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Probiotic supplementation and fast freezing to improve quality attributes and oxidation stability of frozen chicken breast muscle

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ABSTRACT

The study was conducted to determine the effects of probiotic supplementation and fast freezing on quality attributes and oxidation stability of frozen/thawed chicken breast muscle. Broilers (n = 168) were fed with a basal diet or the basal diet plus 250 mg of Sporulin (three strains of *Bacillus subtilis*)/kg of basal diet for 45 days. Pairs of breasts were separated from chicken carcasses and randomly assigned to either slow (−30 °C) or fast freezing (−70 °C) and stored at −30 °C. After thawing at 2 °C for 24 h, half of the thawed breasts from each treatment were stored for an additional 48 h. Probiotic supplementation increased phospholipid content in breasts ($P < 0.05$), but substantially delayed both primary and secondary lipid oxidation products shown by the results from peroxide value and 2-thiobarbituric acid reactive substances ($P < 0.05$). Further, the breasts from probiotic chicken group had a significantly lower nonheme iron content than those from control chicken group. Fast freezing significantly reduced thaw/purge loss and minimized lipid oxidation ($P < 0.05$). Our results suggest that oxidative deterioration and increased purge/thaw loss of frozen chicken breasts can be effectively minimized by probiotic supplementation coupled with fast freezing.

1. Introduction

Freezing is one of the most common preservation methods extending shelf-life of chicken meat products (Ali et al., 2015; Grunert, Stephan, Ehling-Schulz, & Jolher, 2016; Soyer, Özalp, Dalmış, & Bilgin, 2010). While freezing effectively suppresses microbial growth and prevents chemical changes during storage, some defects of meat quality, such as discoloration, inferior water-holding capacity (WHC), and oxidation of lipid and protein, are well-identified challenges associated with freezing/thawing procedures (Ali et al., 2015; Soyer et al., 2010).

In general, meat quality changes in frozen/thawed meat occur during freezing and thawing processes (Li & Sun, 2002). The formation of ice crystals during freezing causes physical and structural damages to muscle tissues and/or cells, in turn, leading to various chemical reactions affecting quality attributes of frozen/thawed meat (Leygonie, Britz, & Hoffman, 2012). In particular, lipid oxidation, which is one of major quality deteriorations in frozen meat products, is attributed to the release of pro-oxidants from muscle cells by ice crystal damage (Leygonie et al., 2012; Soyer et al., 2010). Freezing rates affect the size, location, and distribution of ice crystals, and consequently impact the extent of damage to muscle tissues and/or cells. Thus, it has been suggested that fast freezing could improve color characteristics, WHC (thaw and/or purge loss), and tenderness of frozen/thawed meat products by allowing the formation of small and uniform intracellular ice crystals (Mortensen, Andersen, Engelsen, & Bertram, 2006). However, the effect of fast freezing on lipid oxidation stability of frozen/thawed meat has not been fully studied.

In the poultry industry, the supplementation of microbial dietary probiotics has been recently considered as an alternative way to substitute antibiotics, thereby improving chicken's intestinal homeostasis balance. This could contribute to positive impacts on chickens' feed intake and digestion, immune response, and resistance to disease (Kabir, 2009; Zhang, Zhou, Ao, & Kim, 2012; Zhou,

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Wang, Gu, & Li, 2010). Along with such advantages on chicken growth performance and health, the probiotic supplementation in feed or drinking water could improve the quality attributes of chicken meat, such as WHC, tenderness, oxidation stability, sensory properties and/or microbial growth (Ali, 2010; Liu et al., 2012; Pelicano et al., 2005, 2003; Zhang et al., 2012; Zhou et al., 2010). Thus, it would be reasonable to postulate that probiotic supplementation can reduce or prevent the quality decline of frozen/thawed chicken meat products originated from the freezing/thawing processes. While frozen chicken is a very common type of meat product in the retail market, there is limited information regarding the effects of probiotic supplementation on oxidation stability and meat quality attributes of frozen/thawed chicken meat.

Taken together, we hypothesized that probiotic supplementation coupled with fast freezing will bring synergistic impacts on preventing the quality deterioration of frozen/thawed chicken meat by improving WHC and lipid oxidation stability. Therefore, the objective of this study was to determine the effects of probiotic supplementation (*Bacillus subtilis*) and fast freezing on quality attributes and oxidation stability of frozen/thawed chicken breasts during 0 and 2 days of refrigerated storage after thawing.

