drier at 60 ℃ for 48 h. Dried pomegranate rind was powdered using a mixer grinder. The pomegranate rind powder (5 g) was extracted with 100 mL of 80% ethanol overnight at 40 ℃ in a shaking water bath. The solutions were filtered through 0.45 μm filter membrane and evaporated under vacuum with a rotary evaporator below 50 ℃.

0.5% (*m/V*) glycerol was added to 1.5% (*m/V*) chitosan solution in order to improve its flexibility and extendability. 2% (*m/V*) pomegranate rind powder extract (PRP) were slowly added to the above solution to prepare the film-forming solution. Then, the solution was degassed, cast, and dried to prepare composite CH-PRP film. The pure CH film was prepared without the addition of PRP.

1.2 Preparation of pork meat patties

Fresh pork meat was purchased from a local processor. All muscles were trimmed of visible connective tissues as well as subcutaneous and intramuscular fat. Then, they were ground twice (first ground through a 6 mm grinding plate followed by 4 mm plate). After mincing, meat samples were mixed with 2 wt% sodium chloride by a Kitchen Aid mixer and moulded in Petri dishes to obtain the pork meat patties. The pork meat patties were wrapped with films[14]. Treatments of the present study were as follows: control (control samples under vacuum packaging without chitosan film), CH (under vacuum packaging with pure chitosan film), CH-PRP (under vacuum packaging with chitosan-PRP film). Non-coated and coated samples were packaged in low density polyethylene bags under vacuum. The samples were stored at 4 ±1 ℃ for 20 days and analyzed at 4 days interval (0, 4 th, 8 th, 12 th, 16 th, and 20 th day)[15].

1.3 pH measurement

The pH value of meat sample was recorded using a digital pH meter (PHS-3C, INESA Sciencetific Instrument Co., Ltd, Shanghai, China) using a mixture of 10 g sample and 50 mL distilled water.

1.4 Microbiological analysis

To determine the bacterial count for each sample, a total amount of 10 g sample was collected and placed in a sterile stomacher bag with 90 mL, 0.1% sterile peptone water. The sample was then homogenized for 2 min using a Stomacher and 10 fold serial dilutions (using 0.1% sterile peptone water) were made. 0.1 mL aliquot from each dilution was plated onto standard plate count agar (PCA). The plates were incubated at 37 ℃for 48 h to determine the standard plate count on each sampling day (0, 4 th, 8 th, 12 th, 16 th, and 20 th day). All

microbiological counts were expressed as the log of the colony forming units per gram [$\log_{10}$(CFU/g)] of pork meat patties.

1.5 Thiobarbituric acid reactive substances (TBARS) value

Thiobarbituric acid reactive substances (TBARS) value was determined using a modified extraction method of Witte, Krauze, and Bailey[16]. Briefly, TBARS were extracted in chilled 20% trichloroacetic acid. 2 mL extract was mixed with 2 mL, 0.1% thiobarbituric acid and heated for 30 min. After cooling, the absorbance was determined at 532 nm in a spectrophotometer (T90, Beijing Purkinje general instrument Co., Ltd. Beijing, China). 1,1,3,3, tetraethoxypropane (Sigma, St. Louis, USA) was used as standard. TBARS value was expressed as mg of malonaldehyde/100 g of the meat sample.

1.6 Metmyoglobin (MetMb) assays

The metmyoglobin (MetMb) percentage of the total myoglobin perceptible was determined according to Krzywicki[17]. The sample (5 g) was placed into a 50-mL polypropylene centrifuge tube, and 25 mL ice-cold phosphate buffer (pH 6.8, 40 mM) was added into the tube. Then, the mixture was homogenized for 10 s at 14,000 r/min. The homogenized sample was centrifuged at 10,000 r/min for 15 min at 4 ℃ and the supernatant was filtered with filter paper. The absorbance was read at 700 nm, 572 nm, and 525 nm by scanning the visible spectrum with a spectrophotometer (T90, Beijing Purkinje general instrument Co., Ltd. Beijing, China). The phosphate buffer (pH 6.8, 40 mM) was used as a blank[18]. The percentage of metmyoglobin (%MetMb) was calculated with equation:

$$metMb(\%) = [1.395 - (A_{572} - A_{700})/(A_{525} - A_{700})] \times 100$$

Where A700 was the absorbance at 700 nm, A572 was the absorbance at 572 nm, and A525 was the absorbance at 525 nm.

