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ARTICLE

The Application of Clove Extract Protects Chinese-style Sausages against Oxidation and Quality Deterioration

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Abstract

This study was conducted to evaluate the effects of clove extract (CE) (0.25%, 0.5%, 1%, and 2%) on the oxidative stability and quality deterioration of Chinese-style sausage stored for 21 d at 4°C. The addition of clove extract to sausages significantly retarded increases in Thiobarbituric Reactive Substances (TBARS) values ($p<0.05$), while also controlling the production of protein carbonyls ($p<0.05$). However, the addition of clove extract promoted reduced thiol group content in sausages ($p<0.05$). Sausages amended with clove extract also had decreased L* values ($p<0.05$) and increased a* values ($p<0.05$) when compared with the control. Similarly, texture deterioration was retarded in sausage containing added clove extract when compared with the control during refrigerated storage. Moreover, the addition of clove extract had no negative effects on the sensory properties of sausages. These results suggested that clove extract was effective at protecting sausages from oxidation and quality deterioration during refrigerated storage for 21 d.

Keywords clove, Chinese-style sausage, oxidation stability, color, texture

Introduction

Chinese-style sausages, which are widely consumed in southern China, are typically made of lean meat and 20-40% back fat. When processed using traditional methods, sausage must be naturally air-dried for 10-15 d to produce their characteristic flavor. Accordingly, it is difficult to control quality because of the slow processing times. Therefore, industrial production employs high temperature drying instead of natural air-drying; however, this method causes problems such as fat overflow, a greasy taste and most importantly discoloration and oxidative rancidity. Moreover, lipid oxidation is known to promote the occurrence of protein oxidation, resulting in loss of essential amino acids and detrimental effects on meat quality. Therefore, the degree of lipid and protein oxidation in meat products must be controlled and minimized. It is well known that the addition of antioxidants could effectively delay lipid oxidation of meat products. Synthetic antioxidants are commonly used to retard lipid oxidation during the industrial production of meat products; however, these materials have been reported to have toxic effects. As a result, natural antioxidants have been used instead of synthetic antioxidants and are shown to be an effective method of controlling and retarding lipid oxidation. Indeed many plant and spice extracts have been reported as alternatives to syn-

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thetic antioxidants. Spices are a rich source of total polyphenols with superior antioxidant capacities, and the extracts of various spices such as rosemary, ginger, nutmeg, and oregano have been shown to have potent antioxidant activities (Kong *et al*., 2010; Lara *et al*., 2011; Lu *et al.*, 2011; Sulaiman and Ooi, 2012).

Cloves, the flower buds of *Syzygium aromaticum*, are important plants and aromatic spices that are extensively cultivated in China. Eugenol and caryophyllene are the main ingredients of clove extract, and these compounds are reportedly the most active constituent in the clove and responsible for their antioxidant activity (Radha *et al*., 2014). Some reports have shown that clove extracts can retard the lipid oxidation of meat products (Ito *et al*., 2005; Jayathilakan *et al.*, 2007; McArthy *et al.*, 2001; Xi *et al.*, 2012). However, the effects of the addition of clove extracts to Chinese-style sausage in terms of inhibition of lipid and protein oxidation and impact on texture properties is unknown. Therefore, the present study was conducted to evaluate the effects of different concentrations of clove extracts on lipid and protein oxidation and quality changes during 4°C storage of Chinese-style sausages.

Materials and Methods

Preparation of clove extracts

Dried cloves (*Syzygium aromaticum*) were purchased from a local traditional Chinese pharmacy (China). Aliquots (50 g each) of powdered and dried cloves were mixed into 400 mL of 95% (v/v) edible ethanol for 12 h in enclosed flasks with constant shaking (100 rpm), after which they were filtered through Whatman No. 2 filter paper. The residue was subsequently re-extracted with an additional 200 mL of 95% edible ethanol for an additional 12 h, then filtered. Next, the combined filtrates were concentrated on a rotary evaporator (RE 52CS, Yarong Biochemical Analysis Co., Ltd., China) (50°C) with a vacuum pump and the extracts were freeze dried. Finally, dried extracts were placed in sealed bottles and stored at 4°C before use.

