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.

Keywords: *Bacillus subtilis*, chicken breast, fast freezing, lipid oxidation, probiotics

1. Introduction

Freezing is one of the most common preservation methods extending shelf-life of chicken meat products (Ali, Zhang, Rajput, Khan, Li, & Zhou, 2015; Grunert, Stephan, Ehling-Schulz, & Johler, 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, 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., 2003,2005; Zhou et al., 2010; Zhang et al., 2012). 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-weighted 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., Fayetteville, 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 et al. (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 6,000 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; $\text{WHC (\%)} = (\text{sample weight after centrifugation (g)} / \text{sample weight before centrifugation (g)}) \times 100$.

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; $\text{hue angle} = \tan^{-1}(b^*/a^*)$ (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 (30 g/100 ml) was added to the glass tube and vortex-mixed again for 5 s. Then, 50 µl of iron (II) solution (Shantha & Decker, 1994) was immediately added, vortex-mixed for 5 s, and incubated at room temperature for 5 min. The absorbance of the mixture was read at 500 nm using a spectrophotometer (VWR International, OR, USA), and POV was expressed as milliequivalents of peroxide per kg fat (meq O₂/kg fat).

2.3.7. Phospholipid content

The phospholipid content of frozen/thawed chicken breasts was determined in duplicate according to the method of Stewart (1980) described by Eymard, Carcouët, Rochet, Dumay, Chopin, and Genot (2005). The extracted lipid fraction was diluted to 0.25 mg/ml using chloroform and placed in a 15 ml centrifuge tube. One milliliter of thiocyanate reagent was added to the tube and vortex-mixed for 30 s. The mixture was

centrifuged at $750 \times g$ for 10 min, and the absorbance of the supernatant (from lower phase in the tube) was read at 488 nm. Standard curve was prepared with phosphatidylcholine/chloroform solution (5-50 $\mu\text{g/ml}$). The phospholipid content was expressed as g phosphatidylcholine equivalents per kg fat (g phosphatidylcholine eq/kg fat).

2.3.8. 2-Thiobarbituric acid reactive substances (TBARS)

The TBARS value of frozen/thawed chicken breasts was determined in duplicate according to the method of Buege and Aust (1978) described by Kim et al. (2016). Five grams of sample was homogenized with 15 ml DW and 50 μl of butylated hydroxyl anisole solution (10 g/100 ml) in ethanolic solution (90 ml/100 ml) using the Ultra Turrax at 13,000 rpm for 30 s. One milliliter of the homogenate was mixed with 2 ml of 20 mM 2-thiobarbituric acid (TBA) solution in trichloroacetic acid (TCA) solution (15 g/100 ml). The mixture was heated for 15 min in a water bath (80 °C), cooled for 10 min in ice, and centrifuged at $2,000 \times g$ for 10 min, then filtered through filter paper (Whatman No. 4). The supernatant was placed on 96-well plate; and the absorbance was measured at 538 nm using a microplate spectrophotometer (Epoch, Biotek Instruments Inc., VT, USA). A blank was prepared with 2 ml distilled water and 4 ml TBA/TCA solution. The TBARS was expressed as mg MDA per kg meat (mg MDA/kg meat).

2.3.9. Nonheme iron content

Nonheme iron content of frozen/thawed chicken breasts was determined in duplicate by following the ferrozine method (Ahn, Wolfe, & Sim, 1993). The nonheme iron content was expressed as mg nonheme iron per 100 g meat (mg/100 g).

2.4. Statistical analysis

The experimental design of this study was split-split-plot design, where the whole-plot was for each chicken carcass from two diet treatments (control and probiotic groups), the sub-plot was for each side of chicken breast meat assigned to two freezing rates (slow and fast freezing), and the sub-sub-plot was for the frozen chicken breasts randomly assigned for two different storage periods after thawing (0 and 2 days). All data were analyzed using the PROC MIXED procedure of SAS 9.4 software (SAS, 2012). Least squares means for all traits were separated (F test, $P < 0.05$) by using least significant differences generated by the PDIFF option.

