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Effect of high pressure processing on physicochemical and microbiological properties of marinated beef with reduced sodium content

Isabela Rodrigues [a,b], Marco Antonio Trindade [a], Franciele Roberto Caramit [b], Kezban Candoğan [c], Prashant Raj Pokhrel [b], Gustavo V. Barbosa-Cánovas [b,*]

[a] Laboratory of quality and stability of meat and meat products, Department of Food Engineering, University of Sao Paulo, Brazil
[b] Center for Nonthermal Processing of Food, Department of Biological Systems Engineering, Washington State University, United States
[c] Faculty of Engineering, Department of Food Engineering, Ankara University, Turkey

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ABSTRACT

High pressure processing (HPP) is one of the newer technologies that has shown great potential for manufacturing meat products with reduced sodium content. The aim of this study was to evaluate the effect of HPP applied at different levels in the inactivation of *Listeria innocua* and *Enterococcus faecium* inoculated in marinated beef (*Longissimus lumborum*) with reduced sodium content, as well as its influence on the physicochemical properties of the meat. Samples were inoculated with 10^6 CFU/g of L. *innocua and E. faecium*, and marinated with solutions in different concentrations of NaCl (1 or 2%) and citric acid (1 or 2%) for 18 h and treated with high pressure (300, 450 or 600 MPa). Samples treated with 600 MPa were also evaluated regarding physicochemical stability after 14 days of refrigerated storage. Different marinating solutions were not sufficient to reduce initial microbial loads in the non-pressurized samples, but the combination with high pressure caused six log cycle reductions of both microorganisms. The treatment with 2% salt/2% citric acid was the most effective for each pressure considered for both bacteria. No significant changes were observed in the water activity of the samples, however samples with higher concentration of citric acid showed lower ($p < 0.05$) pH and lower ($p < 0.05$) lipid oxidation after 14 days of storage under refrigeration. Only samples treated with 600 MPa showed an increase ($p < 0.05$) in hardness. The results showed that HPP was able to process a safe meat product with reduced sodium concentration.

Industrial relevance: The result from this study shows the benefits of using HPP as an alternative meat processing technology to develop a meat product with reduce sodium content. Meat processed by this method have better nutritional quality and extended shelf life as compared to conventional processing. The step taken in this study will aid the meat processing industry for the development of microbiologically safe meat product with low sodium content by the application of high pressure processing.

1. Introduction

Since the World Health Organization recommended the reduction of daily levels of sodium intake, salt reduction in foods has become an area of interest to researchers. However, the function of sodium in food products goes beyond sensory aspects, affecting technological properties and food security (Grossi, Søltoft-Jensen, Knudsen, Christensen, & Orlien, 2012). Sodium chloride (NaCl) is an essential ingredient in processed meat products, affecting fat-binding, water-holding capacity (WHC), color, taste and texture (Bak et al., 2012; Duranton, Guillou, Simonin, Chéret, & de Lamballerie, 2012). In addition, because of its ability to reduce water activity, sodium chloride demonstrates a good effect against bacterial growth. To minimize the negative consequences of sodium eduction in meat products, many other technologies have been studied, such as the substitution of sodium chloride for other types of salts or newer processing techniques (Ruusunen et al., 2005; Fulladosa, Serra, Gou, & Arnau, 2009; Grossi et al., 2012; Pietrasik & Gaudette, 2014 and Sharedeh, Gatellier, Astruc, & Daudin, 2015).

High pressure processing (HPP) is a new technology that has been proposed for meat products (Omana, Plastow, & Betti, 2011; Myers, Montoya, Cannon, Dickson, & Sebranek, 2013 and O'Flynn, Cruz-Romero, Troy, Mullen, & Kerry, 2014). According to Torrezan (2003), high pressure processing is an alternative method to ensure the safety of meat products after processing, as well as being a milder treatment as compared with treatments that apply high temperatures, since it allows the obtainment of products with better sensory and nutritional quality, without impairment in reducing microbial loads.