Preparation of sausages containing clove extract

Chilled pork and pork back fat were obtained from a local meat plant in Luoyang, Henan, China. The connective tissue and visible fat were trimmed and the remaining meat was minced through an 8-mm plate. The back fat was manually cut into cubes of about 0.5 cm^3 before use. Collagen casings (diameter of the product 25 mm) were purchased from Luoyang Chundu Meat Product Co. Ltd., Henan, China. The minced lean meat was mixed completely with the seasonings, including salt (3%), sugar (7.5%), monosodium glutamate (0.2%), five-spice powder (0.1%), and white wine (1.5%). To investigate the effects of clove extract on the oxidative stability and texture properties of sausages, five batches of samples were manufactured: 1) no antioxidant (control), 2) 0.25% clove extract, 3) 0.5% clove extract, 4) 1% clove extract, and 5) 2% clove extract. Clove extract was dissolved in edible ethanol before being mixed with the ingredients, after which the ingredients were further homogenized at 500 rpm for 1 min. The back fat cubes (20%) were mixed with the ingredients completely before being stuffed into collagen casings. The control group was manufactured similarly, but edible ethanol was added instead of clove extract. The sausages were hand-linked at 10-cm intervals and dried in an oven for 40 h at 65°C, after which they were cooled to room temperature, vacuum-packaged (DZ-400/2L, Zhengtai Packing Equipment Co., Ltd., China) and stored at 4°C. Each treatment group was randomly sampled at days 0, 7, 14 and 21.

Lipid oxidation

TBARS were measured as described by Coutinho de Oliveira *et al*. (2012), with slight modification. Briefly, samples (1 g) were minced and mixed with 10 mL distilled water, after which the mixtures were homogenized for three periods of 60 s at 10,000 rpm using a Polytron homogenizer. Sausage homogenate (0.2 mL) was transferred to a test tube and mixed with 0.2 mL of 8.1% sodium dodecylsulphate, 1.5 mL 20% acetate buffer (pH 3.5), 1.5 mL of 0.8% (w/v) thiobarbituric acid (TBA) solution and 0.6 mL distilled water. The mixtures were then incubated in a water bath at 95°C for 40 min, after which they were cooled with running tap water. After being added to the tubes with 4 mL of n-butanol-pyridine solution (butanol/pyridine: 15/1) and 1 mL distilled water, the mixtures were vortexed for 30 s, then centrifuged at 4000 rpm for 10 min, after which the absorbance at 532 nm was determined. These values were then compared to those of a standard curve prepared using 1,1,3,3-tetraethoxypropane (TEP). TBARS values were expressed as mg of MDA equivalents/100 g sample.

Protein oxidation

Determination of protein carbonyls

Protein carbonyl content was determined according to

the procedure described by Botsoglou *et al*. (2012). Briefly, 2, 4-dinitrophenylhydrazine (DNPH) was reacted with protein carbonyl chemicals to form protein hydrazones. The reaction products were tested by determining the absorbance at 370 nm, while the absorbance at 280 nm was used to calculate the protein concentrations. A standard curve was prepared using BSA as a standard. The carbonyl groups content was expressed as nmol thiol/mg protein using an adsorption coefficient of 21.0 $mM^{-1} \times cm^{-1}$.

Determination of protein thiol groups

Protein thiol groups were determined by 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) agent according to the method described by Berardo *et al*. (2015). A standard curve prepared from a 200 μM L-cysteine stock solution was used to calculate the protein thiol concentration, while protein concentrations were determined by comparison to a BSA standard curve. The results were expressed as nmol thiol/mg protein.

Color measurement

The internal color of the sausage samples was measured using a colorimeter (Xrite ColorI5, USA). Briefly, samples were put into a circular module with a thickness of 10.0 mm and the values from 30 different samples were used for statistical analysis. A standard plate (L* = 94.0, a* = 0.315, b* = 0.323) was employed to calibrate the instrument prior to measuring. The color values were expressed as L value (L* = 0 darkness, L* = 100 lightness), a value (+60 = red, −60 = green), and b value (+60 = yellow, −60 = blue) based on the CIE color profile system.