1.7 Sensory analysis

The sensory quality of chitosan-based films (color, odor, and overall acceptance) were evaluated by ten trained panelists from Department of Food Science and Technology, Kunming University of Science and Technology, on days 0, 4, 8, 12, 16, and 20 of storage. Sensory evaluation was conducted in individual booths under controlled conditions of light, temperature and humidity. A 5-point descriptive scale was provided to the panelists. A score of 3 or higher in any of the attributes was defined as unacceptable for sale or consumption.

1.8 Release of phenolic content from film into pork meat patties

On days 0, 4, 8, 12, 16, and 20 of storage, films were removed from pork meat patties, and total phenolic content (TPC) of CH-PRP film was determined according to the Folin-Ciocalteu assay (Singleton, & Rossi, 1965) [19]. 50 mg of each film sample was dissolved in 5 mL of methanol. Aliquots of 0.5 mL film extracts were mixed with 2.5 mL of Forint phenol reagent and 2 mL of 7.5% $NaHCO_3$. The tube was allowed to stand for 60 min at room temperature. Absorption at 765 nm was measured using a UV-vis spectrophotometer. TPC was expressed as gallic acid equivalents (mg GAE/g DW). This test was replicated three times for each sample.

1.9 Statistical analysis

SPSS statistical computer software package (SPSS version 13.0) was employed in this study. Analysis of variance (ANOVA) and Duncan's multiple range test were performed to evaluate the significance of differences between mean values. Analyses were run in triplicate for each replicate and the statistical significance was defined at $p<0.05$.

2 Results and discussion

2.1 Change of microbial status and pH value of pork meat patties

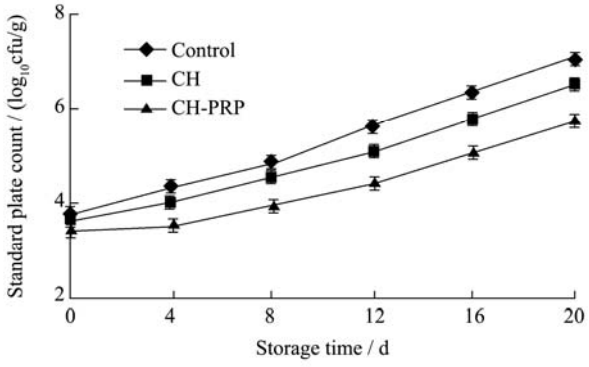

Fig.1 Effect of chitosan films on the standard plate count of pork meat patties during storage.

The microbiological analysis of pork meat patties treated with different chitosan-based films during 20 days storage period was presented in Fig.1. The standard plate count increased throughout 20 days of storage. The standard plate count of CH-PRP treatment group were significantly ($p<0.05$) lower than those in control and CH

treatment groups. At the 4th day, the standard plate count of CH treatment group was significantly ($p<0.05$) different from those in control group.

The microbial growth was more rapid in the control group, where the standard plate count of 5.62 $\log_{10}$CFU/g was found at the 12th day. At this point, the control group had off-odor and discoloration[1]. This value (7.0 $\log_{10}$CFU/g) for the standard plate count was considered as the upper acceptability limit for fresh meat[20]. However, CH and CH-PRP treatment groups never reached this limit value after a storage period of 20 days. Therefore, a microbiological shelf-life extension of 8 days was achieved for CH and CH-PRP treatment groups when compared to the control. This might be because that chitosan-based film could inhibit the growth of spoilage microorganisms and act as biopreservative material in pork meat.

Recently, Giatrakou et al. have reported that the combined use of chitosan and thyme resulted in a shelf-life extension of 6 days, as compared to the control samples[3]. Gómez-Estaca et al. reported that fish coated with chitosan and clove, rosemary or lavender essential oil combination had lower standard plate count than control samples during refrigerated storage[4]. Limit work has been reported on the application of chitosan in combination with pomegranate rind powder extract on fresh poultry products.

