Food Bioprocess Technol (2015) 8:1045–1053
DOI 10.1007/s11947-014-1466-8

ORIGINAL PAPER

Effect of *Coptis chinensis* Franch Addition on the Quality Characteristics of Sausages During Cold Storage

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Received: 27 May 2014 / Accepted: 22 December 2014 / Published online: 18 January 2015

Abstract The effects of *Coptis chinensis* Franch (CCF) powder extract and potassium sorbate addition on the quality characteristics of sausages during cold storage were investigated. Sausages were prepared with potassium sorbate (0.2 %) and CCF extracts (0.2 and 0.4 %) and stored for 4 weeks. Compared to the control, lipid oxidation values dose-dependently decreased in sausages containing CCF before and after 4 weeks of storage. The addition of CCF increased the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity of sausages. Hardness, cohesiveness, and springiness of sausage samples did not show any consistent trends. The chewiness decreased in the control and in sausages containing CCF at 4 weeks of storage, whereas the adhesiveness increased after 4 weeks in sausages containing potassium sorbate and 0.2 % CCF. The lightness, redness, and whiteness dose-dependently decreased in sausages containing potassium sorbate and CCF, whereas yellowness dose-dependently increased before and after 4 weeks of storage. During storage, compared to the control, the total plate count and lactic acid bacteria diminished in sausages with potassium sorbate and CCF. In sensory evaluation, the overall acceptability of sausages was significantly influenced by CCF addition and was lower than that of the control sausages and sausages containing potassium sorbate alone.

Keywords *Coptis chinensis* Franch · Sausage · TBARS · Textural property · Sensory evaluation

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Introduction

Processed meat products contain many additives such as sodium nitrite, sodium chloride, phosphate, sugar, monosodium L-glutamate, or starch. The use of additives contributes to the development of the red color and meat flavor and the inhibition of oxidative rancidity, spoilage, and development of pathogenic bacteria. However, consumers are concerned about the risks to human health from excessive consumption of additives in processed meats. For instance, sodium nitrite is responsible for the unique taste and red color of cured meat products. However, it can be converted to the nitrosating agent NO^+, which can react with biogenic amines to form carcinogenic N-nitrosamines (Honikel 2008). Thus, the concentration of added nitrite has been reduced, and questions on safety have been raised. Sodium chloride may also influence the color and microbial growth in cured meat. However, excessive consumption of sodium chloride in the diet may also have harmful health effects, including increased risk for high blood pressure and stomach cancer. Therefore, alternative ingredients, substitutes, or replacers have been extensively studied to address the concerns of the public. Many natural ingredients exhibit a variety of biological activities, including potent antioxidant, anticancer, and anti-inflammatory activity, blood pressure-lowering effects, inhibition of lipid oxidation, or antimicrobial properties (Hayes et al. 2011).

Coptis chinensis Franch (CCF) is widely used as a herbal medicine in Asian countries (e.g., Korea, China, or Japan). The main components of CCF are berberastine, berberine, columbamine, coptisine, epiberberine, jatrorrhizine, and palmatine (Yuan et al. 2006). Several studies found that CCF possesses antimicrobial activity, which is possibly enhanced by the functional group methylenedioxy or methoxyl at C2 on the phenyl ring (Yan et al. 2008). Jang et al. (2009) reported that four isoquinoline alkaloids (i.e., berberrubine, coptisine, berberine, and palmatine) isolated from CCF exhibited the strongest ·OH scavenging activity, which was closely related to the activity of ferrous ions chelating these molecules. Metal-chelating functional groups such as the hydroxy group at C-9 and the methylenedioxy group at C-9 and C-10 were thought to contribute to the ·OH scavenging activity of the isoquinoline alkaloids (Jang et al. 2009). Hot water extraction of plant substances, which is the most widely used method in oriental medicine, resists heat and is safe compared to other methods of solvent extraction of bioactive compounds in CCF. Natural ingredients possessing antioxidant and antimicrobial properties have the advantage of being readily accepted by consumers as they are considered natural. Although the physiological and pharmacological functions of CCF have been extensively studied, few studies have focused on its effect on the quality of sausages during storage. Therefore, the purpose of this study was to determine the effects of CCF addition on the physicochemical properties of sausages during storage.

Materials and Methods

Materials

Refined salt was obtained from Woo-Il S&F Co. (Ulsan, Korea). Also, sodium nitrite was purchased from Duksan Co. (Gyeongki, Korea). Phosphate, sausage spice, and potassium sorbate were from Taewon Food Co. (Gyeongki, Korea). In addition, sugar was obtained from CheilJedang Co. (Incheon, Korea). Monosodium L-glutamate was purchased from Shinwon Chemical Co. (Seoul, Korea). All other reagents were of the highest grade commercially available.