Texture profile analysis (TPA)

The texture change of sausages was studied using a Texture Analyzer (5544Q6427, Instron Engineering Corporation, USA) as described by Maqsood *et al*. (2012), with some modifications. Briefly, samples were cut to a height of 30 mm prior to analyses and the determinations were subjected twice to compression tests with a Texture Analyzer at room temperature. Each slice of sausage was compressed to 50% of its original height with a cylindrical aluminum probe (50 mm diameter) at 5.0 mm/s. Textural properties of the sausages expressed as hardness, chewiness, gumminess, cohesiveness, and springiness were then calculated according to the TPA curves obtained.

Sensory evaluation

An experienced panel group of nine members evaluated the sausages on days 0 and 21 during refrigerated storage according to the procedures described by Meilgaard *et al*. (1999), with some modifications. Briefly, panelists were asked to evaluate the sausage samples for color, flavor, texture and overall acceptability using a 9-point hedonic scale (1 = dislike extremely; 9 = like extremely). The sausage samples were sliced into pieces 0.20-0.30 cm thick, then placed in containers with covers. Finally, the samples were steamed after boiling the water for 10 min. The samples were cooled to room temperature before being served to the panelists.

Statistical analyses

All tests and samples were conducted in triplicate for data analysis. The data were evaluated by the General Linear Models procedure using the Statistix 8.1 software. Analysis of variance (ANOVA) was conducted to identify significant effects. Significant differences between means were identified by Tukey's test. A $p<0.05$ was considered to indicate significance.

Results and Discussion

Lipid oxidation

The effects of CE on lipid oxidation of Chinese-style sausages during 21 d of refrigerated storage are shown in Fig. 1. There was no significant difference between the TBARS values of each treatment group at day 0 ($p>0.05$). The TBARS values of all groups increased continuously for up to 21 d of storage ($p<0.05$). When compared with the control, the TBARS values of the CE treated group decreased significantly ($p<0.05$) during storage. As the amount of CE increased, the TBARS values decreased significantly ($p<0.05$). The lipid oxidation inhibition effect was highest in 2% CE at all storage times. These results indicate that the addition of CE to Chinese-style sausage had a positive effect on retarding lipid oxidation. Kong *et al*. (2010) reported that clove ethanol extract decreased the TBARS values of raw pork patties during 4°C storage. Moreover, the addition of clove water extract retarded the increase of peroxide value (PV) and TBA of silver carp fillets compared to the control (Maqsood *et al.*, 2012). Naveena *et al*. (2006) found that treatment with lactic acid + clove oil synergistically reduced the TBARS values and microbial counts without affecting color and odor when compared to control and lactic acid-treated buffalo meat steaks. These findings are in accordance with those of the present study and indicate that CE can be employed

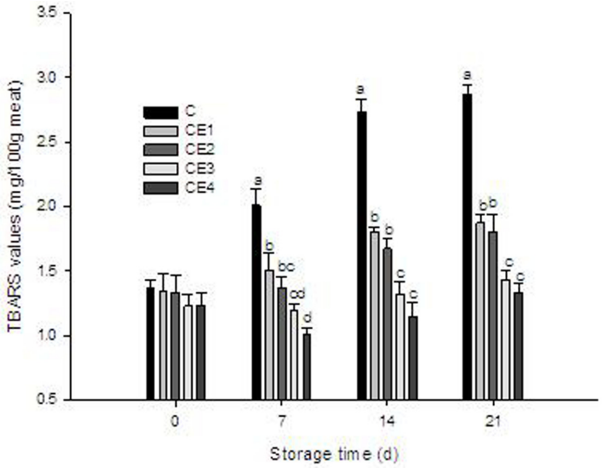

Fig. 1. Changes in TBAR values of Chinese-style sausage added with clove extract (CE) during refrigerated storage. Data are means±standard deviation. n=30. [a-d]Means with different superscript small letters at the same storage time differ significantly (p<0.05). C: Control; CE1: sausage treated with 0.25% clove extract; CE2: sausage treated with 0.5% clove extract; CE3: sausage treated with 1% clove extract; CE4: sausage treated with 2% clove extract.