Al-Zoreky reported that 80% methanolic extract of pomegranate rind powder was a potent inhibitor for *L. monocytogenes*, *S. aureus*, *Escherichia coli* and *Yersinia enterocolitica*[21]. It afforded >1 log10 reduction of *L. monocytogenes* during storage at 4°C. Hayrapetyan et al found that pomegranate peel extract could effectively inhibit *L. monocytogenes* in meat patties at different temperatures[22]. The antimicrobial activity of PRP was related to the presence of phenolic compounds which were likely to be responsible for antibacterial activity. Therefore, the combination of chitosan and PRP could effectively increase the effect of antimicrobial property on pork meat.

Fig.2 showed the effect of chitosan films on pH values of pork meat patties. At the beginning of storage, pH value was not significantly ($p>0.05$) different between the control and the samples with CH treatment. However, pH value of CH-PRP group was significantly ($p<0.01$) lower than those in the control and CH groups. At the 8th day, pH value was significantly ($p<0.05$) different among all the groups. This could be attributed to the greater numbers of bacterial multiplication in the control group[15].

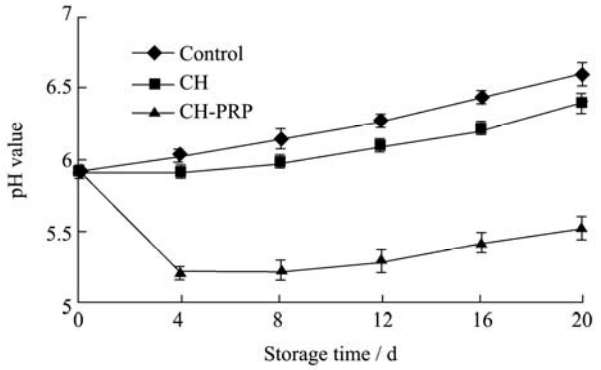

Fig.2 Effect of chitosan films on pH values of pork meat patties during storage.

2.2 Lipid stability (TBARS) of pork meat patties

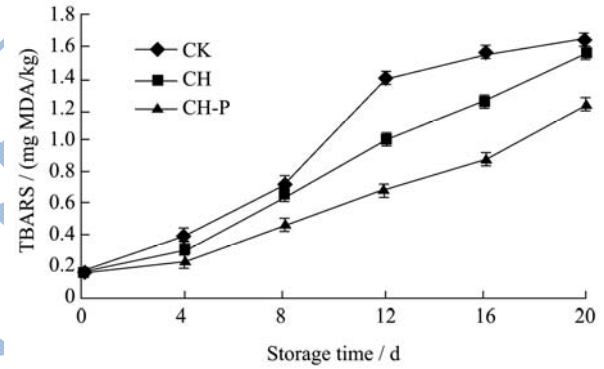

Fig.3 Effect of chitosan films on TBARS of pork meat patties during storage.

The effect of chitosan-based film on the thiobarbituricac idreactive substances (TBARS) values during refrigerated storage of pork meat patties was shown in Fig.3. TBARS test is widely used for assessing the lipid oxidative status of meat. The TBARS value of pork meat patties increased during 20 days of storage in all samples. At the beginning of storage, the TBARS value was not significantly ($p>0.05$) different among all the samples. At 8th day, the TBARS production was significantly ($p<0.05$) inhibited in pork meat patties treated with CH-PRP film, compared with that in the control and CH groups. On the day 12, the TBARS value was significantly ($p<0.05$) different among all the groups. The result suggested that lipid oxidation in pork meat patties could be decreased by the use of chitosan-based film. The mechanism by which this inhibition took place was believed to be related to chelation of free iron, which

released from hemoproteins of meat during storage[23].

Incorporation of pomegranate rind powder extract into chitosan film could enhance the antioxidant properties of the film. The TBARS value of CH-PRP group was significantly ($p<0.05$) lower than that of CH group. The inhibitory effect of CH-PRP film on lipid oxidation might be related to its phenolic constituents and other biochemical compounds in pomegranate rind powder that mainly contribute to the antioxidant activity. Previous studies have reported on the relationship between phenolic constituents and antioxidant activity of pomegranate rind powder. Devatkal et al. reported that pomegranate rind and seed powders were effective as natural functional ingredients in suppressing lipid oxidation in goat meat patties stored at 4±1 ℃ for 12 days[24]. Pomegranate rind powder extracts exhibited a protective effect against lipid oxidation in raw chicken patties during refrigerated storage[25]. These results indicated that CH-PRP film could be used as antioxidant active packaging to protect meat against lipid oxidation.

