Technical paper

The Application of a Compound Natural Preservative Solution to Chilled Beef and Mutton Under Vacuum Packaging During Refrigerated Storage

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A compound natural preservative (CNP) was produced by mixing clove cinnamon extracts with tea polyphenol, chitosan, propolis, nisin and lysozyme. Its effects on microbiological [total aerobic counts (TACs)], chemical [total volatile base nitrogen (TVB-N), 2-thiobarbituric reactive substances (TBARS), total reducing activity (TRA) and pH values], and colorimetric characteristics of chilled beef and mutton that were kept under vacuum packaging during refrigerated storage at 4 ± 1°C were investigated for 4 weeks. Results indicated that the CNP significantly inhibited the growth of microbes, improved TRA values, and decreased TBARS and TVB-N values to some extent especially at the later storage time, but had no obvious advantage in maintaining the color stability of beef and mutton when compared to lactic acid (LA, positive control) and sterile distilled water (DW, negative control). The shelf-lives of chilled beef and mutton were observed to be 3 – 4 weeks for the CNP treatment. The susceptibility of meat species to lipid oxidation was greater in beef than mutton, but spoilage as a consequence of bacteria load was greater in mutton than beef.

Keywords: compound natural preservative, beef, mutton, vacuum packaging, refrigerated storage

Introduction

Many food products are perishable by nature and require protection from spoilage during their preparation, storage and distribution for the desired shelf-life. The spoilage of food is a complicated process involving the interactions of microbial, physical, and chemical changes. Microbial contamination and lipids oxidation in foods during processing and storage are the major causes of food-borne illnesses and loss of shelf-life (Rao et al., 2008). A cold distribution chain is imperative, but it cannot assure the quality and safety of all perishable foods by itself. Therefore, additional preservation techniques such as physical processes (e.g., irradiation and packaging methods), artificial chemical methods (added calcium lactate, sodium succinate, and so on), and natural biological antimicrobial and/or antioxidant ingredients (adding plant essential oils or extracts, etc.) are being used or investigated for their application to food products (Nam et al., 2001; Zhu et al., 2009; Kong et al., 2010).

Meat is recognized as one of the most perishable foods, and consumers prefer meats that are high in polyunsaturated fatty acids (PUFAs) and myoglobin, for its high ferrous iron and phospholipid content (Enser et al., 1996; Rhee et al., 1996). Some researchers have reported that crude clove extracts have the greatest total phenolic contents and ferric-reducing antioxidant power out of 32 tested spices, are also strongly inhibitory of TBARS formation (Wojdylo et al., 2007), and thereby improved the quality and nutritional value of cooked pork patties (Kong et al., 2010). Cinnamon was the second most powerful extract (Kong et al., 2010). Moreover, preservative effects increased with spice concentrations. The incorporation of 1% chitosan was effective at minimizing premature browning and lipid oxidation-induced quality deterioration of ground beef patties (Suman et al., 2011). In addition, meat was contaminated easily by surface...
spoilage microbes or pathogens. The initial microbial load of meat depends on the physiological status of the animal at slaughter and the spread of contamination into slaughter houses during processing, and the temperature and other storage conditions during distribution can also influence the rate of spoilage.

Current research on preservation techniques is focused on the use of natural preservatives (antimicrobial and/or antioxidative substances) with biodegradable packaging materials to control undesirable changes to food characteristics. A combination of chitooligosaccharides (which are produced by treating chitosan with radiation) and lysozymes was more effective against Gram-negative bacteria than when used alone. When tested in a meat model system, the combination treatment resulted in complete elimination of *Escherichia coli*, *Pseudomonas fluorescens* and *Bacillus cereus* and reduced the load of *Staphylococcus aureus* cells in packed inoculum and storage studies (Rao et al., 2008). Chitosan coatings enriched with cinnamon oil enabled the retention of good quality characteristics for longer in fish samples as well as an extended shelf-life during refrigerated storage (Ojagh et al., 2010). The coating effects of tea polyphenol and rosemary extract combined with chitosan could more effectively maintain the good quality characteristics of large crucian carp and could extend its shelf-life by 6–8 days in comparison to the control group during refrigerated storage at (4 ± 1°C) (Li et al., 2012).