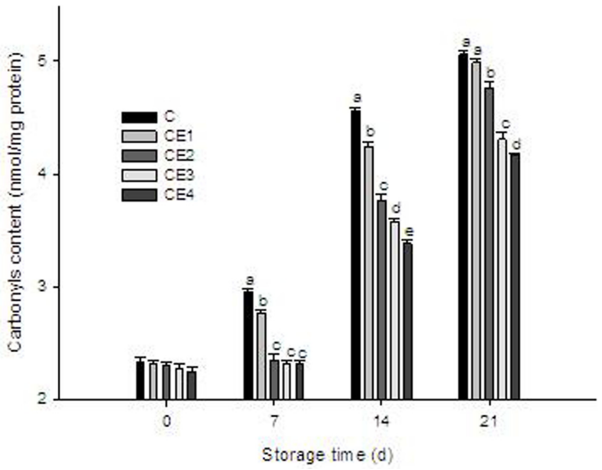

Fig. 2. Changes in carbonyl content of Chinese-style sausage added with clove extract (CE) during refrigerated storage. Data are means±standard deviation. n=30. [a-e]Means with different superscript letters at the same storage time differ significantly (p<0.05). C: Control; CE1: sausage treated with 0.25% clove extract; CE2: sausage treated with 0.5% clove extract; CE3: sausage treated with 1% clove extract; CE4: sausage treated with 2% clove extract.

as a natural antioxidant to improve the storage stability of meat products. The antioxidant activity of CE is due to its phenolic compounds and its hydrogen donating ability (Baghshahi *et al.*, 2014; Wojdyło *et al.*, 2007). The TBARS values of all treatment groups were relatively higher than those reported in several previous studies. This could be because of the high fat content and large addition of sugars (7.5%, basis on the raw meat) to the sausage. During the production and storage of sausages, sugars could be decomposed to aldehydes, which may react with TBA reagent and result in increased TBARS values (Zhang *et al.*, 2013).

Protein oxidation

It is well known that protein oxidation can result in deterioration of meat quality, including water-holding capacity, texture, flavor, color and biological functionality (Lund *et al.*, 2011). Therefore, the amount of protein carbonyls and thiol groups were measured to evaluate the degree of protein oxidation.

Change in protein carbonyl content

The carbonyl compounds content of all treatment groups increased significantly during 4°C storage (p<0.05) (Fig. 2). Control samples showed significantly higher protein carbonyl content than treatment groups on all storage days. The addition of CE had a significant inhibitory effect on the formation of protein carbonyls in Chinese-style sausages. The addition of 0.5%, 1% and 2% CE showed significant inhibitory effects on the production of protein carbonyls during refrigerated storage when compared to the control group. However, the addition of 0.25% CE showed only a limited inhibitory effect and was not effective at day 21. These results indicated that CE has the ability to protect sausages from protein oxidation. Similarly, Chen *et al*. (2016) reported that the addition of CE significantly inhibited carbonyl formation in porcine longissimus myofibrillar proteins. The effectiveness of CE on protein oxidation may be attributed to the phenolic compounds offered by the plant. Our previous study measured the total phenolic content of 13 spices commonly used in China. The results showed that clove extract contains the highest total phenolic content when compared with other spices (Kong *et al*., 2010). Eugenol was found to be the main active phenolic compounds in clove extract. Nam and Kim (2013) found that eugenol has the ability to inhibit lipid peroxidation and scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals. In addition, eugenol could protect DNA from oxidation induced by hydroxyl radicals. Antioxidation of phenolic compounds from plant extracts has been investigated in some reports. Phenolic compounds primarily inhibit protein oxidation by chelat-

ing metal ions, scavenging free radicals, blocking lipid oxidation and combining with proteins to form compounds (Maqsood *et al*., 2012). In addition, phenolic compounds can be bound to proteins by covalent bonds. This type of binding may stabilize and accumulate protein-bound phenoxyl radicals and consequently prevent protein carbonyl formation (Jongberg *et al.*, 2013). However, the exact mechanisms associated with the inhibitory effects of phenolic compounds on protein oxidation remain unclear.