Preparation of *C. chinensis* Franch Extracts

Air-dried CCF was purchased from a herbal market (Kumho market, Seoul, Korea). The samples were washed with running tap water before being chopped into pieces. Then, they were oven-dried at 45 °C for 2 days and ground to a powder. The powder was stored at −20 °C until use. Aqueous extracts of freeze-dried CCF were obtained as follows. CCF powder was suspended and extracted with 1000 mL of water at 100 °C for 3 h. The extracts were filtered through a Whatman no. 2 filter paper and evaporated to dryness. The aqueous extracts were concentrated in a vacuum evaporator at 40 °C. The final extracts were placed in a glass bottle and stored at −20 °C until use. The lyophilized extracts were redissolved in water to a concentration of 1000 μg/mL.

Experimental Design and Sausage Processing

Lean pork (72.40 %) and backfat (11.20 %) were purchased from a local meat-processing plant. Excess fat was trimmed from the meat, and the lean muscle was diced into pieces (approximately 8×4×2 cm) and ground through a 7-mm-diameter orifice using a mincer. Ground meat was cured for 30 min with phosphate (0.24 %) and NPS (1.40 %, NaCl:$NaNO_2$) using a meat mixer and then stored for 24 h at 4 °C. Cured meat was placed in a bowl cutter along with ice (13.80 %), sugar (0.50 %), monosodium L-glutamate (0.06 %), spice (0.40 %), and different ingredients (C: potassium sorbate 0 % and CCF 0 %; T1: potassium sorbate 0.2 % and CCF 0 %; T2: potassium sorbate 0 % and CCF 0.2 %; T3: potassium sorbate 0 % and CCF 0.4 %, respectively). Chopping was continued until the batter temperature reached 10 °C. The emulsified meat batters were stuffed into polyvinylidene chloride (PVDC) casings (50-mm diameter) and placed in a cooking chamber (programmed at 65 °C for 30 min, followed by 75 °C for 30 min and then 80 °C for 20 min). Core sausage temperature was measured with a flexible internal thermometer (Temp 300, Thermo Scientific, MA, USA). After cooling in iced water for 20 min, the sausages were stored at 5 °C until use.

Total Phenol Content

Total phenol content in sample extracts was determined spectrophotometrically according to the Folin–Ciocalteu method (Singleton and Rossi 1965). Because catechin is a polyphenol compound, the total phenol content of the extract from CCF was expressed as microgram catechin equivalents/milligram extract (μg CE/mg). Typically, 150 mL of 1 mg/mL sample, 2.4 L of deionized water, and 150 mL of 0.25 N Folin–Ciocalteu reagent were combined in a plastic vial and then mixed well using a vortex mixer. The mixture was allowed to react for 3 min, and then 300 mL of 1 N Na_2CO_3 solution was added and mixed well. The solution was incubated at room temperature (25 °C) in a dark place for 2 h. The absorbance was measured at 725 nm using a spectrophotometer (Hewlett Packard 8452A, Diode Array, Santa Clara, CA, USA). Additional dilution was conducted if the absorbance value measured was over the linear range of the standard curve ($Y=0.0016X+0.0424$, $R^2=0.9999$).

Total Flavonoid Content

Total flavonoid content was determined using the method of Chun et al. (2003) with minor modifications. Exactly 0.25 mL of sample (1 mg/mL) was added to a tube containing 1 mL of double-distilled water. Subsequently, 0.075 mL of 5 % $NaNO_2$, 0.075 mL of 10 % $AlCl_3$, and 0.5 mL of 1 M NaOH were added at 0, 5, and 6 min, sequentially. Finally, the volume of the reacting solution was made up to 2.5 mL with double-distilled water. The absorbance of the solution was recorded at a wavelength of 410 nm and was detected using Ultrospec 2100 pro spectrophotometers (Amersham Biosciences, Freiburg, Germany). Quercetin, a ubiquitous flavonoid present in many plant extracts, was used as the standard to quantify the total flavonoid content of water extract. Results were expressed as microgram quercetin equivalents/milligram extract (μg QE/mg).

pH

The pH values of sausage homogenate prepared with 3 g of sausage samples and 27 mL of distilled water were determined using a digital pH meter (SevenEasy pH, Mettler-Toledo AG, Schwerzenbach, Switzerland) equipped with an electrode calibrated with phosphate buffer at pH 4.0 and pH 7.0 at room temperature.

