Meat Science 95 (2013) 641–646

Contents lists available at SciVerse ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Antioxidant effects of soy sauce on color stability and lipid oxidation of raw beef patties during cold storage

Hyun-Wook Kim [a], Yun-Sang Choi [a], Ji-Hun Choi [a], Hack-Youn Kim [b], Ko-Eun Hwang [a], Dong-Heon Song [a], Soo-Yoen Lee [a], Mi-Ai Lee [c], Cheon-Jei Kim [a,b,*]

[a] Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 143-701, South Korea
[b] Department of Animal Resources Science, College of Industrial Sciences, Kongju National University, Yesan 340-702, South Korea
[c] World Institute of Kimchi, An annex of Korea Food Research Institute, Bundang 463-746, South Korea

ARTICLE INFO

Article history:
Received 19 October 2011
Received in revised form 24 April 2013
Accepted 7 June 2013

Keywords:
Lipid oxidation
Antioxidant
Discoloration
Soy sauce

ABSTRACT

This study was conducted to evaluate the antioxidant effects of soy sauce on lipid oxidation and color stability of raw beef patties. Raw beef patties were formulated with four solutions such as NaCl (sodium chloride solution), NaCl/SS (1:1 ratio of sodium chloride and soy sauce solution), SS (soy sauce solution), or SS/A (soy sauce solution combined with 0.05% ascorbic acid) in the same salt concentration. Addition of soy sauce resulted in the decreased pH, lightness, and increased yellowness. Treatment SS/A had the lowest percent of metmyoglobin during storage ($P < 0.05$). A reduction ($P < 0.05$) in the 2-thiobarbituric acid, peroxide, and conjugated diene concentration as result of soy sauce addition were observed in treatments SS and SS/A at the end of the storage period. There were no differences ($P > 0.05$) in free fatty acid concentration at the end of storage. The combined addition of soy sauce and ascorbic acid greatly improved ($P < 0.05$) color stability and retarded lipid oxidation.

1. Introduction

Lipid oxidation is one of major reasons for the deterioration of quality of beef products during storage, which leads to changes in the nutritional, texture, and sensory properties (Ladikos & Lougovois, 1990; Mielnik, Olsen, Vogt, Adeline, & Skrede, 2006). To prevent the lipid oxidation, several antioxidants has been used as types of additive or supplement into meat or meat products (Rhee, Lupton, Ziprin, & Rhee, 2003; Valencia, Ansorena, & Astiasarán, 2006). Recently, the use of synthetic antioxidants such as BHA (butylated-hydroxyanisole), BHT (butylated-hydroxytoluene), and TBHQ (tertiary-butylhydroquinone) are of restricted use in many countries because of toxicity issues (Okada et al., 1990). Therefore, evaluation of natural antioxidants has intensified (Sánchez-Escalante, Djenane, Torrescano, Beltrán, & Roncales, 2003).

Soy sauce or soya sauce is a widely used condiment and has been utilized for marinade and seasoning in a variety of meat-based cuisines in East Asia, such as China, Japan, Korea, and Thailand (Nam, Jo, & Lee, 2010). Also, interest on soy sauce as antioxidant is increasing in USA and European countries in recent years (Aoshima & Ooshima, 2009). Soy sauce is a fermented food derived from soybean or wheat. Soy sauce contains salt (approximately 15–20%), water (approximately 50–70%), peptides, isoflavones, free sugar, and organic acids derived from the soybeans during fermentation (Jeon, Sohn, Chae, Park, & Jeon, 2002; Kim, Jo, Yook, Park, & Byun, 2002; Shim et al., 2008). Soy sauce contains several antioxidants such as melanoidins (formed during fermentation), phenolic compounds and free amino acids. According to previous study, addition of soy sauce retards the lipid oxidation of cooked beef (Moon & Cheigh, 1986; Rufián-Henares & Morales, 2007; Wang et al., 2007). Interestingly, soy sauce contains also salt. Rhee and Ziprin (2001) reported that salt acted as a pro-oxidant at concentrations ranging from 0.5 to 2.5% regardless of its various other effects on meat processing. Love and Pearson (1971) found that sodium chloride influences oxidation of triglycerides in meat. Lee, Mei, and Decker (1997) reported that salt influences the activity of the endogenous multi-component antioxidant enzyme due to the presence of electrolytes and its ionic strength. Thus, the addition of salt is an important factor that not only affects microbiological stability, protein solubility, and sensory properties (Martin, 2001), but also alters the oxidative stability of meat products during storage.

