Meat Science 110 (2015) 1–8

Contents lists available at ScienceDirect

Meat Science

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

Dissolved carbon dioxide and oxygen concentrations in purge of vacuum-packaged pork chops and the relationship to shelf life and models for estimating microbial populations

K.R. Adams, S.E. Niebuhr, J.S. Dickson *

Department of Animal Science, Iowa State University, Ames, IA 50011, United States

ARTICLE INFO

Article history:
Received 29 July 2014
Received in revised form 5 March 2015
Accepted 4 June 2015
Available online 11 June 2015

Keywords:
Pork
Dissolved carbon dioxide
Dissolved oxygen
Shelf life
Spoilage

ABSTRACT

The objectives of this study were to determine the dissolved CO_2 and O_2 concentrations in the purge of vacuum-packaged pork chops over a 60 day storage period, and to elucidate the relationship of dissolved CO_2 and O_2 to the microbial populations and shelf life. As the populations of spoilage bacteria increased, the dissolved CO_2 increased and the dissolved O_2 decreased in the purge. Lactic acid bacteria dominated the spoilage microflora, followed by Enterobacteriaceae and *Brochothrix thermosphacta*. The surface pH decreased to 5.4 due to carbonic acid and lactic acid production before rising to 5.7 due to ammonia production. A mathematical model was developed which estimated microbial populations based on dissolved CO_2 concentrations. Scanning electron microscope images were also taken of the packaging film to observe the biofilm development. The SEM images revealed a two-layer biofilm on the packaging film that was the result of the tri-phase growth environment.

1. Introduction

Spoilage is a problem for fresh meat products because fresh meat is contaminated during slaughter and processing with bacteria from the feces, hide, and hooves of the animal (Ayres, 1955). Meat also has a favorable 5.4–6.4 pH, high water activity (0.99), and abundance of low molecular weight compounds, such as glucose and amino acids, for bacteria to utilize (Samelis, 2006). However, spoilage of fresh meat may be managed and extended by lower storage temperature, reduced initial bacterial populations, packaging conditions, and increased carbon dioxide (CO_2) concentrations (Gill, 1986). Lower storage temperature can slow bacterial growth by increasing the lag phase duration and generation time of the bacteria. The greatest increases in lag phase duration and generation time were observed at a storage temperature of −1.5 °C (Gill, 1986; Jeremiah, 1997), but lag phase duration and generation times increased at refrigeration temperatures (less than 4 °C). Freezing the meat would greatly extend the shelf life, but the meat could no longer be marketed as fresh meat.

Initial bacterial populations present at packaging can also influence the length of shelf life for a fresh meat product. For example, fewer than 2 colony forming units (CFU)/g present at packaging resulted in a 7 week shelf life of fresh pork (Holley, Pierson, Lam, & Tam, 2004). Packaging also affects the shelf life of fresh meat, especially if it involves altering the atmosphere by vacuum or modified atmosphere packaging. Overwrap packages have a high oxygen (O_2) content in their package so the shelf life is, on average, 5–7 days (Delmore, 2009; Jeremiah, 1997). The aerobic atmosphere preferentially selects for aerobic and facultatively anaerobic genera such as *Pseudomonas* spp., *Brochothrix thermosphacta*, and Enterobacteriaceae spp. (Doulgeraki, Ercolini, Villani, & Nychas, 2012; Gill, 1986). Modified-atmosphere packages (MAP) may be either high or low oxygen. High oxygen MAP typically have 80% O_2 and 20% CO_2 present and the meat has a similar spoilage microflora as aerobic overwrap packages, with the high oxygen content preferentially selecting for aerobic and facultatively anaerobic genera such as *Pseudomonas* spp., *B. thermosphacta*, and Enterobacteriaceae spp. (Borch, Kant-Muermans, & Blixt, 1996). A typical low oxygen MAP will not be more than 10% O_2, with the remaining balance composed of CO_2, and nitrogen (a typical MAP might be 10% O_2, 20% CO_2 and 70% N_2), and lactic acid bacteria (LAB), Enterobacteriaceae spp., *B. thermosphacta*, and *Pseudomonas* spp. dominate the spoilage microflora (Doulgeraki et al., 2012). Because of the differences in atmosphere and the composition of the spoilage microflora, meat packaged in high oxygen MAP has an average shelf life of 10–21 days, while low oxygen MAP packaged meat is 25–35 days (Delmore, 2009; Jeremiah, 1997). Vacuum-packaging is similar to low oxygen MAP, but it has an anaerobic environment that preferentially selects for facultatively anaerobic bacteria such as lactic acid bacteria, Enterobacteriaceae spp., *B. thermosphacta*, and a few *Pseudomonas* spp. in the beginning of the shelf life (Borch et al., 1996; Doulgeraki et al.,