Thiobarbituric Acid-Reactive Substances

Thiobarbituric acid-reactive substance (TBARS) values were determined using the modified method of Buege and Aust (1978). Sausages (5 g) were weighed in a 50-mL test tube and homogenized with 15 mL of deionized distilled water using a Polytron homogenizer at 1000×*g* for 10 s. The sausage homogenate (1 mL) was transferred to a disposable test tube (3×100 mm), and butylated hydroxyanisole (50 μL, 10 %) and thiobarbituric acid/trichloroacetic acid (TBA/TCA) (2 mL) were added. The mixture was vortexed and then incubated in boiling water for 15 min to develop the color. The sample was cooled in cold water for 5 min, vortexed again, and centrifuged for 15 min at 2000×*g*. The absorbance of the resulting supernatant solution was determined at 531 nm against a blank containing 1 mL of deionized distilled water and 2 mL of TBA/TCA solution. TBARS were calculated from a standard curve of malondialdehyde (MDA), freshly prepared by acidification of TEP (1,1,3,3-tetraethoxypropane) in the range from 0.02 to 0.3 μg/mL (y= 0.8729x+0.0382, r=0.9961) and expressed as milligrams of malondialdehyde per kilogram of sample.

Peroxide Value

The peroxide value (POV) was determined as described in the AOAC (1990). Briefly, 5 g of sample was weighed in a 50-mL glass tube, and to this, 30 mL of acetic acid/chloroform mixture (3:2 *v*/*v*) was added. After incubation at 60 °C for 5 min in a water bath, the mixture was filtered using a no. 1 Whatman filter paper. Subsequently, 0.5 mL of potassium iodide solution was added to the filtrate, which was further analyzed using an automatic titrator equipped with a pH meter and a stirrer. The titration was allowed to run against standard solution of 0.1 N sodium thiosulfate. POV was expressed as milliequivalents (meq) of active oxygen per kilogram of sausages.

DPPH Radical Scavenging Activity

The 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity of sausages was measured using a spectrophotometer (Akowuah et al. 2005). Diluted extract (0.15 mL) was added to 0.9 mL of the methanolic DPPH solution (0.1 mM). After 10 min, the absorbance of the solution was measured at 517 nm. Pure methanol was used as the control. The percentage of DPPH scavenging activity (% SA) was calculated from the equation (1−[absorbance of extract/absorbance of control])×100.

Volatile Basic Nitrogen

One gram of sausage sample and 2–3 drops of phenolphthalein indicator were mixed with 3.5 mL of 20 % NaOH solution in a distillation flask. The apparatus was sealed, and steam distillate was collected in a flask containing 20 mL of 4 % H_3BO_3 and 2–3 drops of methyl red and methylene blue (2:1 mixture). The distillate was continuously collected until the flask was filled to 250 mL. The solution was titrated using 0.01 M HCl to calculate the total volatile basic nitrogen (VBN) in the sample in terms of milligrams of VBN per 100 g sausage, as described by Pearson (1976).

Textural Properties

Textural properties analysis of sausages (2×2×1 cm) was performed using an EZ Test-500 N texture analyzer (TA-XTZ-5, Shimadzu Co., Japan) attached to a cylindrical plunger (5 mm in diameter, 1 mm/s in depression speed) and a 500-N load cell. Texture profile parameters measured included hardness, cohesiveness, springiness, chewiness, and adhesiveness. Textural parameters were measured at room temperature with the following testing conditions: crosshead speed 5.0 mm/s, 50 % compression of the original sample height, surface sensing force 99 g, and threshold 30.0 g. The time interval between the first and second compressions was 10 s. Hardness, cohesiveness, springiness, chewiness, and resilience were calculated from the force–time curves generated for the sample (Intarasirisawat et al. 2014).

Color

The following color coordinates were determined in samples: lightness (L^*), redness (a^*), and yellowness (b^*) of sausages were measured using a Minolta colorimeter (Minolta Chroma Meter CR-300, Minolta Co., Ltd., Ramsey, NJ, USA); before use, the colorimeter was calibrated using a white standard plate (Y=92.8, x=0.3134, and y=0.3193). Sausages were cut in 3-cm-long slices and the surface color of the slices was measured three times for each sample. The whiteness (W) was calculated using the following formula: L^*-3b^* (Park 2005). Color was determined three times for each sample, and mean values were used.

Microorganisms

Microorganisms were analyzed for total plate count (TPC) and number of lactic acid bacteria according to standard procedures (Speck 1992). The TPC and lactic acid bacteria were incubated for 72 h at 37 °C. The relevant colonies on the plates were counted, and the results were expressed as colony-forming units (CFU) per gram of meat sample. The TPCs and lactic acid bacteria were then normalized by logarithm (base 10) transformations.