3. Results and discussion

3.1. Temperature decline

The temperature decline of chicken breasts assigned to either slow or fast freezing is presented in Fig. 1. Freezing rate plays a critical role in forming ice crystals, which is primarily responsible for structural damages to muscle tissues and/or cells. In general, the pattern of ice crystal formation (e.g. size, location, and shape) is determined by passing time through initial freezing point (-1.5 to -7 °C), where nearly 80% of the water in meat is frozen (Kim, Liesse, Kemp, & Balan, 2015). According to Añón and Calvelo (1980), fast freezing (defined as passing the initial freezing point within 10 min) leads to the formation of intracellular ice crystals in muscle fibers. In

this current study, the time to pass the initial freezing point of chicken breasts assigned to fast and slow freezing rates was approximately 3.5 min and 527 min (8.8 h), respectively. Further, to reach the final targeted freezing temperature (-18 °C) from -1.5 °C, chicken breasts assigned to fast freezing took 7.5 min, whereas those assigned to slow freezing took 1,033 min (17.2 h). Therefore, the chicken breast muscles assigned to the fast freezing would likely have much smaller and uniform intracellular ice crystals compared to the chicken breasts assigned to slow freezing counterparts.

3.2. pH and WHC

The effects of probiotic supplementation and fast freezing on pH and WHC (thaw/purge loss and centrifugal WHC) of frozen/thawed chicken breasts at 0 and 2 days after thawing are shown in Table 1. The pH of frozen/thawed chicken breasts was affected by probiotic supplementation, storage after thawing, and their interaction ($P < 0.05$). The pH of breasts from probiotic chicken group (5.91) was significantly lower than those from control chicken group (6.02). Similarly, Ali (2010) reported that breasts (5.76) from *Bacillus subtilis* fed broilers had a significantly lower pH than those from broilers without probiotic feeding (6.00). Refrigerated storage after thawing decreased the pH of frozen/thawed chicken breasts. In particular, a significant decrease in pH was found at breasts from the control group. This result might be due to the release of hydrogen ions from denatured proteins during extended storage after thawing (Leygonie et al., 2012). As a result, the pH value of all frozen/thawed chicken breasts was similar after 2 days of refrigerated storage after thawing ($P > 0.05$), regardless of probiotic supplementation and freezing rates.

The probiotic supplementation had no influence on thaw/purge loss and centrifugal WHC of frozen/thawed chicken breasts ($P > 0.05$). Previous studies reported no impact of *Bacillus subtilis* feeding on WHC of chicken muscles (Kim et al., 2016; Pelicano et al., 2003,2005; Zhang et al., 2012), which was in agreement with our findings. However, fast freezing (3.78%) resulted in a significantly lower thaw/purge loss of chicken breasts compared to slow freezing (8.31%). Furthermore, the extended storage after thawing induced additional thaw/purge loss of frozen/thawed chicken breasts, from 4.44% on 0 day to 7.81% on 2 days ($P < 0.05$). In particular, a substantial increase in purge/thaw loss during storage after thawing was observed for chicken breasts assigned to slow freezing. Conversely, fast freezing resulted in a lower centrifugal WHC of frozen/thawed chicken breasts as compared to slow freezing ($P < 0.05$). This could be attributed to the fact that chicken breasts assigned to fast freezing would have more free water, which was in turn released by centrifugal force. For a similar reason, centrifugal WHC of frozen/thawed chicken breasts significantly increased after 2 days of storage, although the considerable amount of thaw/purge loss already occurred at thawing process. Consequently, the results of the current study confirm that fast freezing reduces water loss of frozen chicken meat during thawing process. Furthermore, it also indicates that slow freezing results in not only excessive water loss upon thawing, but also continued purge fluid during refrigerated storage after thawing.

3.3. Color characteristics

The effects of probiotic supplementation and fast freezing on color characteristics of frozen/thawed chicken breasts on 0 and 2 days after thawing are shown in Table 2.