2.3 Change of Metmyoglobin (MetMb) content of pork meat patties during storage

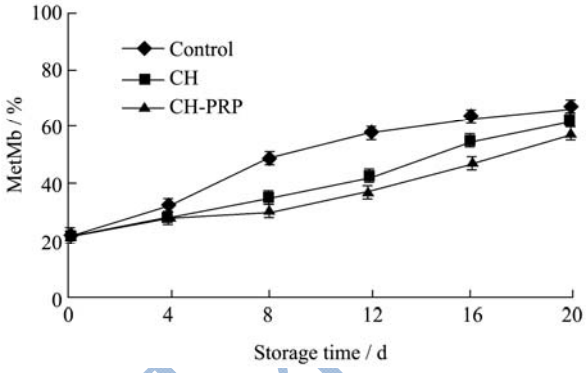

Fig.4 Effect of chitosan films on MetMb content of pork meat patties during storage.

Fig.4 showed the effect of chitosan films on MetMb content of pork meat patties. The metmyoglobin (MetMb) content at the beginning of the storage was 20.7%. As expected, MetMb content of all samples increased steadily during the whole period. This was in agreement with the trend of color in cold-stored pork meat patties. MetMb content of control sample was significantly ($p<0.05$) higher than those of CH and CH-PRP samples after 4 days storage. The incorporation of pomegranate rind powder extract into the chitosan film led to a reduction in MetMb content as compared to sample coated with pure chitosan film after 8 days storage.

It was known that the red color of meat depended upon the concentration of myoglobin (Mb) and its derivatives. The accumulation of metmyoglobin (MetMb, brown in color) was the major factor that resulted in a gradual discolouration (red to brown) of fresh meat[26]. Pomegranate rind powder extract exhibited strong radical scavenging capability. Phenolic compounds in PRP could scavenge the radical generated in meat, providing a possible explanation for the mechanism of MetMb inhibition. Furthermore, chitosan could exert antioxidant activity and their effects were also similar to those of phenolic antioxidants[27].

2.4 Sensory analysis

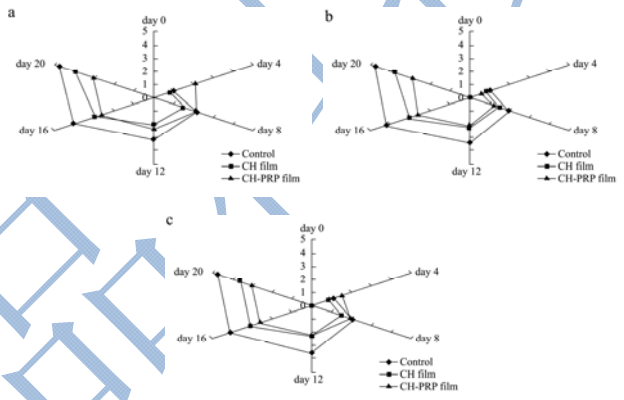

Fig.5 Effect of chitosan films on sensory evaluation of pork meat patties during storage. a-color scores. b-odor scores. c-overall acceptance.

The results of the sensory evaluation (color, odor, and overall acceptance) of pork meat patties, untreated group (control) and treated group (CH film and CH-PRP film) were presented (Fig.5). Color, odor, and overall acceptance scores of pork meat patties, irrespective of treatment, showed a similar pattern of decreasing acceptance.

On the initial day (day 0) of storage, pork meat patties had a pleasant odor and highly acceptable color. The color as expressed in terms of discoloration scores, as shown in Fig.5a, where the higher the scores, the lower the color quality. The sensory color of the samples treated with CH-PRP film was significantly ($p<0.05$) higher than that of control group and CH film group after storage for 4 days. This might be due to the migration of some water-soluble pigments of PRP extract. The discoloration scores of control group were significantly ($p<0.05$) higher than those wrapped with CH and CH-PRP films and reached unacceptable scores by day 12 of the storage.

