Meat Science 129 (2017) 147–152

Contents lists available at ScienceDirect

Meat Science

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

Teriyaki sauce with carvacrol or thymol effectively controls *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium, and indigenous flora in marinated beef and marinade

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ARTICLE INFO

Article history:
Received 19 October 2016
Received in revised form 2 March 2017
Accepted 2 March 2017
Available online 06 March 2017

Chemical compounds:
Carvacrol (PubChem CID: 10364)
Thymol (PubChem CID: 6989)

Keywords:
Teriyaki sauce
Marinated beef
Carvacrol
Thymol
Antibacterial activity
Synergism

ABSTRACT

An effective bactericidal cold-marinating method for beef products is described, exploiting the synergism between soy sauce and natural compounds (carvacrol, CV or thymol, TM) to reduce microbiological risks. Beef slices inoculated with *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium (3.1–3.5 log CFU/g) were marinated in a teriyaki sauce with or without CV and TM (0.3 and 0.5%). After 1, 3, and 7 days at 4 °C, indigenous microflora population, color, lipid oxidation, marinade uptake, and pH of marinated beef and leftover marinade samples were examined. Teriyaki sauce alone did not reduce or inhibit any of the target pathogens or indigenous bacteria, while 0.5% CV- or TM-containing teriyaki sauce inactivated all inocula without recovery within 7 days ($p < 0.05$). The pathogens relocated from the beef into the leftover marinade (3.0–3.4 log CFU/mL) were also completely inactivated. The treatment inhibited growth of indigenous aerobic bacteria ($p < 0.05$) and inactivated coliform bacteria. Physicochemical parameters were not significantly affected ($p > 0.05$).

1. Introduction

Undercooked meat products have been identified as a leading cause of several outbreaks of foodborne illnesses worldwide (Scanga et al., 2000; Sofos, 2008). In particular, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium are frequently associated with outbreaks caused by processed meat products (CDC, 2006, 2014; Choi, Bae, Kim, Kim, & Rhee, 2009; Choi, Kim, Kim, Kim, & Rhee, 2009; Rhoades, Kargiotou, Katsanidis, & Koutsoumanis, 2013). Rangel, Sparling, Crowe, Griffin, and Swerdlow (2005) reported that ~47% *E. coli* O157:H7 outbreaks have been associated with beef products. Since consumers generally prefer medium rare or less-well cooked beef to over-cooked beef (Behrends et al., 2005; Neely et al., 1999; Røssvoll et al., 2014), it is imperative to devise an effective technique for eliminating or controlling those pathogens in commercial beef products at cold temperatures.

Marination, the soaking of food in a seasoned sauce before cooking, is one of many effective flavoring methods, not only enhancing flavor but also improving tenderness and juiciness of meat products (Björkroth, 2005). A number of marinated meat products are currently commercially manufactured and on sale, including barbecue ribs and steaks. Commercially marinated meat products, as ready-to-cook foods, are gaining popularity due to their convenience, whereas their hygiene and safety have not been extensively demonstrated (Jo, Lee, Kang, Shin, & Byun, 2004; Kanatt, Rao, Chawla, & Sharma, 2013). Indeed, coliform bacteria, typical indicators of food hygiene and food safety potential indicators (Brown et al., 2000; Lues & Van Tonder, 2007), were occasionally found in significant amounts (0.3–5.5 log CFU/g, 61.0% detection rate) in sold packaged marinated meat products (Rhee, Ryu, Kang, Kim, & Jang, 2014).

Marination in fermented soy sauce is a typical beef marinating procedure and several manufacturers apply this method in their products. Since international consumption of ethnic foods has been growing because of food consumer globalization and tourism (Mak, Lumbers, & Eves, 2012), beef products marinated in soy sauce, including '*bulgogi*', have become popular worldwide (Jo et al., 2004; Kim, Cha, Chung, Kim, & Chung, 2009). Although the marinade itself is generally regarded as a bacterial growth inhibitor, because of its acidic pH and high

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http://dx.doi.org/10.1016/j.meatsci.2017.03.001
0309-1740/

concentration of salt, preservatives, and spices (Björkroth, 2005; Kargiotou, Katsanidis, Rhoades, Kontominas, & Koutsoumanis, 2011), several researchers report that the marinade alone did not completely control pathogen growth under real processing conditions (e.g., refrigeration) (Masuda, Hara-Kudo, & Kumagai, 1998; Pathania, McKee, Bilgili, & Singh, 2010).

Cured meat products, including marinated beef, traditionally contain synthetic chemical preservatives, such as nitrate and nitrite (Sebranek & Bacus, 2007). However, because of the growing popularity of natural and organic foods, there has been a consumer shift away from chemical preservatives in foods. Antibacterial activity of various plant-derived essential oils (EOs) and their components has long been studied, and they have become popular as natural food additives (Burt, 2004). In our previous study, we discovered a significant synergistic antibacterial interaction between soy sauce and six different EOs [carvacrol (CV), thymol (TM), eugenol, *trans*-cinnamaldehyde, β-resorcylic acid, and vanillin]. CV and TM showed significantly higher bactericidal synergism with soy sauce than other EOs against *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium within 10 min exposure at refrigeration temperatures, even during refrigeration (Moon & Rhee, 2016). Therefore, it is expected that the inclusion of CV and TM in soy sauce beef marination may effectively control potentially hazardous pathogens in meat products and could result in a microbiologically safe and high-quality final product.