No interactions between three main effects on CIE L* (lightness), a* (redness), b* (yellowness) and hue angle (discoloration) were found ($P > 0.05$). There was no significant difference in yellowness of all frozen/thawed chicken breasts. Probiotic supplementation slightly decreased redness and increased hue angle of frozen/thawed chicken breasts ($P < 0.05$). However, the differences between chicken breasts was numerically small and thus practically less meaningful. Similarly, previous studies have reported that *Bacillus subtilis* feeding had no influence on color characteristics of chicken breasts (Kim et al., 2016; Pelicano et al., 2003, 2005; Park & Kim, 2014; Zhang et al., 2012). The extended storage after thawing slightly increased the lightness of frozen/thawed chicken breast muscle ($P < 0.05$). The change in lightness of meat during storage could be affected by water migration and/or distribution through muscle structure alteration (Joo, Kauffman, Kim, & Park, 1999; Mortensen et al., 2006). According to Qiao, Fletcher, Smith, and Northcutt (2001), lightness of chicken breast muscle had a negative correlation with WHC ($r = -0.905$, $P = 0.0008$). Considering the increased thaw/purge loss during refrigerated storage after thawing (Table 1), water migration from intramuscular space to outside could affect light reflectance of surface on frozen/thawed chicken breast muscles, resulting in the increased lightness (Farouk, Wieliczko, & Merts, 2004).

3.4. Lipid content

Lipid contents of breast muscles from control and probiotic chicken groups were 3.42 and 2.95 g/100 g, respectively ($P > 0.05$; Table 3). According to Santoso, Tanaka, and Ohtani (1995), *Bacillus subtilis* supplementation affected abdominal fat accumulation rather than intramuscular fat content, which could support our finding.

The lipid content of frozen/thawed chicken breasts was unaffected by fast freezing rate, storage period and their interaction as expected ($P > 0.05$).

3.5. Lipid oxidation

The effects of probiotic supplementation and fast freezing on lipid oxidation (POV and TBARS) of frozen/thawed chicken breasts on 0 and 2 days after thawing are presented in Fig. 2 and 3, respectively. The breast muscles from probiotic chicken group (2.30 meq peroxides/kg fat) had a significantly lower POV than those from control chicken group (2.75 meq peroxides/kg fat) (Fig. 2a). In addition, POV of chicken breasts assigned to slow freezing rate decreased after 2 days of storage ($P < 0.05$; Fig. 2b), whereas POV of chicken breasts assigned to fast freezing rate remained unchanged ($P > 0.05$).

In terms of TBARS value of frozen/thawed chicken breasts, a three-way interaction among three main effects was found ($P < 0.05$; Fig. 3). The breasts from control chicken group assigned to slow freezing rate exhibited the highest TBARS value ($P < 0.05$), regardless storage time after thawing. Immediately after thawing (0 day), the breasts from probiotic chicken group showed a significantly lower TBARS value than those from control chicken group. On 2 days after thawing, however, a significant increase in TBARS value was observed for breasts from probiotic chicken group assigned to slow freezing rate. This results could be explained by a decrease in primary lipid oxidation products, which was determined by POV (Fig. 2a). Further, breast muscles from probiotic chicken group assigned to fast freezing rate showed a consistently lower TBARS value compared to the other frozen/thawed chicken breasts ($P < 0.05$). Taken together, our findings indicate that probiotic supplementation could

substantially prevent the formation of primary and secondary oxidation products. Moreover, fast freezing could delay the accumulation of secondary oxidation products upon thawing and/or following refrigerated storage after thawing.

In general, remaining unfrozen water in frozen meat products can possibly induce primary lipid oxidation during frozen storage, and subsequently, secondary lipid oxidation occurs during thawing (Leygonie et al., 2012). For this reason, numerous studies mostly focused on the effect of frozen storage temperature and/or duration on lipid oxidation of frozen chicken meat (Botsoglou, Fletouris, Florou-Paneri, Christaki, & Spais, 2003; Grau, Guardiola, Grimpà, Barroeta, & Codony, 2001; Selani, Contreras-Castillo, Shirahigue, Gallo, Plata-Oviedo, & Montes-Villanueva, 2011; Soyer et al., 2010). In our current study, the results indicate that freezing rate could affect the extent of lipid oxidation of frozen/thawed chicken meat. This observation was likely attributed to the fact that fast freezing could minimize the cryo-damages to muscle cells through the formation of intracellular ice crystals. This could inhibit the release of pro-oxidants from muscle cells, such as mitochondrial and lysosomal enzymes and heme (Leygonie et al., 2012). Conversely, Tomás and Añón (1990) reported that fast freezing had no impact on lipid oxidation of frozen/thawed chicken breast muscle, but the precise information of freezing rate was not provided in the literature. To confirm the given positive effect of fast freezing on lipid oxidation of frozen/thawed meat, further studies determining the release of pro-oxidants components from muscle cells under different freezing rates would be warranted.