Change of protein thiol groups

The protein thiol groups content of all samples decreased significantly during refrigerated storage (p<0.05) (Fig. 3). When compared with the control, sausages amended with CE showed significantly higher values at day 0 (p<0.05). However, all sausage samples showed no difference at days 7 and 14 (p>0.05). As the storage time increased, the CE treatment groups showed lower values (p<0.05) than the control group at day 21. Protein oxidation usually results in decreased levels of thiol groups, which can be attributed to the formation of disulphide bonds by oxidation (Lara *et al*., 2011). As a result, changes in thiol content is another important marker reflecting the level of protein oxidation. At day 0, the levels of thiol groups in control samples were lower than in samples treated with CE, indicating that the addition of CE prevented the decrease of thiol groups. These results suggest that CE had an effective inhibitory effect on protein oxidation during the heating of sausages. These results are similar to those reported by Zhang *et al*. (2013), who found that heating promoted the oxidation of protein in Chinese-style sausages, and that the addition of sage decreased the loss of thiol groups. The inhibitory effect of natural antioxidants on the loss of thiol groups has been evaluated in different meat systems. Nieto *et al*. (2013) reported that the addition of essential oils of rosemary and oregano to raw pork patties significantly retarded the loss of thiol groups. Additionally, pomegranate peel extracts were shown to inhibit the loss of thiol groups in beef meatballs (Turgut *et al.*, 2016).

The inhibition of CE on the reduction of protein thiol groups may be related to the scavenging free radical ability of phenolic compounds in CE (Jia *et al.*, 2012). The addition of CE to sausages obviously led to a rapid decline in the content of thiol groups compared to the control during storage (Fig. 2). These results suggest that the addition of CE promoted the reduction of thiol groups in sausages, which is in accordance with variation tendency of protein carbonyl content. These findings are also similar to those reported by Jongberg *et al*. (2011), who suggested that treatment with white grape extract promoted a decrease of thiol groups in beef patties, and that this phenomenon could be due to the ortho-phenolic structures existing in the extract, which are highly likely to adduct with nucleophilic thiols and form thiol-quinone adducts. Eugenol, the main components of CE, was an ortho-phenolic compound that could interact with thiol groups to form adducts; thus, decreasing the concentration of thiol groups. The addition of CE to sausages resulted in a faster loss rate of thiol groups, which may have been due to the balance between the antioxidant and pro-oxidant effects of phenolic compounds in CE. However, the results of changes in the protein carbonyls in sausages showed that CE does have an inhibitory effect on protein oxidation.

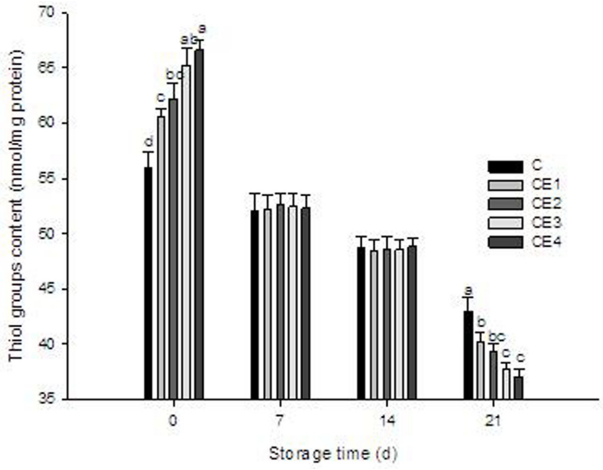

Fig. 3. Changes in thiol group content of Chinese-style sausage added with clove extract (CE) during refrigerated storage. Data are means±standard deviation. n=30. [a-d]Means with different superscript letters at the same storage time differ significantly (p<0.05). C: Control; CE1: sausage treated with 0.25% clove extract; CE2: sausage treated with 0.5% clove extract; CE3: sausage treated with 1% clove extract; CE4: sausage treated with 2% clove extract.

Instrumental color measurement

The color (L*, a* and b*) of sausages treated with and without CE during cold storage at 4°C is shown in Table 1. The L* values of all samples decreased significantly (p <0.05) during refrigerated storage. When compared with the control group, the addition of CE to sausages led to significantly decreased L* values (p<0.05) at day 0. The L* value of the samples decreased with increasing amounts of CE, which could have been caused by the existing purple pigment materials in CE (Nuñez de Gonzalez *et*

al., 2009). The polyphenolic compounds present in CE could be easily oxidized to relevant quinines, which may aggregate to form darker compounds in sausages.