Beef and mutton are traditional muscle foods as well as pork in the north of China. Beef and mutton are more popular in consumers due to contents of higher protein and lower fatty acids than pork. Antimicrobial properties, lipid oxidation-inhibiting effects and color stabilization by different incorporated natural preservatives have been previously reported in ground beef, cooked beef, fresh beef, beef patties, beef burgers, or minced lamb meat/fresh mutton (Suman et al., 2011; Brettonnet et al., 2010; DeJong and Lanari, 2009; Nagai et al., 2006; Hayes et al., 2010; Tang et al., 2006; Trindade et al., 2010; Rao et al., 2008; Jayathilakan et al., 2007). Limited information is available on the effects of compound natural preservatives on chilled beef and mutton under vacuum packaging in refrigerated storage.

Because many natural antimicrobials have a limited spectrum of activity and are effective only at very high concentrations, the aim of this study was to investigate the antioxidant and antimicrobial effects at a lower concentration (1.4%) of the compound natural preservative on the quality of chilled beef and mutton. It was made from clove-cinnamon extracts and incorporated tea polyphenols, chitosan, propolis with nisin and lysozyme, and the tested meat was maintained under vacuum packaging during refrigerated storage at 4 ± 1°C. The objective was to seek a safe and effective natural preservative for ensuring the qualities of chilled meat over the course of its shelf-life, and then to substitute for effective artificial chemical preservative (e.g., 2% lactic acid solution) in the practice production.

**Materials and Methods**

*Materi*l*als*  Chilled beef and mutton (*Longissimus dorsi* muscles) were purchased 24 h post-slaughter from the local supermarket (Taigu, Shanxi province, China) for use in the tests that follow. Clove (*Syzygium aromaticum*) and cinnamon (*Ramulus cinnamomi*) were purchased from the local supermarket (Taigu, Shanxi province, China) and powdered finely before use. Tea polyphenol (purity ≥ 90%, gray-red powder), chitosan (low molecular weight, off-white powder, purity ≥ 99%, 90% degree of deacetylation, viscosity 10 – 1000 cP), Ethanic extracts of propolis, nisin (2%), lysozyme (derived from egg white), and lactic acid were obtained from Enterprise Group Pharmaceutical Co., Ltd. (Beijing, China). All the chemicals and reagents used in this study were of analytical grade and procured from Enterprise Group Pharmaceutical Co.

*Compound natural preservative preparation*  According to preliminary screening results, the compositions and ratios of the compound natural preservative were 6% clove-cinnamon (1:1) extract solution 2.5% (v/v, which is equivalent to mass fraction of clove-cinnamon (w/v) 0.15%), chitosan 0.5% (w/v), tea Polyphenol 0.5% (w/v), propolis 0.1% (w/v), nisin-lysozyme (1:1) 0.15% (w/v) (Sun et al., 2007). That is to say, the concentration of the compound natural preservative was 1.4% (w/v).

The cloves and cinnamon were broken into pieces in a high-speed shatter machine (Power-Driven Super-Speed Smash Machine Model ST112A/B, Sheng-tai Instrument Co. Ltd., Jinan, China). A quantity of 6 g of clove or cinnamon powder was placed in 100 mL of distilled water, cooked at 80°C for 8 h (Constant Temperature Water Bath Model HH-8, Xin-hang Instrument Factory, Jin-tan, China), and stirred constantly and chilled to room temperature, followed by centrifugation at 2000 × g for 15 min (Biofuge Osterode Centrifugal Model D-37520, Kendro Laboratory Products, Germany) to obtain clove or cinnamon extract solution (effective ingredient 6%, w/v). The total phenolics in each spice extract was determined using the Folin-Ciocalteu reagent, 75 mg ± 2.5 mg and 74 mg ± 1.3 mg of gallic acid equivalents per g of dry weight for cloves and cinnamon, respectively. A 12.5 mL volume of 6% clove extract was mixed with 12.5 mL of 6% cinnamon extract, 5 g of chitosan, 5 g of tea polyphenol, 1g of propolis, 0.75 g of nisin, and 0.75 g of lysozyme, and the solution was brought to a volume of 1000 mL with distilled water. The compound natural preserv-
tive solution (CNP, 1.4%, w/v) was thus completed. Lactic acid solution (LA, 2%, w/v, made with 20 g of lactic acid in sterile distilled water to a volume of 1000 mL) was used as a positive control, and sterile distilled water (DW) was used as a negative control.