Previously, we reported no impact of *Bacillus subtilis* feeding on lipid oxidation of chicken breasts during 5 days of postmortem storage (Kim et al., 2016). However, in this current study, the observed antioxidant effect of probiotic supplementation might

be associated with the additional freezing and thawing process. Ali (2010) reported that breasts from *Bacillus subtilis* fed chicken showed a significantly lower TBARS value during 8 days of storage compared to those from non-probiotic-feeding group ($P < 0.05$). In addition, the antioxidant effect of other microbial species feeding on lipid oxidation, including *Saccharomyces cerevisiae* (Aksu, Karaoğlu, Esenbuğa, Kaya, Macit, & Ockerman, 2005; Zhang et al., 2005) and *Aspergillus* spp. (Saleh, Eid, Ebeid, Kamizono, Ohtsuka, & Hayashi, 2011) has been reported. While the obvious inhibitory mechanisms by which microbial probiotic supplementation reduces chicken muscle lipid oxidation has not been fully understood, few studies suggested that a reduction in fat accumulation (Aksu et al., 2005), different depositions of fatty acids (Zhang et al., 2005), and/or production of antioxidant substance (e.g. muscle α -tocopherol) (Saleh et al., 2011) could be related to the given antioxidant effect of probiotic feeding. Thus, further studies evaluating fatty acid composition and antioxidant substance accumulation in muscle from probiotic fed chickens would provide more information to understand the mechanism underlying the antioxidant effect of probiotic supplementation.

3.6. Phospholipid content

The phospholipid content of frozen/thawed chicken breast muscles was affected by probiotic supplementation, freezing rate and storage period, respectively ($P < 0.05$; Table 3). The muscles from probiotic chicken group (6.89 g/100 g fat) had a higher phospholipid content compared to those from control chicken group (3.65 g/100 g fat) ($P < 0.05$). This observation could imply that probiotic supplementation might affect fatty acid synthesis in chicken muscle. In fact, Santoso et al. (1995) reported that

Bacillus subtilis feeding influenced the amounts of serum and muscle phospholipids.

Slow freezing significantly reduced the phospholipid content of frozen/thawed chicken breasts, as compared to fast freezing rate (Table 3). In previous studies, a change in phospholipid content of chicken meat has been extensively evaluated as an indicator of lipid oxidation. Those studies found that a reduction in phospholipids during frozen storage was observed due to phospholipid oxidation and/or lipolytic degradation (Soyer et al., 2010; Pikul, Leszczynski, & Kummerow, 1984). According to Pikul et al. (1984), approximately 90% of malondialdehyde accumulation in chicken muscle, which was determined by TBARS method, was derived from phospholipids since chicken muscle contains high amount of polyunsaturated fatty acids in phospholipids that are highly prone to oxidation (Soyer et al., 2010). In this regard, it would be reasonable to postulate that fast freezing could affect the maintenance of phospholipid content, which would result in the similar preventive effect against primary and secondary lipid oxidation as previously discussed. After 2 days of storage, however, phospholipid content of frozen/thawed breasts increased from 4.50 to 6.04 g/100 fat ($P < 0.05$). This increment might be related to a decrease in other lipid components, such as triglycerides or a reduction in extractability of oxidized lipid components in chloroform/methanol solvent used for fat extraction in the current study (Igene, Pearson, Merkel, & Coleman, 1979). Further studies determining the effect of display storage on phospholipid content along with detailed fatty acid profiles of chicken breasts would be warranted.

