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Headspace oxygen as a hurdle to improve the safety of in-pack pasteurized chilled food during storage at different temperatures

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ABSTRACT

This study investigated the use of headspace oxygen in a model food system to prevent the growth of anaerobic pathogenic bacteria in in-pack pasteurized food at various storage temperatures. Three model food formulations prepared with tryptic soy broth and three agar concentrations (0.1, 0.4 and 1.0%) were sealed without removing the air from the package in high oxygen barrier pouches (OTR = 0.3 cm^3/m^2·day·atm). Important properties influencing bacterial growth, including pH and water activity (a_w) were determined. The oxygen sorption kinetics of each model food were obtained at three different storage temperatures (8, 12, and 20 °C) using an OxySense Gen III 300 system. An analytical solution of Fick's second law was used to determine the O_2 diffusion coefficient. Growth challenge studies at 12 and 20 °C were conducted at three selected locations (top, center and bottom layers) in model foods containing 1% agar. Model foods were inoculated with *Clostridium sporogenes* PA 3679 (300 spores/mL), and were classified as low-acid (pH > 4.5, a_w > 0.85). When the storage temperature decreased from 20 to 8 °C, the oxygen diffusion decreased from 0.82×10^{-9} m^2/s to 0.68×10^{-9} m^2/s. As the agar concentration was increased from 0.1 to 1.0%, the effective oxygen permeability decreased significantly ($p = 0.007$) from 0.88×10^{-9} m^2/s to 0.65×10^{-9} m^2/s. When the inoculated model foods were stored at 12 °C for 14 days, *C. sporogenes* PA 3679 was unable to grow. As the storage temperature was increased to 20 °C, significant bacterial growth was observed with storage time ($p < 0.0001$), and the *C. sporogenes* PA 3679 population increased by around 6 log CFU/g. However, the location of the food did not influence the growth distribution of *C. sporogenes* PA 3679. These results demonstrate that oxygen diffusion from the pouch headspace was primarily limited to the food surface. Findings suggest that the air/oxygen present in the package headspace may not be considered as a food safety hurdle in the production of pasteurized packaged food.

1. Introduction

Consumers today prefer food that requires minimal preparation time compared to conventional meals. They prefer high quality foods that are nutritious, low levels in preservatives, and minimally processed (Peck and Stringer, 2005; Rajkovic et al., 2010). Consumer preference has led to the development of in-package pasteurized foods. These foods are also known as refrigerated processed foods of extended durability (REPFEDs), cook-chill, ready-to-eat, and sous-vide foods (Choma et al., 2000; Daelman et al., 2013; Peck and Stringer, 2005). These types of products are gaining popularity due to the aforementioned consumer preferences (Brunner et al., 2010; Rodgers et al., 2003). For example, the total UK prepared chilled food market increased by 33% from November 2008 to January 2016 (Kantar WorldPanel, 2016).

REPFEDs are a heterogeneous group of food products typified by a variety of ingredients, processing conditions and packaging systems used in their production process. Based on production conditions, REPFED products can be categorized into three groups (Daelman et al., 2013):

1. Products pasteurized in-pack at 90 °C for at least 10 min or equivalent to achieve a 6D reduction of non-proteolytic psychotropic *Clostridium botulinum* spores.
2. Products pasteurized in-package at 70 °C for at least 2 min or equivalent to achieve a 6D reduction of *Listeria monocytogenes*.
3. Products pasteurized out of pack and then packed. These products are not defined by a specific P_{value}, and either the P_{90} or P_{70} pasteurization treatments are possible.

For REPFED products, there are a few microbiological safety

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concerns (Juneja and Snyder, 2008). First, these products are generally formulated with little or no preservatives and have low acid and high moisture content. Second, they undergo minimal thermal processing, are not commercially sterile, and must be refrigerated. Third, vacuum packaging provides a favorable environment for anaerobic and facultative pathogens such as *Clostridium botulinum* and *Bacillus cereus* to grow and produce toxins. Finally, there is a high probability of temperature abuse during distribution and storage.

The storage temperature of chilled foods may vary greatly during manufacturing, distribution, retail, and in-home storage. According to Bruckner et al. (2012), temperatures in trucks during poultry and milk distribution range from −3 °C to 15 °C and 3.6 °C to 10.9 °C, respectively. Additional temperature abuse may occur during retail display. Temperatures above 7 °C are often common in refrigerated display cabinets of convenience stores (Dodds, 1995; Koutsoumanis and Gougouli, 2015; Rybka-Rodgers, 2001; Walker, 1992). Before the stores and homes, there is little or no temperature control after products are purchased. Tamagnini et al. (2008) mentioned that Marklinder et al. (2004) found that 5–20% of foods in general were stored at temperatures above 10 °C in home refrigerators, with maximum temperatures from 11 °C to 18 °C. Koutsoumanis and Gougouli (2015) combined the results of nine surveys conducted in the UK, France, Ireland and Greece, finding that out of over 1000 consumer refrigerators, 64.1% were operating above 5 °C. Therefore, chilled foods may undergo temperature abuse conditions in the cold chain, and low-acid cook-chill foods are unprotected under these circumstances. Since we cannot rely exclusively on the maintenance of refrigerated conditions to assure safety, it is essential to address these challenges.

Non-proteolytic *C. botulinum* and *B. cereus* are spore-forming bacteria with the lowest minimum growth temperatures at 3.3 °C and 4 °C, respectively (ECFF, 2006). Based on this, as along with the ability of non-proteolytic *C. botulinum* and *B. cereus* spores to germinate and produce toxins, the recommended storage and distribution temperatures for cook-chill foods is under 5 °C (ECFF, 2006). If food products are subjected to temperatures above 10 °C for a prolonged time during the cold chain, proteolytic *C. botulinum* and *Clostridium perfringens* are also of concern. In addition, the absence of a competitive microbiota can increase proliferation of these pathogens.