In this study, therefore, we aimed to develop an effective antimicrobial marinating method using soy sauce-based marinade and EO components (CV and TM) to control (1) artificial inoculum of foodborne pathogenic bacteria (*E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium), and (2) indigenous microorganisms (aerobic plate counts and total coliform counts) in both marinated beef and leftover marinades. In addition, physicochemical parameters (color, lipid oxidation, marinade uptake, and pH) were also measured and compared between EO-untreated and EO-treated groups to validate industrial applicability of the developed marinating method.

2. Materials and methods

2.1. Bacterial cell suspensions

Three strains of each species were obtained from American Type Culture Collection. These were: *E. coli* O157:H7 strains ATCC35150, 43889, 43895; *L. monocytogenes* strains ATCC 19111, 19115, 19117; and *S.* Typhimurium strains ATCC 14028, 19585, and DT104 Killer Cow. They were used as test microorganisms. Each strain was stored at −20 °C in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD, USA) with 20% glycerol and sub-cultured on monthly basis. The strains were separately cultured in 10 mL fresh TSB at 37 °C for 18 h, and three strains of the same species were mixed in a plastic 50-mL centrifuge tube (Difco). After centrifugation at 1800 ×*g* for 15 min (Centra-CL2; International Equipment Company, Needham Heights, MA, USA), bacterial pellets were washed twice with 0.85% sterile saline solution. The final pellet was re-suspended in 10 mL 0.85% sterile saline (density ~8–9 log CFU/mL).

2.2. Beef preparation and inoculation

Beef sirloins, sliced to 5 mm thickness, were purchased from a local supermarket and stored at −20 °C. The day before use, beef sirloins were defrosted at 4 °C overnight. Samples were cut into 10 g pieces (30 × 25 × 5 mm) with a flame-sterilized kitchen knife. Presence of *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium in sliced beef was tested by examining 25 g samples according to method described in the Bacteriological Analytical Manual by the United States Food and Drug Administration. Detection of target microorganisms led to rejection of the entire beef sirloin from which the sample was obtained. Thus prepared samples were inoculated with bacterial cell suspensions (see Section 2.1.), as follows. Bacterial suspensions were diluted 10-fold with 0.85% saline to obtain 4–5 log CFU/mL cell densities. The suspensions were dispensed over beef piece surface (final concentration 3–4 log CFU/g inoculated meat). Inoculated beef pieces were incubated at 4 °C overnight to allow the inoculum to attach to the sliced beef surface. After refrigeration, two 10 g sample pieces were randomly selected and used for further experiments. To assess the changes in indigenous bacterium concentration and physicochemical parameters associated with marination (with or without EO addition), uninoculated beef slices were used as experimental samples.

2.3. Marination

Teriyaki sauce (Preservative-Free Kikkoman Teriyaki Sauce, Kikkoman Co., Japan) was purchased from a local market and stored at 4 °C until use. Ingredients in teriyaki sauce were soy sauce, wine, high fructose corn syrup, water, vinegar, salt, spices, onion powder, and garlic powder. CV and TM (Sigma-Aldrich, St. Louis, MO, USA) stock solutions (50×) were prepared by dissolving the appropriate amount of each compound in 98% ethanol. Stock solutions (200 μL) were added to 9.8 mL teriyaki sauce in a sterile plastic 50-mL tube (Difco), to adjust the final EO concentration to 0.3% and 0.5% (v/v). Raw sliced beef samples and samples treated with teriyaki sauce + 2% ethanol were used as negative (unmarinated) and positive controls (teriyaki sauce-marinated), respectively. Beef samples (10 g) were then immersed in the prepared CV- or TM-supplemented marinades and gently agitated with sterile forceps. After fastening the lid of the plastic 50-mL tube, the marinated beef was incubated at 4 °C for 7 days. The experiment was performed in triplicate using different beef sample produced in a different batch.

2.4. Microbiological analysis

Following 1, 3, and 7-day refrigeration, marinated beef and leftover marinades were collected separately. Each marinated beef piece and leftover marinade was placed in a sterile stomacher bag (JS-010, Jin Sung Uni-Tech, Seoul, Korea) and homogenized in 10 volumes of sterile 0.85% saline (230 rpm for 2 min) (Circulator 400, Seward, Worthing, UK). Aliquots (0.1 mL) of the homogenate were serially diluted in 9 mL 0.85% saline 10^4×. Each dilution (0.1 mL) was then spread onto the following selective agar plates: MacConkey sorbitol agar (Difco) for *E. coli* O157:H7 detection; Oxford Agar supplemented with *Listeria* selective supplement (Difco) for *L. monocytogenes* detection; and xylose-lysine-desoxycholate agar (Difco) for *S.* Typhimurium detection. Plate count agar (Difco) and violet red bile agar (Difco) were used for aerobic plate count (APC) analysis and total coliform counts (TCC), respectively. To lower the detection limit to 10 CFU/g beef and 1 CFU/mL marinade, undiluted homogenate (0.2 mL) was spread onto five plates of each agar. All inoculated plates were incubated at 37 °C for 24 h except for PCA (48 h at 37 ± 2 °C), and typical colonies were counted.

2.5. Validation of antimicrobial marination

If none of the inoculum was detected by the plate counting method, recovery of injured bacterial cells was assessed to identify treatment conditions that resulted in a complete inactivation of the inoculum. Beef samples subjected to each condition were separately transferred into sterile stomacher bags and homogenized at 230 rpm for 2 min in 10 volumes of fresh TSB. After 24 h incubation at 37 °C, enriched samples were streaked on selective agar plates, as mentioned in Section 2.4, and further incubated at 37 °C for 24 h. The absence or presence of typical colonies was recorded as a positive or negative result, respectively. The experiments were performed in triplicate.