Pressures in the range of 100 to 800 MPa can be applied to meat products, and pressures above 300 MPa help to inactivate microorganisms,

* Corresponding author.
E-mail address: barbosa@wsu.edu (G.V. Barbosa-Cánovas).

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making the product microbiologically safe (Bak et al., 2012). In addition, high pressure processing does not cause major alterations in taste, flavor or nutrient content of foods, and can be done at low or room temperature (Crehan, Troy, & Buckley, 2000). Application of high pressure processing has been shown to act on myofibrillar proteins in a similar manner to salts, therefore sodium chloride can be reduced on meat products treated with high pressure processing (Omana et al., 2011).

Many researchers have reported on the effect of high pressure in microorganisms in meat products, most of them in ham, turkey and poultry meat (Yuste, Mor-Mur, Capellas, & Pla, 1999, Myers et al., 2013, Garriga, Grebol, Aymerich, Monfort, & Hugas, 2014, Lerasle et al., 2014). *Salmonella* and *Listeria monocytogenes* are the most common bacteria in these studies, probably due to the large involvement of these microorganisms in foodborne illness. *Listeria innocua* is a non-pathogenic indicator microorganism for L. *monocytogenes* (Fairchild & Foegeding, 1993; Yuste et al., 1999). *E. faecium* is a very resistant microorganism used as surrogate for *Salmonella* (Landfeld et al., 2009; Bianchini et al., 2014).

Marinated meat is becoming a popular kind of ready to cook meat product. A marinade is a mixture in which the meat is immersed, injected or massaged, in order to improve flavor, texture, and other sensory attributes, such as color or juiciness (USDA, 2005). In general, salt and acid are essential ingredients for a marinade and provide the desired characteristics mentioned. Moreover, according to Pathania, McKee, Bilgili, and Singh (2010) the growing demand for convenience products and longer shelf life has increased interest in the use of spices, salts and organic acids in marinades, in order to increase food security.

Since consumers have begun to seek products with reduced salt content, it is important to find ways to meet this demand without compromising food safety. The use of high pressure processing seems like a good solution to this problem. Thus, the aim of this study was to evaluate the use of different pressures in combination with different levels of sodium and citric acid in the inactivation of microorganisms and maintenance of the physical and chemical characteristics of marinated beef sirloin.

2. Materials and methods

The meat used in this study (*Longissimus lumborum*) was purchased from a local market in Pullman, WA and the beef was cut in rectangular pieces of 100 g of meat, with approximately 1.5 cm of thickness and 7.0 cm long. A cocktail of *Enterococcus faecium* (ATCC 8459) and *Listeria innocua* (ATCC 51742) was inoculated in the meat to get the final concentration of 10^6 CFU/g of each microorganism. *E. faecium* was used as a *Salmonella enterica* surrogate. The samples were marinated by immersion in solutions with different concentrations of NaCl (1 or 2%) and citric acid (1 or 2%) during 18 h, seasonings were also added in the concentration of 2% onion powder, 2% garlic powder, 1% red crushed pepper and 0.5% black powdered pepper. All the samples were packed under vacuum in Nylon/polyethylene vacuum pouches (Ultravac Solutions, Kansas, USA). After the marination procedure, samples were treated with pressure of 300, 450 or 600 MPa. The equipment used is an EPSI of 2 liters capacity manufactured by Engineered Pressure Systems Inc. Haverhill, MA 01835. This equipment has an Electrohydraulic Intensifier Pump (Hochdruck-Systeme GmbH, AP 10-0670-1116, Sigless, Austria). The compression fluid used was a mixture of 5% Mobil Hydrasol 78 water solution (Hydraulic systems fluid company, Houghton International Inc., Valley Forge, PA, USA). After searching specialized literature, two processing times were identified to treat the samples: 1 and 5 min. After a few trials it was decided 5 min was the appropriated time to validate our hypothesis.