Preparation of meat samples: Chilled beef and mutton (with the fascia removed) were cut into blocks (200 ± 10 g each) and randomly divided into three groups, and three replicates were made for each group. The three groups were soaked in DW, LA (2%), or CNP (1.4%) for 2 min, allowed to stand for 15 min, weighed, and packed in sterile vacuum packaging (Vacuum Packaging Machine Model DZQ400-2D, Dingli Packaging Machinery Wenzhou Co. Ltd., China) and stored in the refrigerator at 4 ± 1°C. A 2.5 mL quantity of filtrate was added to 2.5 mL of sterile distilled water, and the pH value was measured using a digital pH meter (Precision pH meter Model 211, Leici Instrument Shanghai Co. Ltd., China) in 15 mL of distilled water, and the pH value was measured using the following equation (Ma and Xiong, 2011):

$$\text{pH} = \log_{10} \frac{V_1}{V_2}$$

where the volume of hydrochloric acid that was used by 1 mL of filtrate was expressed as $V_1$, the volume of hydrochloric acid in which 1 mL of boric acid was used up was expressed as $V_2$, and a quantity of 2.5 g of meat sample was expressed as $m$. Here, “14” is the atomic mass of a nitrogen atom.

Analysis of samples

1. Microbial analysis: TACs of the meat samples were analyzed using a dilution plate counting method to illustrate aerobic plate count detection in accordance with the Microbiological Examination of Food Hygiene of the GB/T from the Chinese national standard (GB/T 4789.2-2010). A 10 g meat sample was homogenized with 90 mL of 0.9% sodium chloride (NaCl)-water solution and stirred for 30 min. Multiple serial dilutions were made with this sample. Three 1 mL aliquots of each dilution were transferred to Petri dishes containing 15 mL of plate count agar (PCA, Base Bio-Tech, Hangzhou, China) for each sample. The TACs values were determined by counting the number of colony-forming units after incubation at 37°C for 24 h.

2. Measurement of pH: The pH values were measured to conform to the pH section from the Meat and Meat Products chapter of the GB/T from the Chinese national standard (GB/T 9695.5-2008). A 5 g sample of ground meat was homogenized for 30 s (High-Shear Disperser Emulsification Machine, Fluko Equipment Shanghai Co., Ltd, China) in 15 mL of distilled water, and the pH value was measured using a digital pH meter (Precision pH meter Model 211, Leici Instrument Shanghai Co. Ltd., China).

3. Determination of total volatile basic nitrogen (TVB-N): TVB-N values were estimated using the half-micro diffusion method to follow the preferred analysis protocol for the hygienic standard of meat and meat products from the GB/T of the Chinese national standard (GB/T 5009.44-2003). A 2.5 g meat sample was blended in 25 mL of distilled water, stirred frequently, chilled at room temperature for 30 min and filtered. The filtrate was stored in a refrigerator at 4 ± 1°C. A 1 mL quantity of 3% aqueous boric acid (H3BO3) was mixed with an indicator that was produced from the dissolution of 0.1 g of methyl red and 0.1 g of methylene blue in 100 mL of ethanol that was added to the inner compartment of a glass dish (Long-Yuan Glass Production Factory, Beijing, China), while 1 mL of filtrate was added to the outer compartment of the dish and 1 mL of potassium carbonate (K2CO3) saturation solution was added to the other side (so that the two solutions in the inner and outer compartments could not touch). The glass dish was immediately closed tight, swirled gently to blend the filtrate with the potassium carbonate solution, and stored in a constant temperature incubator for 2 h at 37°C. Afterwards, the boric acid solution was titrated with a 0.1062 mol/L hydrochloric acid (HCl) solution. The TVB-N value (mg N/100 g of meat sample) was determined according to the consumption of hydrochloric acid. The equation was as follows:

$$\text{TVB-N (mg/100g)} = (V_1 - V_2) \times 0.1062 \times 14 \times 10000 / m$$

where the volume of hydrochloric acid that was used by 1 mL of filtrate was expressed as $V_1$, the volume of hydrochloric acid in which 1 mL of boric acid was used up was expressed as $V_2$, and a quantity of 2.5 g of meat sample was expressed as $m$. Here, “14” is the atomic mass of a nitrogen atom.