Fatty Acid Composition

Lipids were extracted with chloroform and methanol as described previously (Folch et al. 1957) and concentrated using an evaporator (Zymark Turbovap 500; Hopkinton, MA, USA). The fatty acid composition was determined as described previously (Hur et al. 2004). For lipid hydrolysis, an aliquot of lipid extract (30 mg) and 3 mL of 4 % H_2SO_4 in methanol were combined in a screw-capped test tube. The test tube was then placed in boiling water (100 °C) for 20 min and subsequently cooled at room temperature. The resultant free fatty acids were methylated with 1 mL of 14 % boron trifluoride in methanol at room temperature for 30 min. Next, 1 mL of water and 5 mL of hexane were added, and the samples were vortexed and centrifuged at 500×g for 10 min. The upper organic solvent layer was used to determine the fatty acid composition. Fatty acid methyl esters were analyzed on a gas chromatograph equipped with an on-column injector port and flame-ionization detector. A Silar capillary column (30×0.32×0.25 mm; Shimadzu, Tokyo, Japan) was used to separate the fatty acid methyl esters. The initial gas chromatography oven temperature was 140 °C, which was increased at 2 °C/min to a final temperature of 230 °C. The temperatures of the injector port and detector were set at 240 and 250 °C, respectively. Fatty acid methyl esters (1 mL) were injected into the split injection port (100:1 split ratio). The flow rate for the He carrier gas was 50 mL/min. Each fatty acid was identified by comparing its retention time with a standard.

Sensory Evaluation

Sensory evaluation was performed by a panel of 25 semi-trained tasters. Panel development followed the prescreening, screening, training, and performance evaluation phases as described previously (Cross et al. 1978). The panel evaluated each treatment within each replicate in triplicate, and the evaluation was performed using samples at room temperature. Triplicate responses were taken to monitor the inherent texture variability associated with the same sample. One slice, 0.5-cm thick and 5 cm in diameter, was cut into six pie-shaped wedges and presented to each panelist. The panelists chose three of the most characteristic wedges in order to avoid a sample containing large pieces of connective tissue. The color, aroma, flavor, springiness, juiciness, and overall acceptability were evaluated according to a 9-point scale (9=very good and 1=very bad).

Statistical Analysis

Statistical analyses were conducted on three batches of sausages. Data for each batch of sausages on physicochemical characteristics, texture properties, meat color, microorganisms, fatty acid composition, and sensory evaluation were analyzed using ANOVA with the SAS software (SAS Inst. Inc., Cary, NC, USA). Significant differences ($p<0.05$) between mean values of samples were determined for physicochemical characteristics, texture properties, meat color, microorganisms, fatty acid composition, and sensory evaluation.

Results and Discussion

The total phenolic and flavonoid content of CCF were 24.10 and 24.92 μg/mg extract, respectively (data are not shown). However, sausages with the extract of CCF did not differ significantly in terms of total polyphenol and flavonoid content (data are not shown).

The results of physicochemical characteristics of sausages after storage are presented in Table 1. The pH significantly ($p<0.05$) decreased in sausages containing CCF or potassium sorbate compared to the control before and after storage.

In particular, CCF showed antioxidative activity and reduced growth of microorganisms. TBARS and POV as lipid oxidation values dose-dependently decreased in sausages containing CCF compared to the control before and after storage for 4 weeks. Moreover, potassium sorbate addition in sausages also decreased the lipid oxidation values. For the same concentration of CCF and potassium sorbate, the oxidation values

Table 1 Effect of *Coptis chinensis* Franch addition on the physicochemical characteristics of sausages during storage

Item	Treatment	Storage (weeks)	
		0	4
pH	C	5.92±0.06Ab	6.16±0.01Aa
	T1	5.87±0.01Bb	6.05±0.01Ba
	T2	5.86±0.02Bb	6.01±0.03Ba
	T3	5.83±0.01Bb	5.93±0.01Ba
TBARS (mg/100 g)	C	0.37±0.02Aa	0.33±0.02Ab
	T1	0.21±0.01B	0.20±0.01B
	T2	0.20±0.02Ba	0.16±0.01Cb
	T3	0.15±0.01Ca	0.13±0.01Db
POV (meq/kg)	C	0.73±0.04Aa	0.67±0.03Ab
	T1	0.43±0.03B	0.40±0.03B
	T2	0.40±0.03Ba	0.33±0.02Cb
	T3	0.29±0.01Ca	0.26±0.01Db
DPPH (%)	C	12.60±1.14Da	5.80±0.84Db
	T1	15.20±0.84Ca	12.00±0.71Cb
	T2	35.00±1.58Ba	27.00±1.58Bb
	T3	37.40±1.67Aa	29.80±1.30Ab
VBN (mg %)	C	6.84±0.07b	7.92±0.62Aa
	T1	6.86±0.17	7.08±0.29B
	T2	6.84±0.26b	7.86±0.06Aa
	T3	6.92±0.19b	7.30±0.27Ba

Means with different roman letters (A–D) in the same column significantly differ with $p<0.05$. Means with different roman letters (a, b) in the same row significantly differ with $p<0.05$

obtained were similar. However, after 4 weeks of storage, the values were lower for samples treated with CCF.