The control group showed higher a* values (p>0.05) than samples amended with CE at day 1 (Table 1). As the storage time increased, the a* value of the control group decreased significantly. When compared to the control, samples treated with CE had a higher a* value (p<0.05) and remained steady during refrigerated storage. It is noted that CE could had a preventative effect on the discoloration of sausage samples during 4°C storage. Kong *et al*. (2010) reported that treatment of pork patties with clove extract significantly reduced the loss in a* value compared with the control group (p<0.05) during a 7-d storage period.

Several researchers have reported that lipid oxidation of meat products resulted in deterioration of redness (Hayes *et al.*, 2011; Jia *et al.*, 2012; Jung *et al.*, 2012). When compared with the control, the CE treatment groups had higher b* values (p<0.05). The b* values of the CE treatment groups decreased slightly during storage. Falowo *et al.* (2014) reported that the prevention effect of natural plants on discoloration of meat products was due to the antioxidant actions of phenolic compounds. The inhibitory effects of phenolic compounds on lipid and protein oxidation of meat products have been widely reported (Fasolato *et al.*, 2016; Maqsood *et al.*, 2015); therefore, these preventive effects of CE on the discoloration of pork sausages could be due to the phenolic compounds they contain.

Effect of CE on textural properties of Chinese-style sausages

The textural properties of Chinese-style sausages without and with CE during chilled storage are shown in Table 2. At the beginning of refrigerated storage (day 0), the textural properties of all sample groups showed no significant differences (p>0.05). As the storage time increased, the hardness values of the control samples increased significantly (p<0.05). However, the hardness values of the CE treatment groups showed no significant difference during the storage time (p>0.05). The hardness values of all treatment groups decreased as the amount of CE added increased. The textural properties of the meat products had a significant effect on consumer acceptability. Taken together, these results suggested that CE had an inhibitory effect on the increasing hardness of sausages during chilled storage. These findings are similar to those of Zhang *et al*. (2013), who indicated that the addition of sage retarded changes in the hardness of Chinese-style sausages during 4°C storage. The hardness of sausages increased as the refrigerated storage time increased, which was probably because of destruction of the emulsion stability in response to separation of fat and water from the protein substrate (Estévez *et al.*, 2005). Protein oxidation may have a negative effect on the hardness of meat products (Lorido *et al.*, 2016). Therefore, natural antioxidants may retard changes in the hardness of meat products by reducing emulsion destabilization and decreasing protein cross-linking through their inhibitory effects against protein oxidation (Zhang *et al*., 2013). In addition, antioxi-

Table 1. Effect of clove extract (CE) on color characteristics of Chinese-style sausages during storage

Items	Storage time	Treatment				
		C	CE1	CE2	CE3	CE4
L*	Day 0	42.44±0.50^{aA}	40.40±0.57^{aB}	38.44±0.50^{aC}	37.22±0.30^{aCD}	36.64±0.40^{aD}
	Day 7	41.23±0.30^{bA}	38.67±0.47^{bB}	36.70±0.58^{bC}	34.88±0.33^{bD}	34.62±0.30^{bD}
	Day 14	38.79±0.30^{cA}	36.95±0.15^{cB}	35.38±0.37^{cC}	33.39±0.42^{cD}	32.21±0.58^{cE}
	Day 21	36.33±0.56^{dA}	34.97±0.27^{dB}	33.58±0.48^{dC}	31.28±0.32^{dD}	31.09±0.04^{dD}
a*	Day 0	8.12 ±0.04^{aA}	8.05±0.04^{aAB}	7.99±0.04^{abBC}	7.96±0.02^{bcBC}	7.91±0.04^{dC}
	Day 7	7.67±0.03^{bC}	7.85±0.04^{bB}	7.95±0.04^{bA}	8.04±0.03^{aA}	8.02±0.03^{cA}
	Day 14	7.49±0.04^{cB}	8.02±0.05^{aA}	8.05±0.04^{aA}	8.02±0.03^{aA}	8.10±0.02^{bA}
	Day 21	7.37±0.03^{dC}	8.01±0.02^{aB}	8.04±0.03^{abB}	7.99±0.02^{abB}	8.21±0.02^{aA}
b*	Day 0	12.05±0.04^{a}	14.48±0.28^{a}	14.82±0.26^{a}	15.86±0.17^{a}	16.78±0.27^{a}
	Day 7	11.47±0.21^{b}	13.68±0.28^{ab}	13.78±0.29^{b}	15.17±0.23^{ab}	15.52±0.27^{b}
	Day 14	11.12±0.15^{b}	13.12±0.14^{b}	13.22±0.29^{b}	14.46±0.41^{bc}	14.27±0.28^{c}
	Day 21	10.12±0.10^{c}	11.44±0.50^{c}	12.24±0.36^{c}	13.55±0.51^{c}	13.34±0.25^{d}

C: Control; CE1: sausage treated with 0.25% clove extract; CE2: sausage treated with 0. 5% clove extract; CE3: sausage treated with 1% clove extract; CE4: sausage treated with 2% clove extract.