4. Spectrophotometric determination of thiobarbituric acid reactive substances (TBARS): The lipid oxidation of meat samples was monitored by determining the concentration of thiobarbituric acid reactive substances during storage. A 5 g quantity of meat sample (ground meat) was homogenized in 15 mL of 7.5% TCA (which was mixed with 0.1% BHA and 0.1% EDTA) for 30 s. The homogenate was filtered. A quantity of 2.5 mL of filtrate was added to 2.5 mL of 0.02 mol/L thiobarbituric acid at boiling temperature (100°C) for 40 min and cooled in ice immediately. Afterwards, 5 mL of chloroform (CHCl3) were added, and the mixture was centrifuged at 2000 × g in 2 °C for 10 min. The spectrophotometric absorbance of the pink supernatant was measured at 532 nm. TBARS values were calculated as mg of TBA-reactive substances (TBARS) per 100 g of meat sample using the following equation (Ma and Xiong, 2011):

$$\text{TBARS (mg / 100 g)} = (A_{532} / W) \times 92.4,$$

where $A_{532}$ is the absorbance of the pink supernatant at 532 nm, $W$ is the weight of the meat sample (g), and “92.4” is a constant derived from the sample dilution factor and the molar extinction coefficient (156,000 M-1 cm-1) of the pink supernatant (TBARS). All assays were conducted in triplicate on every sample in storage.

5. Determination of total reducing activity (TRA): TRA was measured in meat samples using the method of Suman...
et al. (2004). Triplicate meat samples in 2 g quantities were homogenized (High-Shear Disperser Emulsification Machine, Fluko Equipment Shanghai Co., Ltd., China) with 10 mL of 25 mM PIPES (piperazine-n, n-bis-2-ethane-sulfonic acid) buffer, and 5 mL of homogenate was transferred to a 10 mL volumetric flask. Two mL of 5 mM potassium ferricyanide was mixed with the homogenate and chilled in a refrigerator at 2°C for 1 h and stirred for every other 15 min. Then, 0.1 mL of 0.5% ammonium sulfamate and 0.2 mL of 0.5 M lead acetate were added, and the mixture was allowed to stand at room temperature for 5 min. A 2.5 mL volume of 20% trichloroacetic acid was added, and the solution was brought to its total volume (10 mL) with distilled water. After 5 min, the solution was filtered through Whatman No. 42 filter paper. A 25 mM solution of PIPES was prepared. Solution absorbances were measured at 420 nm using a WFJ2100 UV-visible spectrophotometer (Lengpu Instrument Co. Ltd., Shanghai, China). The TRA was expressed as the A420 of 25 mM PIPES minus the A420 of sample filtrate, that is to say, TRA = 1-A. 6. Measurement of color The effects of preservatives on the color properties of meat samples were evaluated by an automatic Color Difference Meter (TCP2, Aoyike Photoelectric Instrument Co. Ltd., Beijing, China) with a 3.18 mm aperture set to illumination D at 65 and 10° standard observer angle. The instrument was calibrated using a white standard plate. The system of the International Commission on Illumination (CIE) was used, and L*-values (lightness), a*-values (redness) and b*-values (yellowness) were collected for each sample. Triplicate meat samples were analyzed to obtain an average colorimetric value. 7. Statistical analysis The experimental factors included two meat species, three preservative treatments and five storage times. The data were statistically analyzed with an analysis of variance (ANOVA) using SAS 9.1.3 (SAS Institute Inc., 2003), followed by a Duncan's multiple range test. Differences among three preservative treatments or five storage times were considered to be statistically significant when p < 0.05 (at a 95% confidence level). The graphs were plotted using Sigma Plot 10.0 (Systat Software Inc., 2006). The average values were reported along with the standard deviations (± Standard Deviation). Results and Discussion Microbial analysis Muscle foods are susceptible to microbial contamination, which sometimes leads to food-borne illnesses. It has been estimated that approximately one-third of the world’s food production is lost annually to microbial spoilage (Li et al., 2012). Table 1 shows the TACs values for all of the meat sample treatments, which increased significantly with storage (p < 0.05). With respect to factors such as slaughter equipment, ambient environment and human handling, fresh meat was quickly contaminated by surface bacteria such as intestinal bacteria. Preservatives, especially CNP, significantly inhibited the initial aerobic count of meat by 2.58 (beef) and 2.91 (mutton) log CFU/mL. At the same storage times, the preservatives significantly inhibited aerobic counts compared to DW (p < 0.05), and the inhibiting effect of CNP was stronger than LA. In comparison to chilled mutton, the TACs values for chilled beef in refrigerated storage were reduced significantly, which is in agreement with a report by Nagai et al. (2006). The beef and mutton that were treated with DW attained a TACs value of 6.02 and 6.96 log CFU/mL on week 3, respectively, while they attained 7.47 and 7.13 log CFU/mL on week 4, which outnumbered the microbiological acceptability limit of 7 log CFU/mL for raw meat (GB/T 4789.2-2010, China), indicating a microbiological shelf-life of approximately 3 weeks for DW treatments. It was concluded that the solutions of CNP and LA were significantly delaying microbial growth (p < 0.05) in comparison to the DW, and the CNP solution could especially extend the shelf-life of beef to 4 weeks and extend that of mutton to close to 4 weeks.
Previous studies showed that tea polyphenols had broad antimicrobial properties and could effectively inhibit most food-borne pathogens and spoilage organisms (Kumudavally et al., 2008). Chitosan effectively inhibited the growth of not only Gram-positive and Gram-negative bacteria but also yeasts and molds (Serrano and Bañón, 2012). Nisin and Ethanol extract of propolis successfully inhibited viable Gram positive bacteria counts or Escherichia coli development (Ercolini et al., 2010; Tosi et al., 2007). Lactic acid had the same inhibitory effect on the growth of spoilage bacteria and cold-tolerant pathogens in pork (Greer and Dilts, 1995). This research showed that the antimicrobial property of 1.4% CNP solution was stronger than 2% LA solution because of the integrated superiority of several natural antimicrobials, which could extend the shelf-life of chilled beef and mutton for approximately 3 – 4 weeks.