Samples without high pressure processing (control) were prepared to evaluate the effect of the solutions. After treatment, all of the samples were analyzed for water activity, pH, color, shear force, cooking loss, lipid oxidation, microbiological loads, and sodium content. The samples treated with 600 MPa were also evaluated after 14 days of storage at 4 °C. For each treatment three repetitions were made and the analyses were made in triplicates.

2.1. Bacteria growth and counting

Both microorganisms studied in this work were purchased from ATCC. The culture of L. *innocua* and *E. faecium* was grown in brain-heart infusion broth (BHI) and tryptic soy broth (TSB) respectively at 37 °C under agitation (218 rpm). The cell was harvested when the culture reaches the early stationary phase, where they are strong. The time to reach early stationary phase for L. *innocua* and *E. Faecium* was 18 and 10 h, respectively. The cultures were frozen in glycerol solution (20%) until use and before inoculation, they were unfrozen and mixed with 100 mL of TSB. An amount of TSB + culture were taken to inoculate the samples to the final concentration of 10^6 CFU/g of product.

For first dilution, 25 g of product were homogenized in 225 mL of peptone water for 2 min. After that, decimal dilutions were made. Enterococcosel™ Agar (Bile Esculin Azide Agar, BD™ BBL) was used for plating *E. faecium* and Oxford Listeria Agar Base was used for *Listeria innocua.* Plates were incubated at 37 °C for 48 h. Counts of the microorganisms were expressed as log colony forming units (CFU)/g sample.

2.2. Physicochemical properties

Water activity was determined by AQUALAB equipment (Decagon Devices, Pullman, WA) and pH values were taken by using a pH analyzer (Mettler Toledo, FE20/EL20, Columbus, OH, USA), both of them were measured in triplicate.

Samples of each treatment were subjected to color evaluation with a spectrophotometer (Konica Minolta, CM-5, New Jersey, USA). Lightness (L^*), redness (a^*, red ± green) and yellowness (b^*, yellow ± blue) values were measured according to the CIE Lab system, using the illuminant D65 with 10° of observation angle and gap cell of 30 mm. The average of six readings by samples were taken. The color was also measured for raw meat (without marinade solutions and without HPP).

For measuring shear force and loss of water by cooking, initially the samples were cooked in an oven (180 °C) until internal temperature reached 72 °C. Samples were weighed before and after cooking, and the cooking loss (CL) percentage was calculated with Eq. (1), as follows:

$$CL = (\text{Initial weight} - \text{final weight})/\text{Initial weight} \times 100 \quad (1)$$

After cooking, samples were cut into pieces of 2 × 1 × 1 cm (6 pieces) and the shear force was measured by using a texture analyzer (TA-XT2, Stable Micro Systems, Scarsdale, N.Y., U.S.A.) calibrated with a speed of 10 mm/s, distance of 40 mm and 5 kg of force.

Lipid oxidation was measured by the determination of thiobarbituric acid reactive substances (TBARS) according to the methodology described by Vyncke (1970). This methodology measures lipid oxidation by the quantity of substances that reacts with thiobarbituric acid. These substances are originated during the oxidation of the lipids in the product. First, TBARS were extracted from the meat by homogenization with trichloroacetic acid (Sigma Aldrich, USA) (5 g of meat and 25 mL of acid) and filtered, then thiobarbituric acid (Sigma Aldrich, USA) was added to the extract (5:5 v/v) and the solution was placed in a water bath (98 °C/40 min) to promote the reaction. The final solution was read in a spectrophotometer (538 nm) and the results calculated from the calibration curve prepared with 1,1,3,3 – Tetraethoxypropane (Sigma Aldrich, USA). A total of six readings were taken for each sample.

2.3. Sodium concentration

Flame atomic absorption spectrometry was used to measure salt content in the samples. Portions of 0.2500 g of dried sample were digested by using a microwave digester system with 4.5 mL of nitric acid and 0.5 mL of hydrogen peroxide. After completed digestion,