The aims of this study were (1) to evaluate the antioxidative effects of soy sauce against lipid oxidation and discoloration of raw beef patties, and (2) to compare of lipid oxidation and discoloration of patties exposed to sodium chloride or soy sauce at the same salt concentration.

2. Materials and methods

2.1. Formulation and processing of raw beef patties

Fresh Hanwoo (Korean native cattle) meat (M. *semimembranosus*) and trimmings (50% fat) were purchased from a local processor 48 h

* Corresponding author at: Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 143-701, South Korea. Tel.: +82 2 450 3684; fax: +82 2 444 6695.
E-mail address: kimcj@konkuk.ac.kr (C.-J. Kim).

0309-1740/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.meatsci.2013.06.006

postmortem. All subcutaneous and inter-muscular fat and visible connective tissue were removed from the fresh beef muscle. Lean meat and trimmings were initially ground through Ø-8 mm plate using a meat grinder (PM-70, Mainca, Barcelona, Spain). The ground meat and trimmings were divided into four portions for each experiment prior to the addition of the sodium chloride or soy sauce solutions. Commercial soy sauce (Fermented soy sauce, Sempio Foods Co., Seoul, Korea) was purchased from the local market. Table 1 shows the physicochemical properties of the soy sauce. The soy sauce solutions were diluted in ice water to a final salt concentration of 10% (w/v). Sodium chloride solutions 10% (w/v) were prepared by dissolving sodium chloride in ice water. Each portion of beef meat (60%) was mixed with each solution (20%), and the trimmings (20%) and ascorbic acid (0.02%, only SS/A treatment) were added (w/w), and subsequently reground through a 3 mm plate. Table 2 shows the details of the raw beef patties used in this study. Each mixed sample was divided into 4 in smaller portions (about 100 g each) and allocated to the 4 periods. Batches were processed into 100 ± 1 g patties with 100 mm in diameter and 15 mm in thickness using patty presses (small ground press, Spikomat Ltd., Nottinghamshire, UK). Each raw beef patty was sealed in PE/Nylon film bags, stored at 4 ± 1 °C for 10 days. Patties were evaluated for lipid oxidation and color stability.

2.2. Analysis of raw beef patties

2.2.1. pH measurement

The pH of 5-g samples mixed with 20 ml distilled water for 60 s in a homogenizer (Ultra-Turrax T25, Janke and Kunkel, Staufen, Germany) at 8000 rpm speed and pH was determined with a pH meter (Model 340, Mettler-Toledo GmbH, Schwerzenbach, Switzerland).

2.2.2. Color measurements

The instrumental color of each samples was determined using a colorimeter (Minolta Chroma meter CR-210, Osaka, Japan; illuminate C, calibrated with a white plate, CIE $L^* = +97.83$, $a^* = -0.43$, $b^* = +1.98$). Values for CIE L^* (lightness), CIE a^* (redness), and CIE b^* (yellowness) were recorded.

2.2.3. Metmyoglobin concentration

Metmyoglobin concentration from each sample was determined using a modified procedure of Krzywicki (1979). Samples were blended with 5 volumes of cold 0.04 M phosphate buffer at pH 6.8 for 10 s in a homogenizer (Model AM-7, Nihonseiki Kaisha Ltd., Tokyo, Japan). After standing at 1 °C for 24 h, the mixtures were centrifuged at 3500 *g* at 4 °C for 30 min. The supernatant was further clarified by filtration through Whatman No. 1 filter paper. The absorbance of filtrate was measured at 525, 572, and 700 nm using a spectrophotometer (Optizen 2120 UV plus, Mecasys Co. Ltd., Daejoen, Korea). The percent of metmyoglobin was calculated using the formula:

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

where A_λ = absorbance at λ nm.