**Total volatile basic nitrogen (TVB-N) analysis**

The TVB-N value is one of the most widely used indicators for meat deterioration. As a result of the degradation of proteins and non-protein nitrogenous compounds, which are chiefly caused by spoilage microbial activity, some volatile basic nitrogenous substances were produced, e.g., ammonia and primary, secondary and tertiary amines (dimethylamine, trimethylamine). Other similar volatile basic nitrogenous compounds were also produced (Li et al., 2012), and consequently, the meat deteriorated. Fig. 1 indicates that the values of TVB-N increased gradually in all of the meat sample treatments during the 4-week storage. For beef, TVB-N values increased from an initial value of 7.58 to 14.51 mg/100 g of meat sample with the DW treatment, from 7.27 to 12.00 mg/100 g of meat sample with the LA treatment, and from 5.65 to 9.13 mg/100 g of meat sample with the CNP treatment at the end of the storage period (Week 4). During the whole 4-week storage time, the TVB-N values of three treatments were maintained within the acceptability limit level of 20 mg /100 g of fresh meat (GB/T 5009.44-2003, China). In mutton, the TVB-N values of the three treatments increased from a higher initial value of approximately 12.07 – 13.84 to 20.44 – 22.13 mg/100 g of meat sample, which was above the acceptability limit level at week 2 of storage. This result suggests that mutton was more easily perishable than beef. At the same storage time, TVB-N values of CNP were lower than that of LA and DW for the same meat species. For beef, the preservatives (CNP and LA) significantly reduced protein degradation to produce greater TVB-N values than DW \( (p < 0.05) \) for week 2 – 3, and the difference of CNP with LA was significant \( (p < 0.05) \) at the 4\textsuperscript{th} week. However, the differences for mutton among three treatments were not significant \( (p > 0.05) \) until the 3\textsuperscript{rd} week.

