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Effect of HPP treatment on the safety and quality of beef steak intended for sous vide cooking

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ABSTRACT

High Pressure Processing (HPP), a non-thermal microbial inactivation technology, can be potentially used as hurdle during processing while enhancing the taste and nutrition of muscle foods. In this study, the effect of HPP treatment on safety and quality of beef steak intended for sous vide cooking was determined. Beef steaks were inoculated with *E. coli* 0157:H7 via pin pad and then seared, vacuum packaged, and subjected to HPP. HPP treatment at 450 MPa for 15 min resulted in reductions of 4.74 log CFU/g in *E. coli*. At 600 MPa, *E. coli* levels saw reductions of 6.13 log CFU/g, after 10 min. HPP can achieve microbial reductions of *E. coli* exceeding the 5-log definition of pasteurization, allowing the potential for the creation of convenience products with reduced potential risk in home sous vides cooking. HPP treatment at 450 MPa and 600 MPa did not significantly change the properties of most seared beef steaks in term of pH, water activity, moisture content, expressible moisture. Additionally, while the degree of lipid oxidation (via TBARs) decreased significantly ($p < 0.05$) in Post-HPP samples after sous vide cooking for 2 h, there was not significant difference among pressure treatment ($p > 0.05$).

1. Introduction

Sous vide (SV) processing, a French cooking technique of immersing vacuum packaged foods in water held at stable temperature, has been actively studied by researchers since the 1990s (Mossel & Struijk, 1991; Schellekens, 1996). This method is typically defined as a "low-temperature-long-time" cook, which has several benefits: creating a consistent and appealing texture, retaining favorable color, preventing evaporative losses of moisture and flavor during cooking and the inhibition of cross-contamination during storage (Church & Parsons, 2000; Keller, Benno, Lee, & Rouxel, 2008; Myhrvold, Young, & Bilet, 2011). Sous vide cooking has become more popular and widely used in restaurants and culinary schools during the last decades (Kamozawa & Talbot, 2010, pp. 25–200). Equipment costs initially limited access by home users, but the recent influx of inexpensive "clamp-on" circulators has opened the door to an increase in home consumers trying out the technology. And, as they typically have less training and experience than culinary professionals, there are potential safety issues related to inadvertent or intentional shortening of cooking time, especially given that no official guidelines directed at practitioners exist (O'Bryan, Crandall, Martin Griffis and Johnson, 2006).

Previous attempts on SV cooked meat-based dishes have primarily been focused on its use as a possible strategy for controlling microbial growth, including that of *Clostridium botulinum, Bacillus cereus, Clostridium perfringens, Listeria monocytogenes, Salmonella* spp and *Escherichia coli* O157: H7(Borch & Arinder, 2002; Sutherland & Porrit, 1997). However, little work has been done on the applications of other technologies prior to SV cooking, which could either help to reduce cooking times, reduce the risk of illness, and/or to extend the shelf life of the product. Muscle foods, and especially beef products, have been linked to multiple, sometimes fatal, outbreaks of *Escherichia coli* O157: H7 and have been responsible for many associated recalls in the US (CDC, 2016). And while the primary risk related to intact cuts from muscles are on the surface, significant food safety risks are possible if the food undergoes processing which could carry pathogenic microorganisms from the surface or environment into the inner parts of the cut, as is common industry practice, during mechanical tenderization and brine injection (Gupta, 2012; Luchansky, Phebus, Thippareddi, & Call, 2008). This serious concern has led *Escherichia coli* O157: H7

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to be considered as an adulterant in non-intact beef blade tenderized and brine injected beef since 1999 (FSIS, 1999). Thus, alternative processes such as HPP, a non-thermal microbial inactivation technology, are a desirable option for the industry, especially as these have been studied and are currently used commercially for post-processing pasteurization to control Listeria in cooked, ready-to-eat(RTE) products (Campus, 2010; Simonin, Duranton, and De Lambaallerie, 2012; Torres and Velazquez, 2005). While the use of HPP is desirable from a microbial standpoint, less research on fresh intact beef by HPP has been undertaken, primarily due to the significant detrimental changes in quality under certain pressures by HPP. For example, one study showed that the color of fresh beef was significantly changed, with lightness increasing when exposed to pressures greater than 200 MPa (Carlez, Veciana-Nogues, & Cheftel, 1995), while others found that tenderness decreased and lipid oxidation rates increased in beef subjected to different times and pressures by HPP (Bolumar, Skibsted, & Orlien, 2012; McArdle, Marcos, Kerry, & Mullen, 2010, 2011; Suzuki, Watanabe, Iwamura, Ikeuchi, and Saito, 1990). Since SV cooking is well known to result in a tender product, and partial pre-cooking (searing) creates stable surface colors, the combination of these techniques with HPP may result in an interesting and meaningful application of HPP. Thus in this study, we aimed to first determine the efficacy of HPP on the destruction of *Escherichia coli* O157: H7 in internally contaminated, seared samples intended for sous vide cooking, and then to quantify the effect of the combination technique to the different aspects of beef quality.