Values are mean±SD (n=30).Values with a different letter (a-d) within a column of the same batch are significantly different (p<0.05). Values with a different letter (A-D) within a row of the same storage day are significantly different (p<0.05).

Table 2. Effect of clove extract (CE) on textural properties of Chinese-style sausages during refrigerated storage

Items	Storage time	Treatment				
		C	CE1	CE2	CE3	CE4
Hardness (N)	Day 0	86.40±2.05^{dA}	86.80±2.10^{aA}	85.73±1.46^{aA}	85.57±0.95^{aA}	84.93±1.36^{aA}
	Day 7	91.43±1.20^{cA}	85.93±1.43^{aB}	85.67±0.97^{aB}	84.47±1.58^{aB}	84.13±1.43^{aB}
	Day 14	94.60±0.90^{bA}	86.53±0.90^{aB}	85.97±0.96^{aBC}	85.37±1.06^{aBC}	84.47±0.95^{aC}
	Day 21	98.37±0.95^{aA}	88.47±0.95^{aB}	87.37±0.93^{aBC}	86.83±1.52^{aBC}	86.30±1.42^{aC}
Springiness (cm)	Day 0	0.71±0.20	0.72±0.15	0.73±0.12	0.71±0.23	0.72±0.15
	Day 7	0.73±0.13	0.74±0.16	0.74±0.15	0.74±0.21	0.73±0.14
	Day 14	0.72±0.14	0.74±0.11	0.74±0.16	0.72±0.18	0.73±0.12
	Day 21	0.72±0.11	0.73±0.21	0.73±0.11	0.73±0.17	0.71±0.17
Cohesiveness (ratio)	Day 0	0.45±0.13	0.44±0.09	0.47±0.11	0.45±0.11	0.45±0.12
	Day 7	0.46±0.15	0.45±0.18	0.46±0.08	0.45±0.10	0.45±0.11
	Day 14	0.47±0.09	0.46±0.19	0.47±0.13	0.46±0.17	0.46±0.09
	Day 21	0.46±0.10	0.48±0.16	0.48±0.12	0.46±0.14	0.47±0.14
Gumminess (N)	Day 0	29.26±1.03^{dB}	32.13±1.78^{bA}	31.20±1.18^{bAB}	32.52±1.33^{aA}	31.40±1.11^{aAB}
	Day 7	35.52±1.04^{cA}	36.86±1.28^{aA}	32.29±0.85^{bB}	32.30±1.15^{aB}	32.52±0.91^{aB}
	Day 14	37.48±1.03^{bA}	37.29±0.95^{aA}	32.62±0.90^{bB}	32.58±0.92^{aB}	32.84±1.55^{aB}
	Day 21	39.62±0.74^{aA}	38.16±1.74^{aA}	34.65±1.23^{aB}	33.64±1.04^{aB}	33.10±1.68^{aB}
Chewiness (N×cm)	Day 0	22.38±1.19^{cA}	21.34±1.13^{bA}	20.97±0.42^{bA}	21.44±0.90^{bA}	21.71±0.98^{aA}
	Day 7	23.77±0.77^{bcA}	23.33±1.08^{aA}	23.57±1.15^{aA}	22.60±0.94^{abA}	22.22±1.55^{aA}
	Day 14	25.58±1.33^{bA}	24.52±0.87^{aAB}	22.91±0.97^{aB}	23.41±1.51^{abB}	22.59±0.97^{aB}
	Day 21	30.51±1.27^{aA}	24.67±0.78^{aB}	23.60±0.86^{aB}	23.66±0.98^{aB}	23.71±0.98^{aB}