Because TVB-N is produced mainly by the bacterial decomposition of meat muscle, the initial TVB-N value was related to the relatively low value of TACs and with higher values of TVB-N with storage, in contrast to lower values of TACs, and lower values of TVB-N with preservative treatments (CNP and LA). The TVB-N value of CNP-treated samples was lower with the LA treatment during the whole storage period. Kumudavally et al. (2008) demonstrated that an ethanolic extract of green tea significantly inhibited the formation of biogenic amine (proteolytic degradation products) of fresh mutton at ambient temperature \( (25 \pm 2\degree C) \).

**Thiobarbituric acid reactive substances (TBARS) analysis**

Lipid oxidation is one of the main factors that limits the quality and acceptability of lipid-containing foods. The TBARS value has been broadly used to describe the degree of lipid oxidation, and the presence of TBARS is due to second stage auto-oxidation, during which peroxides are oxidized to aldehydes and ketones. Lipid oxidation of meat samples was monitored by determining the quantity of thiobarbituric acid reactive substances (TBARS) in storage. The values of TBARS are shown in Fig. 2. The results showed that with the exception of CNP-treated beef, TBARS production in the other treatments increased gradually with storage time \( (p < 0.05) \). The curves of LA and DW treatments were similar and their TBARS values were higher than that of the CNP treatment at the same storage time, and the differences became more significant after the 2\textsuperscript{nd} week \( (p < 0.05) \).

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**Fig. 1.** Changes in TVB-N values of chilled beef and mutton during refrigerated storage. Meat samples were dipped in distilled water (DW), 2% lactic acid solution (LA) or 1.4% compound natural preservative solution (CNP) and then stored at 4 ± 1\degree C for 4 weeks. Each point is the mean of triplicates, and the bars denote ± 1 standard deviation.

**Means within each storage period with the same letter are not significantly different \( (p > 0.05) \).

**Means within each treatment with the same letter are not significantly different \( (p > 0.05) \).**
Previous studies showed that lactic acid could significantly reduce the bacterial load along with a significant increase in the values of hexanal, 2-pentyl furan and MDA, and lipid oxidation increased with increases in acid concentrations (Ogden et al., 1995; Sundar and Zhang, 2006). However, the clove extract, which had eugenol as its major component, had the highest free radical-scavenging ability among the 32 spice extracts tested (Wojdyło et al., 2007) and also exhibited stronger inhibitory ability on the TBARS formation as well as greater ferric-reducing power in cooked pork patties (Kong et al., 2010). Nagai et al. (2006) demonstrated that propolis not only had higher hydroxyl radical scavenging activity (over 77% inhibition) but also had the strongest superoxide-scavenging activities among the ascorbic acid, α-tocopherol and honey species that were tested. Chitosan significantly inhibited lipid oxidation and premature browning of ground beef (Suman et al., 2011). This study indicated that the CNP solution had a stronger antioxidant capacity than the LA solution (p < 0.05) in chilled beef and mutton, and the antioxidant activity was greater in beef than in mutton.

The susceptibility of meat species to lipid oxidation was greater in beef than mutton, and the same result was obtained by Jayathilakan et al. (2007). One possible explanatory factor was the difference in the phospholipids, polyunsaturated fatty acids (PUFA) and/or free iron contents between the two meat species (Rhee et al., 1996). Ferric and ferrous irons catalyze the decomposition of lipid peroxides to more volatile aldehydes and ketones (Baron and Andersen, 2002). Beef muscles had greater PUFA and phospholipid concentrations and/or a higher ferric/ferrous iron concentration than mutton (Enser et al., 1996), and thus were oxidized more easily.

**Total reducing activity (TRA)** Myoglobin (Mb) is commonly found in three forms: deoxymyoglobin (DeoMb), oxymyoglobin (OxyMb), and metmyoglobin (MetMb), and the relative proportions of these compounds determine the color of fresh meat. Each molecule of Mb contains a single iron atom that may bind one molecule of O2 when the iron is in the ferrous state (Mb-Fe2+). Mb-Fe2+ undergoes spontaneous oxidation to the ferric state (Mb-Fe3+) to form MetMb, which cannot bind O2 (Zhu et al., 2009). The total reducing activity (TRA) of MetMb was one factor that influenced interconversion among DeoMb, OxyMb, and MetMb.