The addition of CCF or potassium sorbate increased the DPPH radical scavenging activity of sausages. However, VBN as the protein degradation value of sausage samples did not show any consistent trend before and after storage.

The decrease of TBA and POV as lipid oxidation values was probably due to the presence of CCF, which was reported to have antioxidative ability and to retard fat oxidation. Numerous studies reported that phytochemicals such as phenolic compounds had a major contribution to antioxidant activity. In general, free radical scavenging and antioxidant activity of phenols (e.g., flavonoids, phenolic acids) mainly depends on the number and position of hydrogen-donating hydroxyl groups on the aromatic ring of the phenolic molecules and is also affected by other factors such as glycosylation of aglycones and additional H-donating groups (–NH, –SH) (Cai et al. 2004). Phenolic compounds serve as antioxidants by donating hydrogen atoms to radical species and being oxidized to phenoxyl radicals themselves (Jongberg et al. 2013). Therefore, the prevention of lipid oxidation by breaking radical reaction chains is considered the most important antioxidant effect of CCF in sausage samples because it contains flavonoid or phenolic components, which might be responsible for the apparent antioxidative activity. In summary, the addition of the phenol-rich CCF could inhibit the formation of secondary lipid oxidation products and improve the shelf life of sausages. In contrast, the ability of CCF to inhibit oxidation seems to be more pronounced against lipid oxidation than protein oxidation because VBN as the protein deterioration value was not significantly different among the sausage samples of this study. Although the amino acid residues lysine, arginine, proline, and threonine are prone to oxidation in meat products (Estévez 2011), we assumed that CCF addition in sausages did not affect the protein or amino acid degradation.

The results on texture properties of sausages after storage are presented in Table 2. The hardness, cohesiveness, and springiness of sausage samples did not show any consistent trend. The chewiness decreased in the control and in sausages containing CCF after 4 weeks of storage, whereas the adhesiveness increased after 4 weeks in sausages containing potassium sorbate and 0.2 % CCF. The meat color of sausages after storage is shown in Table 3. The L^*, a^*, and W dose-dependently decreased in sausages containing CCF, whereas b^* dose-dependently increased before and after 4 weeks of

Table 2 Effect of *Coptis chinensis* Franch addition on the texture properties of sausages during storage

Item	Treatment	Storage (weeks)	
		0	4
Hardness (kg)	C	5.75±0.61	6.10±0.22
	T1	6.42±0.66	7.20±1.15
	T2	6.58±0.80	6.70±0.84
	T3	5.58±0.66	6.40±0.82
Cohesiveness (%)	C	6.17±0.68	6.20±0.91
	T1	6.25±0.61a	5.00±0.71b
	T2	6.83±0.41	6.80±0.76
	T3	5.83±0.75	6.70±0.91
Springiness (mm)	C	6.00±0.84	6.60±0.82
	T1	5.58±0.80	5.90±0.89
	T2	5.92±0.92b	7.20±0.45a
	T3	6.17±0.82	6.50±0.87
Chewiness (kg, mm)	C	20.67±2.88a	17.40±0.89b
	T1	18.67±3.01	17.20±0.84
	T2	22.33±1.75a	16.80±0.45b
	T3	21.25±1.60a	17.20±0.84b
Adhesiveness	C	5.58±0.66	6.20±1.10
	T1	5.33±0.61b	7.20±0.84a
	T2	4.67±0.93b	6.90±1.02a
	T3	5.42±0.74	6.40±0.82

Means with different roman letters (a, b) in the same row significantly differ with $p<0.05$

Table 3 Effect of *Coptis chinensis* Franch addition on the meat color of sausages during storage

Item	Treatment	Storage (weeks)	
		0	4
L^*	C	79.10±0.40A	78.80±0.87A
	T1	78.57±0.29Ab	79.00±0.26Aa
	T2	77.07±0.18B	76.63±0.71B
	T3	75.53±0.68C	75.45±0.77C
a^*	C	7.90±0.15Ba	7.62±0.21Ab
	T1	8.11±0.15Aa	7.35±0.21Ab
	T2	3.17±0.09C	3.41±0.33B
	T3	3.18±0.17Ca	2.90±0.17Cb
b^*	C	7.21±0.12Db	7.55±0.11Da
	T1	7.68±0.15C	7.78±0.07C
	T2	31.54±0.21Ba	30.92±0.11Bb
	T3	41.03±0.25Aa	40.13±0.19Ab
W^a	C	57.48±0.56Aa	56.16±0.81Ab
	T1	55.53±0.55B	55.65±0.31A
	T2	−17.56±0.81Cb	−16.11±0.90Ba
	T3	−47.55±0.93Db	−44.95±1.05Ca

