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The effect of the packaging system and storage time on myofibrillar protein degradation and oxidation process in relation to beef tenderness

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ABSTRACT

This study investigated the impact of packaging systems on the degradation and oxidation of beef proteins regarding beef tenderness of *longissimus lumborum* (LL) and *biceps femoris* (BF) muscles stored in vacuum skin packaging (VSP), a modified atmosphere with high oxygen concentration (MAP), and combined of these two methods (VSP + MAP). A significant decrease in the Warner-Bratzler shear force (WBSF) in VSP at D14 and D28 for LL was observed compared to BF. A significant effect of packaging system on troponin-T (Tn-T) and desmin degradation was shown ($p \leq 0.001$). A high concentration of oxygen in MAP and VSP + MAP affected protein oxidation, which was reflected in myosin oxidative cross-linking. An increase of WBSF values detected in steaks packed in VSP and VSP + MAP systems could be caused by the intensification of protein oxidation. Furthermore, BF was more susceptible to oxidation compared to LL. The VSP + MAP packaging system has resulted in the maintenance of a bright, red color, however has not improved the beef tenderness.

1. Introduction

The process of food packaging in the meat industry is developing rapidly. In the case of the retail beef market, vacuum (*e.g.* vacuum skin packaging (VSP) method) and modified atmosphere packaging (MAP) are most commonly used for packaging of fresh beef. For the last few decades, the vacuum skin packaging method, which allows better exposure of the product, has generated the most interest in the meat industry and in the consumer sector (Kang, Kang, Seong, Park, & Cho, 2014). Meat color is an important factor, as it determines a purchase decision. An assessment of color changes resulting from the storage system plays a significant role in affecting consumers' perception of meat quality (Liu et al., 2014). The color of the meat is influenced by many factors, such as the concentration and chemical form of heme pigments, mainly myoglobin (Franco et al., 2012; McMillin, 2008). Vacuum packaging may prolong the shelf life of meat, although a lack of oxygen results in an unattractive color change, which results from a transformation of myoglobin into dark-red deoxymyoglobin (Li, Lindahl, Zamaratskaia, & Lundström, 2012). For that reason, modified atmosphere packaging with high oxygen content (70–80% O_2) is more often applied, so as to maintain the bright red color that is more desirable and preferred by the consumer (Franco et al., 2012; Kerry, O'Grady, & Hogan, 2006; Zakrys-Waliwander, O'Sullivan, Walsh, Allen, & Kerry, 2011). Nevertheless, oxygen concentration is strongly connected to shelf-life, as well as to other qualitative aspects of meat stored in modified atmospheres (Fuentes, Estévez, Ventanas, & Ventanas, 2014; Lund, Heinonen, Baron, & Estévez, 2011). A high oxygen concentration is one of the main reasons for lipid (Zakrys, Hogan, O'Sullivan, Allen, & Kerry, 2008) and protein oxidation (Kim, Huff-Lonergan, Sebranek, & Lonergan, 2010), which contributes to the deterioration of sensory qualities (Jakobsen & Bertelsen, 2000; Jayasingh, Cornforth, Brennand, Carpenter, & Whittier, 2002), the decrease of nutritive values (Zakrys-Waliwander et al., 2011), and the technological properties of meat (Clausen, Jakobsen, Ertbjerg, & Madsen, 2009; Cruzen et al., 2015). Lipid oxidation leads to a deterioration of sensory qualities due to rancidity and the development of a warmed-over flavor (WOF), while the changes taking place within proteins are connected to the deterioration of textural aspects of the meat. Protein oxidation can influence the physical and chemical properties of proteins, including their solubility, hydrophobicity, water-holding capacity, gelation functions, and even meat tenderness and nutritional value (Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004a, 2004b; Zakrys-Waliwander, O'Sullivan, Allen, O'Neill, & Kerry, 2010). During *post mortem* storage, muscle has a decreased ability to maintain its antioxidant defense system, which may result in an increased accumulation of reactive oxygen and nitrogen species (Renerre, Dumont, & Gatellier, 1996). Since thiol groups of cysteine residues are highly susceptible to oxidative modification and

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results in the formation of larger protein complexes, the rate of oxidation was observed mainly *via* protein polymerization.

Beef usually attains suitable tenderness with 10 days of vacuum aging after slaughter, but it becomes brown and remains a dark, unattractive color for consumers during retail displays in this type of package (Fu et al., 2014). On the other hand, maintaining the meat's bright red color is possible when oxygen presence is taken into account. Therefore, modified atmosphere packaging (MAP) with a high oxygen concentration is used as a tool for preventing dark meat color formation during retail storage (McMillin, 2008). Both of these systems have a number of advantages and drawbacks. Therefore, producers should investigate new solutions for meat packaging technologies, which provide high quality to satisfy the ever increasing and changing consumers' expectations. One solution may be to use a combination of both these methods, *i.e.* VSP and MAP to achieve suitable beef tenderness while maintaining a desirable bright red color. The aim of this study was to assess the influence of two packaging systems, namely VSP, MAP and combinations of these two systems (VSP + MAP) on myofibrillar protein degradation, oxidation and polymerization processes in relation to beef tenderness for two muscles, *longissimus lumborum* and *biceps femoris*.