3.5. Nonheme iron content

The nonheme iron content of frozen/thawed chicken breast muscles was affected

by only probiotic supplementation ($P < 0.05$; Table 3), where the breasts from probiotic chicken group (0.078 mg/100 g) had a significantly lower nonheme iron content than those from control chicken group (0.199 mg/100 g). Previously, Min, Nam, Cordray, and Ahn (2008) reported that raw chicken breast muscle contained 0.130 mg/100 g, which was a similar range to our result, and suggested that an increase in free ionic iron, namely nonheme iron, greatly accelerated lipid oxidation. According to Rhee, Ziprin, and Ordonze (1987), nonheme plays a prooxidative role in catalyzing the hydroperoxides, resulting in secondary oxidation products. While there has been no available literature on the effect of probiotic supplementation on iron content and status in chicken muscle, our results suggest that the probiotic supplementation could be involved in the reduced formation of nonheme iron, resulting in an increase in lipid oxidation stability of breast muscles, in parts. Further studies determining the effect of probiotic feeding on initial heme/nonheme iron contents in chicken breasts and their changes under oxidative conditions would be warranted.

4. Conclusion

The results from our study found that probiotic supplementation had a significant inhibitory effect on lipid oxidation of frozen/thawed chicken breasts. Moreover, fast freezing considerably reduced thaw/purge loss and suppressed the formation of primary and secondary lipid oxidation products of frozen/thawed chicken breasts. Despite high phospholipid content, it is of interest to note that probiotic supplementation prevented the formation of both primary and secondary oxidation products. The observed positive impact of probiotic supplementation could result in leading a synergistic effect when coupling with fast freezing.

In conclusion, our results suggest that probiotic supplementation coupled with fast freezing could effectively minimize oxidative quality deterioration and improve WHC of frozen/thawed chicken breasts. Further studies on determining underlying inhibition mechanisms on lipid oxidation by probiotic supplementation should be warranted.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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Table 1. Effect of probiotic supplementation, freezing rate, and storage period on pH and water-holding capacity (WHC) of chicken breasts

Main effects				WHC (%)	
Probiotic feeding ^A	Freezing rate ^B	Storage period ^C	pH value	Thaw/purge loss	Centrifugal WHC ^D
Control group	Fast	0 day	6.13	3.60	75.80
		2 days	5.94	4.62	77.32
	Slow	0 day	6.11	5.30	78.73
		2 days	5.91	11.37	82.78
Probiotic group	Fast	0 day	5.94	2.73	74.83
		2 days	5.87	4.84	82.15
	Slow	0 day	5.92	6.14	78.49
		2 days	5.92	10.43	81.67
SEM ^E			0.749	1.264	1.853
<i>Control vs probiotic</i>					
Control group			6.02a	6.22	78.66
Probiotic group			5.91b	6.03	79.28
<i>Slow freezing vs fast freezing</i>					
Slow freezing			5.96	8.31a	80.42a
Fast freezing			5.97	3.78b	77.53b
<i>0 day storage vs 2 days storage</i>					
0 day of storage after thawing			6.02a	4.44b	76.96b
2 days of storage after thawing			5.91b	7.81a	80.98a
<i>Significance of main effects and interactions (P value)</i>					
Probiotic supplement effect (P)			0.0052	NS ^F	NS
Freezing rate effect (F)			NS	<0.0001	0.0260
Storage period effect (S)			0.0036	<0.0001	0.0027
P × F			NS	NS	NS
P × S			0.0444	NS	NS
F × S			NS	0.0082	NS
P × F × S			NS	NS	NS

^AProbiotic feeding: basal diet for control group and basal diet plus 250 ppm Sporulin (1.0×10^6 cfu/g feed, containing *Bacillus subtilis*) for probiotic group.

^BFreezing rate: slow freezing was performed in a -30 °C conventional freezer, whereas fast freezing was conducted in a liquid nitrogen chamber (-70 °C).

^CStorage period: storage periods in a 2 °C cooler (0 and 2 days) after thawing.

^DHigh value means an excellent WHC against centrifugal force.

^ESEM: standard error of the mean.

^FNS: non-significance.

a,b Different letters within each main effect are significantly different ($P < 0.05$).