Means with different roman letters (A–D) in the same column significantly differ with $p<0.05$. Means with different roman letters (a, b) in the same row significantly differ with $p<0.05$

[a] $W=L^*-3b^*$

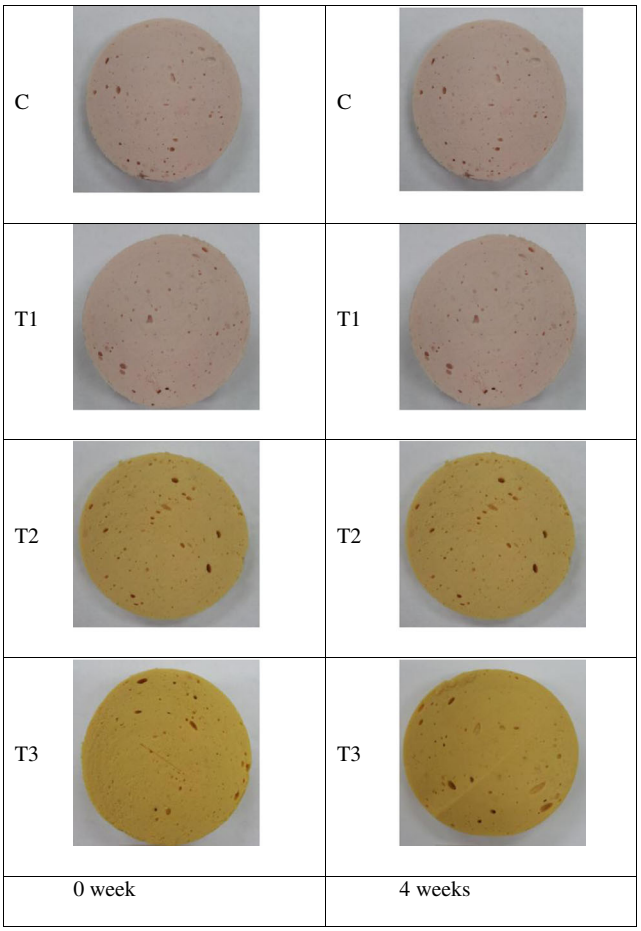

Fig. 1 Sections of the processed sausages analyzed in this study

storage. For potassium sorbate, the L^* parameter was similar to that of the control samples, a^* and b^* increased but only before the storage, and W decreased also before the storage. Several authors have shown that low L^* values in meat are related to a high intensity of the red color (Timón et al. 2014).

CCF addition largely influenced sausage color during storage, mainly because CCF itself is yellow, thus dramatically increasing the yellowness of sausages in sausage samples containing CCF (Fig. 1). Moreover, this color change could negatively affect the sensory evaluation score. Especially, the flavor and overall acceptability of sausage samples decreased during storage. Therefore, decoloration or bleaching of CCF should be a prerequisite to prevent the alteration of color or sensory characteristics.

Moreover, the texture properties of meat products are closely related to protein content, myofibrillar protein degradation, muscle types, or connective tissue content. However, the results of texture properties did not show any consistent trend during storage, indicating that CCF addition did not largely affect the texture properties, probably because the amount of CCF added in the sausages was too low to change the protein properties during storage.

The microorganisms present in sausages after storage are listed in Table 4. The total plate count and lactic acid bacteria of all sausage samples significantly increased with storage periods. During storage, the total plate count and lactic acid bacteria diminished in sausages containing potassium sorbate and CCF compared to the control. Especially, the total plate count and lactic acid bacteria were significantly lower in sausages containing potassium sorbate after 4 weeks of storage.

Table 4 Effect of *Coptis chinensis* Franch addition on the microorganisms present in the sausages during storage

Item	Treatment	Storage (weeks)	
		0	4
TPC ($\log_{10}$ CFU)	C	4.12±0.29Ab	6.20±0.31Aa
	T1	2.74±0.34Cb	4.62±0.37Ca
	T2	3.24±0.34Bb	5.66±0.47Ba
	T3	2.98±0.36BCb	5.02±0.41Ca
Lactic acid bacteria ($\log_{10}$ CFU)	C	3.06±0.36Ab	5.18±0.31Aa
	T1	1.78±0.34Bb	3.66±0.43Ca
	T2	2.24±0.38Bb	4.68±0.53ABa
	T3	2.02±0.28Bb	4.28±0.79BCa

Means with different roman letters (A–C) in the same column significantly differ with $p<0.05$. Means with different roman letters (a, b) in the same row significantly differ with $p<0.05$