2. Materials and methods

2.1. Animal management and sample collection

One hundred and sixty-eight 1-day-old chicks (Ross 708 male broiler) were obtained from a commercial hatchery (Miller Poultry, Orland, IN), which were group-weighed and randomly placed in 12 pens at the Poultry Research Facility of Purdue University, based on their average body weight. The animal management procedures were approved by the Purdue Animal Use and Care Committee (PACUC Number: 1111000262). The pens were randomly assigned to two diet groups for 45 days as follows; basal diet for control group and the basal diet plus 250 mg of Sporulin (1.0×10^6 cfu/g feed, containing three strains of *Bacillus subtilis*)/kg of basal diet for probiotic group. The information on the commercial probiotic product (Sporulin) was provided by the manufacturer (Pacific Vet Group-USA, Inc., Fayettville, AR, USA), and the feeding level followed the recommendations of the manufacturer. The formulation of basal diet was based on the recommendations for nutrients by the NRC (1994). The environment and feeding regimes was followed by the conditions described by Kim, Yan, Hu, Cheng, and Kim (2016). On day 45, two birds were randomly taken from each pen, commercially harvested, and air-chilled in a 2 °C carcass room for 24 h. Both sides of breast muscles (*M. Pectoralis minor*) were removed from each chicken carcass at 1 day postmortem, weighed, and individually vacuum-packaged in a polyamide/polyethylene bag.

2.2. Freezing and thawing procedure

Pairs of breast muscles were randomly assigned into two different freezing rates (slow and fast freezing), respectively. Slow freezing was performed in a −30 °C conventional air freezer, whereas fast freezing was conducted in a liquid nitrogen chamber, where ambient temperature maintained at −70 °C (12 CF cabinet freezer, RS Cryo Equipment Inc., USA). A decline in internal core temperature of the breasts was monitored until reached at - 20 °C by using a digital temperature logger (OctTemp2000, MadgeTech, Inc., Warner, NH) with a thermocouple (T-type, Omega Engineering, Stamford, CT). After the freezing process, all frozen chicken breasts were immediately placed in the −30 °C conventional freezer and stored for 12 months. The frozen chicken breasts were thawed in a 2 °C cooler for 24 h (0 day of refrigerated storage after thawing), to determine extended storage effect on quality attributes of frozen/thawed breasts, and half of frozen/thawed samples from each treatment were continuously placed in the same cooler for additional 48 h (2 days of refrigerated storage after thawing). The frozen/thawed chicken breasts were blotted with a paper towel, weighed, and used for further analyses.

2.3. Analyses of frozen/thawed chicken breasts

2.3.1. pH

Three grams samples of frozen/thawed chicken breasts were taken from the same location and homogenized with 27 ml of distilled water (DW) using a homogenizer (Ultra-Turrax T25, Janke & Kunkel IKA-Labortechnik, Staufen, Germany) at 6000 rpm for 30 s. The pH value of frozen/thawed chicken breasts was determined in triplicate using an electronic pH meter (Sartorius Basic Meter PB-11, Sartorius AG, Germany).

2.3.2. Thaw/purge loss

Thaw/purge loss (%) of frozen/thawed chicken breasts was determined by calculating the differences in the weight before freezing and after thawing.

2.3.3. Centrifugal WHC determination

WHC of frozen/thawed chicken breasts was determined in duplicate according to the centrifugal method described by Kim et al. (2016). The WHC (%) was estimated by calculating the percentage breast weight before and after centrifugation; WHC (%) = (sample weight after centrifugation (g)/sample weight before centrifugation (g)) × 100.

2.3.4. Instrumental color measurement

Surface color (five random locations on bone side) of frozen/thawed chicken breasts was determined using a Hunter MiniScan EZ colorimeter (Hunter, Reston, VA, USA) equipped with a 25 mm (diameter) measuring. The setting for the illuminant was D65 source and the observer was standard 10°. Calibration of the instrument was conducted using black and white calibration tiles, according to the standard manual. CIE L*, a*, and b* values were recorded. Hue angle values were calculated using the following equation; hue angle = $\tan^{-1}(b^*/a^*)$ (AMSA, 2012).

2.3.5. Lipid extraction and content

To determine lipid content, peroxide value, and phospholipid content, lipid fraction was extracted from frozen/thawed chicken breasts using a chloroform/methanol solvent system (2:1, ml:ml) (Lee, Trevino, & Chaiyawat, 1996). The lipid content per sample was determined in triplicate according to the procedure described by Soyer et al. (2010). Five grams of chicken breast was homogenized with 50 ml of the chloroform/methanol solvent in a Waring blender. The mixture was filtered through filter paper (Whatman No. 1), and the filtrate was divided into two layers using 20 ml of NaCl solution (0.5 g/100 ml). The chloroform phase was taken, and chloroform was evaporated on a hot plate. Lipid content was calculated as follows; lipid content (%) = [(amount of extracted lipid (g)/sample weight (g)) × (volume of chloroform (ml)/10 ml) × 100].

2.3.6. Peroxide value (POV)

POV of frozen/thawed chicken breasts was determined in duplicate following the method of the International Dairy Federation (IDF) described by Soyer et al. (2010). The extracted lipid fraction (150 mg) was placed in a disposable glass tube and mixed with 9.8 ml of the chloroform/methanol solvent mixture. After vortex-mixing for 5 s, 50 μl of ammonium thiocyanate solution