2. Materials and methods

2.1. Processing and packaging of beef samples

The experiment was conducted on muscles obtained from Simmental × Holstein-Friesian crossbred bulls (SM × HF; n = 9) reared on one farm and slaughtered at the age of 20–21 months in a local slaughterhouse. Live weight of bulls ranged from 670 to 686 kg. Carcasses were classified using the SEUROP classification scale for conformation (S – superior; E – excellent; U – very good; R – good; O – fair; P – poor). The fat cover classification (1 – low; 2 – slight; 3 – average; 4 – high; 5 – very high) was used. Hot carcass weights ranged from 346 to 369 kg. The carcasses were graded as R− to R+ with fat cover of 2+ to 3+. The muscles *longissimus lumborum* (LL) and *biceps femoris* (BF) were excised (remove of external fat and visible connective tissue) from both half-carcasses 24 h after slaughter and were cut starting from the proximal side into six 2.5 cm thick steaks (sample weight: 250 g ± 15 g), perpendicularly to the long axis of each muscle (after excluding the first steak). Then steaks from both half-carcasses were assigned randomly to three packaging systems:

a) Vacuum skin packaging (VSP): the steaks were packed on PET/PE support trays (135 × 250 × 20 mm, Cryovac® RSB03X56; Sealed Air Corporation, New Jersey, US). The film used was 100 μm thick polyethylene, with a maximum O_2 permeability of 2 cm^3/m^2/24 h/bar at 23 °C with a relative humidity of 0% (Cryovac® TS201; Sealed Air Corporation, New Jersey, US). The packaging and sealing of the film was performed at 200 °C with a Cryovac® VS26 packager (Sealed Air Corporation, New Jersey, US) and stored for 14 and 28 days.
b) Modified atmosphere packaging (MAP): steaks were packaged and stored for 14 days (MAP D14) with a high oxygen concentration gas mixture – 80% O_2 20% CO_2. The steaks were placed on PET/PE trays (parameters: 187 × 137 × 50 mm), and the film used was a 44 μm thick PET/CPP + AF laminate with maximum oxygen permeability not exceeding 10 cm^3/m^2/24 h/bar at 23 °C with a relative humidity of 0% (EC04, Corenso, Helsinki, Finland). Samples were packed with an M3 packaging machine (Sealpack, Oldenburg, Germany).
c) A combination of vacuum skin packaging and MAP packaging (VSP + MAP): the steaks were aged in vacuum for 14 days and subsequently repackaged into MAP and stored for 14 days.

To avoid microbial contamination, steak preparation and repackaging procedures from VSP into MAP were carried out in 4 °C. The cold store and knives were previously sterilized by means of appropriate disinfectants and were irradiated with a UV lamp for 12 h before further procedures. From the control and from each packaging system, one steak was used for WBSF and CL analysis and one steak was divided into three portions to perform physiochemical analysis. Irrespective of the packaging system, all the steaks were stored under refrigeration

(2 ± 1 °C) for the period assumed for the given packaging system. Control samples were steaks cut out from the muscles 24 h after slaughter, vacuum packaged and evaluated the next day. At the end of steak storage, samples of about 10 g were cut, repacked into vacuum bags, and kept at −80 °C until further protein analysis.

2.2. pH determination

Meat pH was measured by a penetration probe at three different locations per steak using a pH electrode (TESTO 205, Germany). The electrode was calibrated with buffers (pH 4.0 and 7.0) prior to measurement. Values were documented to ensure that steaks had normal pH levels and were suitable for packaging (*i.e.* pH < 5.8).

2.3. Instrumental color measurement in CIE L* a* b* system

Instrumental color measurement in CIE L* a* b* system of bovine meat stored in different packaging systems was performed using a Minolta CR-400 chromatometer (Konica Minolta, Inc., Tokyo, Japan), according to Wyrwisz, Półtorak, Poławska, et al. (2012), and the values of L*, a*, b* were recorded. The diameter of the measuring head was 8 mm. The device was calibrated on a white standard plate (L* = 98.45, a* = −0.10, b* = −0.13). Illuminant D65 (color temperature: 6500 K) and a standard observer (2°) were used. Ten measurements were conducted on each sample (steak), in every quarter and in the central part of the steak. Data was collected immediately after opening the packages and within a 30-minute blooming period under refrigerated conditions (2 ± 1 °C).

2.4. Warner-Bratzler shear force measurement

Instrumental measurement of Warner-Bratzler shear force (WBSF) was performed with a universal testing machine (Instron, 5965 model, MA, USA) with a Warner-Bratzler add-on device, according to Wyrwisz, Półtorak, Zalewska, Zaremba, & Wierzbicka (2012). Parts of the steaks (100 g ± 10 g) from each packaging system and storage period were cooked individually in closed PA/PE bags immersed in a water bath (Memmert, WNE 14, Germany) at 80 °C to achieve a final internal temperature of 71 °C, and were subsequently cooled down in cold water and stored overnight at 3 ± 1 °C. Six cylindrical samples (1.27 cm in diameter and 2.5 ± 0.2 cm in length) were cut using a V-shaped blade. Shear force direction was perpendicular to the orientation of muscle fibers. The test was carried out with the measuring head at a constant speed (500 N capacity) – 200 mm/min, at a standardized sample temperature of (2 ± 1 °C).

2.5. Cooking loss

The extent of cooking loss (CL) was determined through measurement of sample mass before (M_i) and after heat treatment (M_f), following cooling to ambient temperature. Heat treatment was performed as in section 2.4.

2.6. SDS-PAGE and Western Blotting analysis for protein degradation

Myofibrils extracted from the samples after different aging periods were prepared according to the procedure described by Fritz, Swartz, and Greaser (1989) with the modifications of Kołczak, Pospiech, Palka, and Łącki (2003). A sample (2.5 g) without visible fat and connective tissue was homogenized with an Ultra Turrax homogenizer (IKA T18