In this study, bacterial growth decreased with CCF addition during storage. This result may be due to CCF having antibacterial activity. The benefits of CCF have recently gained attention because it is a rich source of polyphenolic compounds, including berberine. Fan et al. (2008) reported that berberine in CCF exhibits diverse antibacterial activity and possesses a wide antimicrobial spectrum. Superoxide dismutase (SOD) catalyzes the dismutation of the superoxide radical into H_2O_2 and O_2, thus being involved in the first line of resistance against ROS (Zhang et al. 2011). SOD might be an important site of action of berberine, and O_2^- might inhibit bacterial growth (Zhang et al. 2011). Vattem et al. (2004) reported that the antimicrobial activity of the extracts was correlated with the antioxidant activity. Soluble phenols are thought to exert their antimicrobial effect by causing hyperacidification at the plasma membrane interface of the microorganism, which potentially results in the disruption of the H^+-ATPase required for ATP synthesis (Vattem et al. 2004). It has been suggested that phenolic compounds destabilize the cytoplasmic membrane and, in addition, act as

Table 5 Effect of *Coptis chinensis* Franch addition on the fatty acid composition in the sausages during storage

Fatty acids	Storage (weeks)	Treatments			
		C	T1	T2	T3
Capric acid (C10:0)	0	0.08±0.00	0.08±0.00	0.08±0.00	0.08±0.01
	4	0.08±0.00	0.08±0.00	0.08±0.00	0.08±0.00
Lauric acid (C12:0)	0	0.08±0.00	0.08±0.00	0.08±0.00	0.08±0.00
	4	0.08±0.00	0.08±0.00	0.08±0.00	0.08±0.00
Myristic acid (C14:0)	0	1.42±0.01	1.43±0.01	1.41±0.01	1.38±0.01
	4	1.41±0.01	1.41±0.00	1.40±0.01	1.37±0.00
Palmitic acid (C16:0)	0	24.83±0.59	25.22±0.03	24.45±0.20	24.70±0.17
	4	24.83±0.18	24.86±0.01	24.47±0.26	24.57±0.02
Palmitoleic acid (C16:1)	0	2.34±0.02	2.25±0.03	2.37±0.02	2.14±0.02
	4	2.26±0.02	2.19±0.30	2.32±0.29	2.12±0.01
Magaric acid (C17:0)	0	0.34±0.01	0.34±0.00	0.35±0.00	0.34±0.01
	4	0.33±0.01	0.33±0.01	0.36±0.01	0.35±0.00
Magaolic acid (C17:1)	0	0.36±0.03	0.38±0.02	0.39±0.01	0.36±0.02
	4	0.34±0.02	0.36±0.02	0.38±0.02	0.34±0.01
Stearic acid (C18:0)	0	13.66±0.52	14.07±0.60	12.93±0.30	14.05±0.80
	4	13.68±0.69	13.93±0.32	13.17±0.57	14.02±0.52
Oleic acid (C18:1)	0	43.57±0.46	42.28±0.06	43.95±0.46	42.65±0.41
	4	43.79±0.42	43.15±0.01	43.73±0.90	42.94±0.02
Linoleic acid (C18:2, n-6)	0	11.59±0.09	12.16±0.06	12.30±0.10	12.48±0.09
	4	11.50±0.11	11.94±0.01	12.33±0.13	12.41±0.01
Linolenic acid (C18:3, n-3)	0	0.62±0.01	0.65±0.01	0.64±0.01	0.66±0.01
	4	0.61±0.01	0.63±0.01	0.64±0.01	0.66±0.00
Arachidic acid (C20:0)	0	0.02±0.00	0.02±0.01	0.01±0.01	0.01±0.00
	4	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.01
Eicosenoic acid (C20:1)	0	1.09±0.01	1.04±0.01	1.03±0.01	1.06±0.01
	4	1.08±0.01	1.03±0.01	1.03±0.02	1.05±0.01
Eicosatrienoic acid (C20:4)	0	0.01±0.00	0.01±0.00	0.01±0.00	0.00±0.00
	4	0.00±0.00	0.00±0.00	0.00±0.0	0.00±0.00
SFA	0	40.42±0.33	41.24±0.11	39.31±0.31	40.65±0.28
	4	40.42±0.28	40.70±0.01	39.57±0.80	40.49±0.03
MUFA	0	59.58±0.33	58.76±0.11	60.69±0.31	59.35±0.28
	4	59.58±0.28	59.30±0.01	60.43±0.80	59.51±0.03
PUFA	0	12.22±0.09	12.82±0.07	12.95±0.11	13.14±0.09
	4	12.11±0.12	12.57±0.01	12.97±0.15	13.07±0.01

Values are mean±SD (n=3)