C: Control; CE1: sausage treated with 0.25% clove extract; CE2: sausage treated with 0.5% clove extract; CE3: sausage treated with 1% clove extract; CE4: sausage treated with 2% clove extract.
Values are mean±SD (n=30).Values with a different letter (a-d) within a column of the same batch are significantly different (p<0.05). Values with a different letter (A-C) within a row of the same storage day are significantly different (p<0.05).

dants may maintain the integrity of muscle membranes by inhibiting lipid oxidation and thereby prevent moisture loss from muscle fibers and changes in the texture of sausages (Estévez *et al.*, 2006). During refrigerated storage, the effects of CE addition on the gumminess and chewiness values of all samples were in accordance with the hardness; however, the springiness and cohesiveness of all samples showed no significant difference. These results indicated that CE has the ability to retard degradation of sausage texture by exerting antioxidant effects.

Effect of CE on sensory evaluation of Chinese-style sausages

The effects of CE on the sensory evaluation score of Chinese-style sausages at days 0 and 21 of storage are shown in Table 3. The addition of CE decreased the color values of sausages (p>0.05) at days 0 and 21, which is in accordance with the changes in the L* values. These chan-

Table 3. Effect of clove extract (CE) on sensory evaluation score of Chinese-style sausages during refrigerated storage

Storage time	Treatment	Color	Flavor	Texture	Overall-acceptability
Day 0	C	7.21±0.51	7.03±0.66	7.08±0.34	7.01±0.47
	CE1	7.12±0.56	7.15±0.73	7.09±0.42	7.13±0.36
	CE2	6.93±0.71	7.24±0.65	6.99±0.47	7.18±0.51
	CE3	6.84±0.70	7.22±0.54	7.04±0.50	7.22±0.49
	CE4	6.72±0.62	7.21±0.84	7.05±0.36	7.16±0.58
Day 21	C	7.31±0.41	7.18±0.64	7.11±0.27	6.92±0.39
	CE1	7.22±0.38	7.25±0.87	7.12±0.34	7.25±0.46
	CE2	7.04±0.39	7.44±0.79	7.02±0.38	7.28±0.52
	CE3	7.03±0.28	7.92±0.88	7.05±0.31	7.13±0.59
	CE4	6.97±0.40	7.81±0.84	6.99±0.43	7.17±0.61

C: Control; CE1: sausage treated with 0.25% clove extract; CE2: sausage treated with 0.5% clove extract; CE3: sausage treated with 1% clove extract; CE4: sausage treated with 2% clove extract. Values are mean±SD (n=30).

ges were probably a result of the darker color of the clove extract itself. However, the addition of CE had no negative impact on the flavor attributes of the sausages. In addition, the flavor evaluation scores of CE treatment groups were slightly higher than those of the control group (p> 0.05), indicating that CE treatment improved the flavor attribute of the sausages. Moreover, CE treatment appeared to have no effect on the texture scores relative to the control, although the instrumental detection results showed that the hardness decreased with increasing amounts of CE. Similarly, the overall-acceptability of sausages amended with CE was slightly higher than that of the control (p>0.05). Taken together, these findings indicated that CE could be used as natural antioxidants in sausages to prevent quality deterioration without any negative effects on consumer perception. Based on the results of TBARS and protein oxidation, control group showed higher lipid and protein oxidation than the CE treated groups. However, the sensory evaluation result showed no significant differences between control and CE treated groups on 0 and 21 days of storage. This may be associated with the formulation of Chinese-style sausages which contained 1.5% white wine. Feng *et al*. (2016) also reported that addition of red wine (5%) appears to provide some potential advantages for processed meats, particularly with respect to sensory profiles.

Conclusions

Addition of CE to sausages effectively inhibited lipid and protein oxidation, even though CE promoted a decrease in the thiol groups content of sausages during refrigerated storage. Moreover, the addition of CE has preventive effects on the discoloration of sausages. CE treatment also had the ability to prevent the deterioration of sausages texture properties. Further, sensory panel groups found that Chinese-style sausages added with CE had better flavor and overall-acceptability scores. However, there was no significant difference in any of the sensory attributes of sausages treated with CE relative to the control. Taken together, these results indicate that CE could be used as a natural antioxidant additive for meat products to effectively inhibit oxidation and quality deterioration.

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