2. Materials and methods

2.1. Culture propagation

Escherichia coli O157: H7 (ATCC 35150) was obtained from the BSL-3 microbiology lab at the University of Nebraska-Lincoln (Lincoln, NE, USA). Prior to use, cultures were maintained individually at deep freezer (−70 °C) in glycerol-TSB mix (800 ml glycerol/L culture). After thawing, cultures for experiments were reactivated by performing two serial transfers with one loop to 10 mL tryptcase soy broth (TSB, Becton-Dickinson and Co., Franklin Lakes, NJ USA) with incubation 35 ± 2 °C for 24 h after each transfer. The resulting culture (100 μL) was then spread onto tryptcase soy agar (TSA, Becton-Dickinson and Co., Franklin Lakes, NJ USA) and incubated at 35 ± 2 °C for another 24 h to produce a bacterial lawn. One milliliter of 1 g/L peptone water was added to each agar surface to suspend the cells. Cells were then scraped gently using a sterile plate spreader. The culture was collected in a 50 mL conical tube (Fisher Scientific, Fair Lawn, NJ, USA) and then transferred to a 96 well, Flat Bottom plate, with Low Evaporation Lid (Microtest Tissue Culture, Franklin Lakes, NJ USA) to facilitate inoculation by pin pad.

2.2. Sample preparation and inoculation

M. triceps brachii (*long head*); were obtained from a commercial abattoir and cut into steak portions (85±5 g, 2.54 cm thickness) at the Loeffel Meat Laboratory (University of Nebraska-Lincoln, Lincoln, NE, USA). For microbial analysis, steaks were inoculated internally using a 96 long-pin pad which had been dipped into the inoculum held in the aforementioned 96-well plate, with each steak receiving 2 inoculations, one on each of the larger sides. Then sample was seared using a direct heat device (Searzall, Booker and Dax Lab, New York, NY, USA) for 1 min on each side. During searing, steaks were placed on an aluminum foil covered pan. Post searing, samples were immediately sealed in polypropylene pouches under vacuum using a commercial sealer (VACMASTER Model VP215, ARY Consumer Goods, St. Louis, MO, USA) and stored for no longer than 24 h at 4 °C prior to HPP treatment. For quality attribute tests, the beef steaks were seared using a conveyor oven (Lincoln 1240, Welbilt New Port Richey, FL, USA), set to 260 °C for 5 min, transferred into polypropylene pouches and submerged in an ice bath to halt cooking. Preliminary tests were conducted to confirm that the internal temperatures of the samples did not exceed 25 °C and the color and texture of the internal meat were still raw-like.

2.3. High pressure processing treatment

For microbial analysis, HPP treatments were performed using high pressure processing system (ISO Lab, Stansted Fluid Power, Stansted, UK). The processing fluid, a mixture of ethylene glycol and water, was kept at 4 °C prior to pressurizations to limit the influence of adiabatic heating, and pressures and temperatures were recorded in real-time. The samples were treated under different two different pressure (450 MPa, 600 MPa) with 6 different treatment times (2s*, 1, 3, 6, 10, 15 min). Control samples were also prepared as above, but were not subjected to HPP treatment. After HPP treatment, samples were first chopped into small pieces using a sterilized knife and then transferred into polypropylene stomacher pouches along with 25 ml of 1 g/L peptone water. These bags were then stomached for 45 s, and serial dilutions of the liquid were made prior to plating on APC and ECC petrifilms (The 3 M Company, Maplewood, MN, USA) and incubated according to manufacturer instructions to enumerate the survival microorganisms.

Samples for quality testing were processed using a larger commercial scale unit (Hiperbaric 55, Hiperbaric USA, Miami, FL, USA) located in the UNL Food Science Pilot plant. Samples were vacuum packaged and stored at 4 °C for at least 24 h prior to HPP treatment. Samples were then pressurized at 450 MPa and 600 MPa with a holding time of (2 s*, 3, 6, 10 15 min).

For each quality trial, 48 samples were prepared for both HPP treatment and HPP&SV cooking. A single randomly selected sample was sliced into small pieces which were used for a_w, pH and moisture content and color measurement. Two additional randomly selected samples were used for TBARs, WBSF and water holding capacity.

2.4. Warner Bratzler shear force (WBSF)

Samples from each treatment were *sous vide* cooked for 2 h using a clamp-on circulating water heater (ANOVA Precision cooker, Anova Culinary LLC, Houston, TX, USA) set at 55 °C. Samples were then transferred to a walk-in cooler at 2–5 °C overnight before coring. Six cylindrical cores (Diameter- 1.27 cm) were removed from each cooked steak parallel to the muscle fibers using a drill press to ensure uniform in diameter. Cores were then analyzed using a Texture Analyzer (Model TMS-PRO, Food Technology Corp., Sterling, VA, USA) fitted with a Warner-Bratzler blade and slotted base. A crosshead speed of 250 mm/min was used and the mean peak shear force (in kg) of the 6 cores was determined for each steak.

2.5. Color measurements

The internal color of all samples was determined instrumentally using a hand-held colorimeter (CR-300, Konica-Minolta, Ramsey, NJ, USA) with D65 illuminant, using diffuse illumination and 0°viewing angle (specular component included). Prior to measuring, the colorimeter was calibrated according to manufacturer instructions using a provided white ceramic calibration plate. Samples were measured prior to searing, after searing, after HPP and After SV. CIE L*a*b* values were recorded for three locations