SFA saturated fatty acid, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid

proton exchangers, thereby reducing the pH gradients across the cytoplasmic membrane (Gallucci et al. 2009). The resulting collapse of the proton motive force and depletion of the ATP pool eventually lead to cell death (Gallucci et al. 2009). Thus, we assumed that the antimicrobial effect of CCF in sausages might be due to phenolic toxicity to microorganisms during storage. Another plausible reason for bacterial growth inhibition may be the decrease in pH brought about by CCF addition. In general, a high pH also encourages the growth of organisms such as *Brochothrix thermosphacta*, *Shewanella putrefaciens*, or enterobacteriaceae. In high-pH vacuum-packed meat, bacteria levels are only around 60 % of the total microbial population compared with 80–90 % in meat of pH <5.8. Shewanella and enterobacteriaceae also metabolize amino acids when the glucose is exhausted and produce offensive odors and flavors. Bacteria are very sensitive to the hydrogen ion concentration (pH) because their enzymes are affected by pH to stabilize the cell membrane or ion exchange, and many putrefying bacteria of meat products grow better at neutral pH. Because low pH in sausages may originate from CCF itself, pH decrease by CCF addition in sausages may be one of the main reasons for the antibacterial growth effect in this study.

The fatty acid composition of sausages after storage is shown in Table 5. The fatty acid composition was not significantly different in all sausage samples during storage. A slight decrease in many fatty acids of the sample containing CCF was observed at 4 weeks of storage, while a decrease was noticeable in the control and the extract containing CCF. The decrease in EPA and DHA, especially in the control, might be due to their susceptibility to oxidation. CCF prevented the loss of essential fatty acids, particularly polyunsaturated fatty acid (PUFA). The results suggested that CCF was able to retard the oxidation of unsaturated fatty acids in emulsion sausages to some extent. The sensory evaluation score of sausages after storage is shown in Table 6. Color, aroma, and springiness of sensory evaluation were not significantly differently affected by the addition of potassium sorbate and CCF. However, sausages containing potassium sorbate and CCF resulted in a lower score for flavor than the control sausages. Overall, the acceptability of sausages containing CCF was lower than that of control sausages and sausages with potassium sorbate addition.

Similarly, in this study, the protein degradation of sausage samples during storage may be affected to a lesser extent by CCF addition. Moreover, CCF addition may not influence the fatty acid composition because CCF is a phytochemical and contains no lipid-based components.

In this study, we found that CCF addition influenced the quality characteristics of sausages during cold storage for 4 weeks.

Table 6 Effect of *Coptis chinensis* Franch addition on the sensory evaluation of the sausages during storage

Item	Treatment	Storage (weeks)	
		0	4
Color	C	7.42±0.49	7.90±1.02
	T1	7.92±0.74	7.90±1.02
	T2	7.50±0.63	7.50±0.71
	T3	7.75±0.76	7.50±0.71
Aroma	C	7.58±0.49	7.70±0.84
	T1	7.50±0.55	7.10±0.89
	T2	6.92±0.74	7.70±0.67
	T3	7.17±0.68	7.30±0.84
Flavor	C	8.25±0.61A	8.30±0.67A
	T1	7.92±0.74A	7.60±0.55AB
	T2	5.92±0.66B	6.70±0.84B
	T3	5.42±0.66Bb	6.70±0.84Ba
Springiness	C	7.17±0.75	7.40±0.55
	T1	6.83±0.41	7.10±0.89
	T2	6.50±0.55	6.60±0.42
	T3	6.83±0.82	6.20±0.76
Juiciness	C	7.33±0.61	6.70±0.97
	T1	7.25±0.76	6.80±0.76
	T2	7.00±0.55	6.30±0.84
	T3	7.08±0.80a	5.80±0.84b
Overall acceptability	C	7.67±0.41A	7.90±1.02A
	T1	7.75±0.52A	7.40±0.89AB
	T2	6.75±0.69B	6.30±0.84B
	T3	6.92±0.58B	6.30±0.84B

Means with different roman letters (A, B) in the same column significantly differ with $p<0.05$. Means with different roman letters (a, b) in the same row significantly differ with $p<0.05$

Conclusions

In this study, lipid oxidation values dose-dependently decreased in sausages to which CCF was added. Moreover, the addition of CCF increased the DPPH radical scavenging activity of sausage samples. Hardness, cohesiveness, and springiness of sausage samples did not show any consistent trend. During storage, the total plate count and lactic acid bacteria diminished in sausages containing potassium sorbate and CCF compared to those of the control samples. In sensory evaluation, the overall acceptability of sausage samples containing CCF was significantly lower than that of the control sausage samples. Therefore, we conclude that CCF addition is not an effective way to improve the sensory evaluation of sausages, but may beneficially affect lipid oxidation and reduce the growth of microorganisms and phenolic or flavonoid content in sausages, which are considered good natural sources of dietary antioxidants.

Acknowledgments This study was supported by the Gyeongnam National University of Science and Technology Grant 2014.

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