For samples in CH-PRP film group, the discoloration scores were acceptable throughout the storage. Release of PRP phenolic compound could inhibit the microbial growth and lipid oxidation during storage and thereby leading to the lighter discoloration of the CH and CH-PRP samples.

In this study, the odor as expressed in terms of off-odor scores, as shown in Fig.5b, where 1 represented the lowest intensity of an off-odor. The off-odor scores of samples wrapped with control, CH film, and CH-PRP film, increased numerically during storage. The off-odor scores of control sample increased significantly ($p<0.05$) after refrigerated storage for 8 days. No significant ($p>0.05$) difference in the off-odor scores between CH film treatment and CH-PRP film treatment for 12 days. Significant ($p<0.05$) difference was observed in the off-odor scores for control, CH, and CH-PRP groups for 16 days storage. Unacceptable off-odor scores (score $\geqslant 3$) were observed in control samples and CH samples by day 12 and 16, respectively. For samples in CH-PRP film group, the off-odor scores were acceptable throughout the storage.

The consumer acceptability of meat products containing added phytochemicals is of high importance in the development of functional meat products[28]. The overall acceptance was expressed in terms of unacceptable scores, where the higher the scores, the more unacceptable the overall acceptance. The unacceptable scores (Fig.5c) of control samples was significantly ($p<0.05$) higher than those of CH and CH-PRP samples by day 12. Unacceptable overall acceptance scores (score $\geqslant 3$) were observed in control samples and CH samples by day 12 and 16, respectively. Higher unacceptable scores of CH-PRP samples on the initial day (day 0, day 4, and day 8) of storage, was because that PRP extract made the pork meat patties slightly darker. The samples wrapped with CH-PRP film could maintain acceptable sensory quality throughout the storage.

The results revealed that incorporation of PRP extract into chitosan film could enhance the antioxidant and antimicrobial properties of the CH-PRP film and thus extended the shelf life of pork meat patties.

2.5 Release of phenolic content from film into pork meat patties

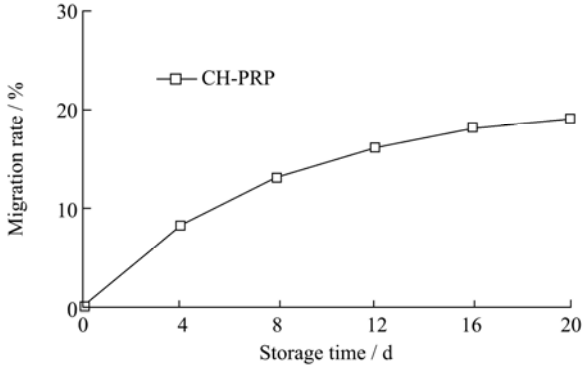

Fig.6 Release of phenolic content from CH-PRP film into pork meat patties during storage.

The use of active packaging film with antimicrobial or antioxidant agents could be more efficient than adding active agents directly into the food stuff since they might gradually migrate from the package to the food, thereby maintaining higher concentration when most necessary. So, it was very important to determine release behavior of phenolic content from food stuff. Release of phenolic content from CH-PRP film into pork meat patties during storage was shown in Fig.6. A gradual release was observed from the chitosan film containing PRP. Compared with the available literature, it appeared that the migration rate of active agents was much lower than oregano essential oil-impregnated chitosan film and Zataria multiflora Boiss essential oil-incorporated chitosan film[29]. It might be due to the stay of active agent at the surface of pork meat patties (in the close vicinity of the film). When the CH-PRP film was removed for analysis from the surface of pork meat patties, phenolic compounds which were in the close vicinity of the CH-PRP film was also removed. However, phenolic compounds which were in the close vicinity of the CH-PRP film also provided the antioxidant activity of food packaging material. These results provided justification for the application of chitosan film incorporated with PRP in food packaging.

3 Conclusion

The present study demonstrated the effectiveness of chitosan film incorporated with PRP extract on the antimicrobial properties and lipid oxidation of pork meat patties during storage at 4±1 ℃ for 20 days. Chitosan film incorporated with PRP extract could be a good alternative for preserving quality and extending the shelf life of pork meat patties.

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