2.2.4. 2-Thiobarbituric acid (TBA) measurements

Lipid oxidation was assessed in triplicates for each sample by the 2-thiobarbituric acid (TBA) method of Tarladgis, Watts, Younathanm, and Dugan (1960) with minor modifications. A 10 g sample was blended with 50 ml distilled water for 2 min and then transferred to a distillation tube. The cup used for blending was washed with additional 47.5 ml of distilled water, which was added to the same distillation flask with 2.5 ml 4 N HCl and a few drops of an antifoam agent (KMK-73, Shin-Etsu Silicone Co., Ltd., Korea). The mixture was distilled and a 50 ml distillate was collected. Five ml of 0.02 M 2-thiobarbituric acid in 90% acetic acid (TBA reagent) was added to test tube containing 5 ml of the distillate and mixed well. The tubes were capped and heated in a boiling water bath for 30 min to develop chromogen and cooled to room temperature. The absorbance was measured at 538 nm, against a blank prepared with 5 ml distilled water and 5 ml TBA-reagent, using a UV/VIS spectrophotometer (Optizen 2120 UV plus, Mecasys Co. Ltd., Korea). The TBA values were calculated as mg MDA/kg meat. The formula was:

$$\text{TBA(MDA mg/meat kg)} = (\text{optical density of sample} - \text{optical density of blank}) \times 7.8.$$

Table 1
Physicochemical properties of soy sauce.

pH	CIE L^*-value	CIE a^*-value	CIE b^*-value	Salinity (%)
4.83 ± 0.03 [a]	8.64 ± 0.32	−0.32 ± 0.34	1.89 ± 0.20	16.00 ± 0.02

[a] All values are mean ± standard deviation of three replicates ($n = 3$).

Table 2
Formulation of raw beef patties.

Treatments[a]	Solution (salt in solution, total salt based on the total sample weight)
NaCl	Control, NaCl solution[b] (10%, 2%)
NaCl/SS	NaCl + soy sauce solutions (1:1 ratio, 10%, 2%)
SS	Soy sauce solution (10%, 2%)
SS/A	Soy sauce + 0.05% ascorbic acid solution (10%, 2%)

[a] All sample models contain 60% minced beef, 20% each solution, and 20% beef trimmings, and were stored for 0, 3, 7, and 10 days at 4 ± 1 °C.
[b] Each of the solutions was diluted by ice water to adjust total salt concentration.

2.2.5. Total lipid extraction

Total lipids were extracted from samples according to Folch, Lee, and Stanley (1957) by using a chloroform and methanol solvent system (the ratio of 2 to 1). Lipid extracts were evaporated and concentrated using a rotary evaporator (rotary evaporator N-1000, EYELA, Tokyo, Japan), and packed into a micro-tube (2 ml).

2.2.6. Determination of conjugated dienes (CD)

Formation and alteration of conjugated dienes (CD) were determined as described by Sirinivasan, Xing, and Decker (1996) with minor modifications. Samples (0.5 g) were suspended in 5 ml of distilled water and homogenized to form a smooth slurry. A 0.5 ml aliquot of this suspension was mixed with 5 ml of extracting solution (hexane and isopropanol, 3 to 1 ratio) during 1 min. After centrifugation at 2000 *g* for 5 min, absorbance of the supernatant was read at 233 nm. Concentration of conjugated dienes was calculated using the molar extinction coefficient of 25,500 M^{-1} cm^{-1} and the results were expressed as μmol/mg of sample.

2.2.7. Peroxide values (POV) of lipid extracted from raw beef patties

The peroxide value (POV), which is an indicator of early lipid rancidity, was measured to determine the degree of lipid oxidation (Juntachote, Berghofer, Siebenhandl, & Bauer, 2007). The peroxide values (POV) of the lipid extracted from the samples were measured by AOAC (1995), and calculated as follows:

$$\text{POV(meq of peroxides/kg of meat)} = (S\text{–}B) \times F \times N \times 1000/W$$

where, S = titration amount of sample; B = titration amount of blank; F = titer of 0.01 N sodium thiosulfate; N = normality of sodium thiosulfate; and W = sample weight (g).

2.2.8. Free fatty acid (FFA) content of lipids extracted from raw beef patties

The acid values of the lipid extracted from the samples were measured to determine formation of free fatty acids, the percentage of