* Corresponding author at: 2372 Kildee Hall, Iowa State University, Ames, IA 50011, United States.
E-mail address: jdickson@iastate.edu (J.S. Dickson).

http://dx.doi.org/10.1016/j.meatsci.2015.06.003
0309-1740/

2012). Vacuum-packaging, due to its anaerobic environment, can have a shelf life of 45–90 days (Delmore, 2009; Jeremiah, 1997).

The shelf life of vacuum-packaged meats is also affected by the CO_2 concentration present in the package, which is a metabolic by-product of microbial metabolism. Carbon dioxide is known to inhibit bacteria by affecting the cell membrane permeability, decarboxylating enzymes, and acidifying the intracellular pH (Dixon & Kell, 1989; Gill, 1986). Carbon dioxide can inhibit bacteria because it is soluble in water at refrigeration temperatures, forming dissolved CO_2, and carbonic acid (Dixon & Kell, 1989; Gill, 1986). Carbon dioxide is also soluble in the fresh meat tissue at a rate of 960 mL of CO_2/kg of fresh meat at 1 atm, 0 °C, and pH 5.5 (Gill, 1988). This rate is similar for pork, beef, and lamb and it can be affected by pH and storage temperature (Gill, 1988). CO_2 will dissolve into the fresh meat product, forming carbonic acid and inhibiting the spoilage bacteria by acidifying the intracellular pH and affecting cell membrane permeability.

CO_2 inhibits different types of bacteria at different rates. LAB have the highest resistance to CO_2 because they produce CO_2 as a by-product of cellular respiration, whereas *Pseudomonas* has the least resistance (Dixon & Kell, 1989). *B. thermosphacta* and Enterobacteriaceae have intermediate resistance to CO_2 (Dixon & Kell, 1989; Nowak, Rygala, Oltuszak-Walczak, & Walczak, 2012).

The standard method of determining microbial populations in packaged meat is a method is time-consuming, destructive, and expensive to conduct (Bruckner, Albrecht, Petersen, & Kreyenschmidt, 2013; McDonald & Sun, 1999; McMeekin & Ross, 1996). A total mesophilic aerobic bacterial enumeration requires three days to complete, and delivers historical data on the microbial population which was in the product 72 h earlier. Because of this, there is interest in developing a method of estimating microbial populations based upon an more rapid instrument measurement (Bruckner et al., 2013; McDonald & Sun, 1999; McMeekin & Ross, 1996). This instrument measurement would focus on a by-product of microbial metabolism (Hammes & Hertel, 2006). Potentially, a microbial metabolic byproduct could estimate microbial populations and be used to estimate shelf life of packaged meats. There are very few studies conducted which determine the interaction of microbial metabolic byproducts and microbial populations using a meat system and in the context of shelf life. However, previous work by Devlieghere and Debevere (2000) and Devlieghere, Debevere, and Impe (1998) used Brain–Heart-Infusion media, or a similar broth system, to determine how dissolved CO_2 affected certain types of spoilage bacteria.

The objectives of this study were to determine the dissolved CO_2 and O_2 concentrations in the purge of vacuum-packaged pork chops during storage, and to determine the relationship between dissolved CO_2 and O_2 concentrations to the microbial populations and shelf life. The hypothesis was that dissolved CO_2 concentrations will increase and dissolved O_2 concentrations will decrease inside the vacuum-package, and that the concentrations of the dissolved gases could be used to estimate microbial populations. In addition, scanning electron microscopic images were taken to document the development of the microflora over time in the packaged meat.