The TRA values of different treatments and meat species during refrigerated storage are shown in Fig. 3. Both the DW and LA treatment TRA values increased slowly with storage. One possible reason was that the meat surface had lower oxygen pressure under the vacuum packaging, which caused the decrease of OxyMb but accelerated a reduction in the activity of MetMb, and TRA was consequently improved to a limited extent. The CNP solution significantly enhanced the initial values of TRA for both chilled meats, and during the entire storage, the TRA values of the CNP treatments were at higher levels in comparison to the two controls (p < 0.05). It was reported that the addition of reducing agents, e.g. ascor-
bic acid, increased surface $a^*$ values and TRA and decreased the lipid oxidation of ground beef, and the addition of ascorbic acid accelerated MetMb reduction by donating electrons to the ferric state of heme and subsequently resulted in the conversion of ferric myoglobin to ferrous myoglobin (Sepe et al., 2005). In contrast, a reduction in the pH of lactic acid-treated samples enabled the denaturation of the globin protein moiety that protects the heme, and the iron in the heme of myoglobin is more likely to be oxidized from the ferrous ($Fe^{2+}$) to ferric ($Fe^{3+}$) state, resulting in an increase in metmyoglobin formation but a decrease in TRA value (Ogden et al., 2006). Including some reducing polyphenols in the CNP solution of this study, such as tea polyphenol, eugenol (clove extract), and cinnamaldehyde (cinnamon extract), led to an increase in the TRA values of meat samples, as well as the CIE $L^*$ and $a^*$ values (Table 2), to some extent.

Table 2 shows that CIE $L^*$ and $a^*$ values decreased significantly ($P < 0.05$) with increased storage for all of the meat sample treatments. The LA solution could not protect the color from fading from the meat samples. The same result was obtained by Sundar et al. (2006). The external surface of mutton that was treated with CNP demonstrated greater $L^*$ values than mutton treated with LA and DW ($P < 0.05$). Despite other differences between the meat species, the differences in $a^*$ values among the three treatments were not significant ($P > 0.05$) after the same storage times. This study suggests that the CNP solution had no obvious advan-

### Table 2. The values of color differences for chilled beef and mutton in storage.

<table>
<thead>
<tr>
<th>Chilled meat</th>
<th>Color</th>
<th>Treatments</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Beef</strong></td>
<td>$L^*$</td>
<td>DW</td>
<td>33.46 ± 1.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.07 ± 1.75&lt;sup&gt;b,a&lt;/sup&gt;</td>
<td>31.74 ± 0.86&lt;sup&gt;b,a&lt;/sup&gt;</td>
<td>30.85 ± 1.23&lt;sup&gt;b,a&lt;/sup&gt;</td>
<td>28.84 ± 1.00&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LA</td>
<td>36.30 ± 1.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.60 ± 1.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.99 ± 0.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.90 ± 0.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.59 ± 0.29&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CNP</td>
<td>39.82 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.54 ± 0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.44 ± 0.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.60 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.75 ± 1.21&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>$a^*$</td>
<td>DW</td>
<td>6.48 ± 1.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.23 ± 0.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.13 ± 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.75 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.32 ± 0.61&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td></td>
<td>LA</td>
<td>7.42 ± 2.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.29 ± 1.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.50 ± 1.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.97 ± 1.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.88 ± 1.30&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td></td>
<td>CNP</td>
<td>8.72 ± 1.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.87 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.56 ± 1.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.01 ± 0.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.13 ± 1.40&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>$b^*$</td>
<td>DW</td>
<td>3.79 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.57 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.13 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.61 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.69 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td>LA</td>
<td>2.17 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.25 ± 0.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.14 ± 0.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.25 ± 1.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.57 ± 0.98&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td>CNP</td>
<td>4.20 ± 0.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.14 ± 0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.78 ± 0.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.93 ± 0.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.04 ± 0.30&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td><strong>Mutton</strong></td>
<td>$L^*$</td>
<td>DW</td>
<td>41.87 ± 0.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.65 ± 0.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.85 ± 2.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.68 ± 0.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.53 ± 2.40&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td>LA</td>
<td>42.88 ± 0.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.27 ± 1.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.26 ± 1.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.46 ± 0.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.64 ± 0.49&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>CNP</td>
<td>44.67 ± 1.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.63 ± 1.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.82 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.75 ± 0.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.05 ± 2.50&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>$a^*$</td>
<td>DW</td>
<td>14.77 ± 0.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.16 ± 0.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.17 ± 0.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.40 ± 1.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.68 ± 0.68&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
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<td>LA</td>
<td>14.89 ± 0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.87 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.58 ± 0.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.70 ± 0.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.76 ± 1.03&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
<td>CNP</td>
<td>15.26 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.84 ± 0.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.82 ± 2.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.73 ± 2.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.54 ± 1.15&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
<td>$b^*$</td>
<td>DW</td>
<td>12.30 ± 1.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.92 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.01 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.88 ± 0.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.52 ± 0.58&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td>LA</td>
<td>12.00 ± 0.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.07 ± 0.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.66 ± 0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.14 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.40 ± 1.03&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>CNP</td>
<td>11.94 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.84 ± 1.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.47 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.59 ± 0.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.00 ± 0.81&lt;sup&gt;a&lt;/sup&gt;</td>
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Meat samples were dipped in distilled water (DW), 2% lactic acid solution (LA) or 1.4% compound natural preservative solution (CNP) and then stored at 4 ± 1°C for 4 weeks.