However, the use of oxygen provides an alternative for in-package pasteurized food. Bacteria vary widely in their ability to use and tolerate oxygen (Prescott et al., 2002). *Clostridium botulinum* is an anaerobic bacteria that does not use oxygen for growth and eventually dies in the presence of oxygen (Prescott et al., 2002). Anaerobic bacteria do not have the elaborate system of defenses that aerobic bacteria have, since the system relies on a series of special enzymes in large quantities. These include super dismutase, catalase, and peroxidase, which can scavenge toxic compounds that form in an oxygen-rich atmosphere (Jasso-Chávez et al., 2015; Johnson, 2009). Anaerobic bacteria produce these enzymes in very small amounts, or not at all. Thus, the variability in oxygen tolerance of obligate anaerobes may be influenced by the amount of those enzymes that they can produce.

In order for oxygen to suppress the growth of *C. botulinum* in food, it should be able to dissolve in the food surface and diffuse throughout the product. In this study, we addressed these challenges by assessing the use of oxygen to improve the food safety design of cook-chill foods during temperature abuse. Oxygen diffusion in food model/packaging systems was observed at 8, 12 and 20 °C. Three food models with different matrices were compared. The growth of *Clostridium sporogenes* PA 3679 as a surrogate of *Clostridium botulinum* was monitored within the food (top, center and bottom layers).

2. Materials and methods

2.1. Food model preparation and properties measurements

Tryptic soy broth (TSB) culture medium was used as a model food.

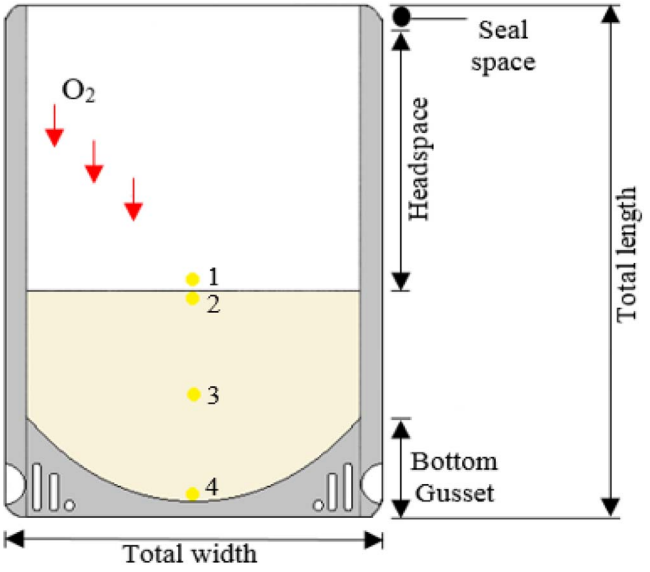

Fig. 1. Pouch dimension and set up. Pouch dimension: W × L × BG (13 cm × 18.5 cm × 3.5 cm). Oxydot 1 is located just above the food model surface, Oxydot 2 is located just below the food model surface, Oxydot 3 is located at the center of the food model column, Oxydot 4 is located at the food bottom layer. Distance between oxydots 2–3 and 3–4 is 3 cm. Sealed space was approximately 0.8 cm. Total height of food column is 7.5 cm.

The medium was prepared according to the manufacturer's instructions (Bacto, BD™) and was supplemented with different agar concentrations (0.1, 0.4, and 1.0% w/v). The resulting model foods were in liquid, semisolid and solid at temperatures below 45 °C. pH and water activity (a_w) were determined with a potentiometer (Mettler Toledo, EL 20) and a vapor sorption analyzer (Decagon Devices, Inc. VSA1042), respectively.

2.2. Food/packaging system set up

For oxygen sorption kinetics, light-sensitive oxygen sensors (OxyDot) were adhered at selected locations in the pouches to monitor O_2 concentration at the headspace, as well as within the food during storage time (Fig. 1). The adhesive used to glue the oxydots was RTV108-12C (Momentive, MIL-A-46106B Compliant) high performance silicone sealant. A small amount was applied to the inside of the pouch at selected locations. Using a vacuum pen, the oxydots were picked up with the coating side in contact with the pen, and then gently pressed to the adhesive inside the pouches. The adhesive was allowed to dry completely for 8 h, and then the model foods were dispensed for further analysis.

One batch of each formulation was dispensed in triplicate in 250 mL volumes in 13 cm × 18.5 cm × 3.5 cm stand up multilayered plastic pouches (Kurarister™ CF, Kuraray Company of America, Inc.). Next, the open edge of the pouches was sealed without a vacuum, using a manual impulse heat sealer (Hang bag sealer MP-12, Midwest Pacific®, Rocky Mount, MO, USA). This provided a water-tight and air-tight seal for an effective subsequent sterilization procedure by means of retorting. This thermal process allowed for preparation of the model foods without background microbiota, which could disturb the measurement of oxygen concentration in the different food/packaging systems. The oxygen transmission rate (OTR) of the film used in the pouch was 0.3 cm^3/m^2·day·atm. The pouches had a laminated structure with three layers of polyethylene terephthalate (12 μm thickness), biaxial oriented polyamide (15 μm) and cast polypropylene (50 μm) films.

2.3. Storage conditions and oxygen measurements

After sterilization, the pouches were stored at 8, 12, and