2. Materials and methods

2.1. Meat sample preparation

Sixty bone-in thick cut pork *Longissimus thoracis et lumborum* (LTL) chops were purchased from a retail storage the day of initial packaging, with a post-mortem age of 3–14 days. The bones were removed at the laboratory with a flame-sterilized knife before being placed into a vacuum-package (B470T, Cryovac Sealed Air Corporation, Duncan, SC); oxygen transmission rate of 3–6 cm^3/m^2, 24 h, 1 atm, at 4.4 °C and 0% relative humidity, and a water vapor transmission rate of 0.5–0.6 g at 37.7 °C (100% relative humidity, 100 in^2, 24 h). Three pork chops were placed into each vacuum-package. Before sealing, a pair of empty, autoclaved 3 × 22 mm flat-bottom glass test tubes, with a 3 × 6 mm flea magnetic stir bar in each tube, were placed into an autoclaved 0.95 × 2.54 × 10.16 cm Teflon stand (Fig. 1). These glass tube and Teflon stands were placed aseptically into the vacuum-package with the meat. The tubes were placed upright in the package approximately 7.6 cm away from the pork chops. The vacuum-packages were then vacuum-sealed (975–980 mm Hg vacuum) with a Multivac C350 (Kansas City, MO). After sealing, the vacuum-packages were stored in a 4 °C cooler for up to 60 days. Over time, the exudate from the meat collected in the sterile tubes in the package.

2.2. Dissolved oxygen and carbon dioxide concentration measurements

Two vacuum-packages were randomly selected on days 0, 5, 15, 30, 45, and 60 for dissolved gas concentration measurements. The vacuum-packages were placed into a biosafety cabinet and allowed to temper to 20-22 °C for 10 min. After 10 min, the packages were placed onto a stir plate (Dataplate Digital Hotplate/Stirrer Series 730, Thermolyne Corporation, Dubuque, IA), set to an stirring rate of 300 rpm and allowed to mix for 1 min. The outside of the package near the glass tubes was wiped with ethanol and allowed to evaporate before sterilized scissors were used to cut a cross aseptically into the top of one glass tube. After opening the package, a probe designed to measure dissolved oxygen in a liquid system (Hach LDO101 probe; Hach HQ30d meter, Ames, IA) was slipped inside one of the glass tubes until halfway submerged. The dissolved oxygen concentration was measured four times before the measurements were averaged for an average dissolved oxygen concentration. The dissolved oxygen probe was calibrated before use according to the manufacturer's directions for a 100% oxygen concentration.

Dissolved carbon dioxide was measured using an electrode designed to determine dissolved CO2 in liquid systems (Thermo Scientific Orion

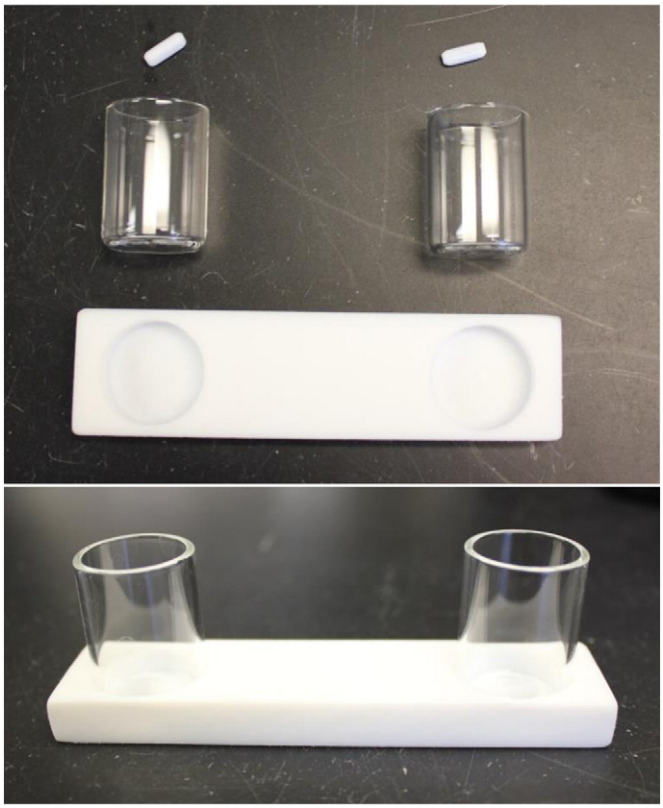

Fig. 1. Purge Collection Tubes. Two 3 × 22 mm flat-bottom glass test tubes, with 3 × 6 mm flea magnetic stir bar in each tube, seated in a 0.95 × 2.54 × 10.16 cm Teflon stand.