A, B and C; $P < 0.05$, between different treatments; a, b, c, d and e; $P < 0.05$, between different storage times.
tage over the LA solution with respect to the color stability of chilled beef and mutton.

The pH analysis Changes in the pH values of meat samples during storage are shown in Fig. 4. During storage, the pH values of beef decreased in the early stages and then increased gradually. In contrast, the pH values of mutton increased in the early stages and then decreased gradually. The initial decrease of pH of the beef might be related to the accumulation of lactic acid, which is a product of glycolysis, because the maturation process (development of rigor mortis) of beef was not finished at 24 h after slaughter, while the increase seen during late storage may be caused by the growth of spoilage bacteria, leading to the accumulation of alkaline substances (e.g., ammonia and trimethylamine). In contrast, the mutton pieces were close to maturity (maximum development of rigor) at 24 h after slaughter. After storage week 3, the pH values of mutton decreased dramatically, the reason likely being that anaerobic bacteria became predominant under the vacuum packaging and produced a large amount of lactic acid.

This study also showed that the CNP solution enabled chilled meat to maintain higher pH levels (5.9 < pH < 6.6) during storage week 3. It was reported that the irradiation of meat receiving a high-pH (> 6.2) treatment resulted in less color change and lower lipid oxidation than a low-pH (< 5.4) treatment (Nam et al., 2001). In addition, the TVB-N value was correlated with the pH of the product (Noseda et al., 2010). As a consequence of the presence of many alkaline polyphenols in this study such as tea polyphenol, eugenol (clove extract), and cinnamaldehyde (cinnamon extract) in the CNP solution, the CNP treatment had a higher pH during storage, thereby maintaining higher TRA values but lower TBARS values. Considering the values of TACs, TVB-N, TBARS, TRA and pH, the CNP solution could extend the shelf-life of chilled meat for 3 weeks (for mutton) or greater than 4 weeks (for beef).

Conclusion With growing concern over the presence of chemical residues in foods, the demand for nontoxic natural preservatives is increasing. The present study showed that preservative treatment with either 1.4% compound natural preservative [a mix of 12.5 mL clove (Syzygium aromaticum) extract (6%, w/v) and 12.5 mL cinnamon (Ramulus cinnamomi) extract (6%, w/v) with 5 g of chitosan, 5 g of tea polyphenol, 1 g of propolis, 0.75 g of nisin, and 0.75 g of lysozyme] or 2% lactic acid could effectively retard microbial growth, delay the formation of TVB-N and lipid oxidation, postpone deterioration, maintain color stability to some extent and extend the shelf-life of chilled beef and mutton for 3 – 4 weeks during refrigerated storage. Compound natural preservative was both safer and more effective than the chemical preservative (lactic acid) for maintaining chilled beef and mutton during refrigerated storage.

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References