Table 2. Effect of probiotic supplementation, freezing rate, and storage period on color characteristics of chicken breasts

Main effects			Color characteristics			
Probiotic feeding ^A	Freezing rate ^B	Storage period ^C	CIE L* (lightness)	CIE a* (redness)	CIE b* (yellowness)	Hue angle (discoloration)
Control group	Fast	0 day	51.27	5.79	17.55	71.67
		2 days	54.62	5.17	18.00	74.03
	Slow	0 day	52.13	5.85	17.68	71.70
		2 days	54.65	5.35	17.88	73.33
Probiotic group	Fast	0 day	54.07	4.73	17.93	75.27
		2 days	54.89	5.24	18.06	73.84
	Slow	0 day	52.82	4.60	17.37	75.18
		2 days	55.67	4.67	18.06	75.44
SEM ^D			1.371	0.651	0.839	1.899
<i>Control vs probiotic</i>						
Control group			53.17	5.54a	17.78	72.68b
Probiotic group			54.36	4.81b	17.85	74.93a
<i>Slow freezing vs fast freezing</i>						
Slow freezing			53.82	5.12	17.75	73.91
Fast freezing			53.71	5.23	17.88	73.70
<i>0 day storage vs 2 days storage</i>						
0 day of storage after thawing			52.57b	5.24	17.63	73.45
2 days of storage after thawing			54.96a	5.11	18.00	74.16
<i>Significance of main effects and interactions (P value)</i>						
Probiotic feeding effect (P)			NS ^E	0.0306	NS	0.0235
Freezing rate effect (F)			NS	NS	NS	NS
Storage period effect (S)			0.0013	NS	NS	NS
P × F			NS	NS	NS	NS
P × S			NS	NS	NS	NS
F × S			NS	NS	NS	NS
P × F × S			NS	NS	NS	NS

^AProbiotic feeding: basal diet for control group and basal diet plus 250 ppm Sporulin (1.0×10^6 cfu/g feed, containing *Bacillus subtilis*) for probiotic group.

^BFreezing rate: slow freezing was performed in a -30 °C conventional freezer, whereas fast freezing was conducted in a liquid nitrogen chamber (-70 °C).

^CStorage period: storage periods in a 2 °C cooler (0 and 3 days) after thawing.

^DSEM: standard error of the mean.

^ENS: non-significance.

a,b Different letters within each main effect are significantly different ($P < 0.05$).

Table 3. Effect of probiotic supplementation, freezing rate, and storage period on lipid, phospholipid and nonheme iron contents of chicken breasts

Main effects			Lipid (g/100 g)	Phospholipid (g/100 g fat)	Nonheme iron (mg/100 g)
Probiotic feeding ^A	Freezing rate ^B	Storage period ^C			
Control group	Fast	0 day	3.33	3.37	0.190
		2 days	3.81	6.01	0.181
	Slow	0 day	3.77	1.52	0.219
		2 days	2.77	3.69	0.207
Probiotic group	Fast	0 day	2.85	8.23	0.084
		2 days	3.19	9.25	0.074
	Slow	0 day	3.21	4.89	0.083
		2 days	3.55	5.19	0.070
SEM ^D			0.439	0.748	0.034
<i>Control vs probiotic</i>					
Control group			3.42	3.65b	0.199a
Probiotic group			2.95	6.89a	0.078b
<i>Slow freezing vs fast freezing</i>					
Slow freezing			3.07	3.82b	0.145
Fast freezing			3.29	6.71a	0.132
<i>0 day storage vs 2 days storage</i>					
0 day of storage after thawing			3.54	4.50b	0.144
2 days of storage after thawing			2.93	6.04a	0.133
<i>Significance of main effects and interactions (P value)</i>					
Probiotic feeding effect (P)			NS ^E	<0.0001	<0.0001
Freezing rate effect (F)			NS	<0.0001	NS
Storage period effect (S)			NS	0.0189	NS
P × F			NS	NS	NS
P × S			NS	NS	NS
F × S			NS	NS	NS
P × F × S			NS	NS	NS

^AProbiotic feeding: basal diet for control group and basal diet plus 250 ppm Sporulin (1.0×10^6 cfu/g feed, containing *Bacillus subtilis*) for probiotic group.

^BFreezing rate: slow freezing was performed in a -30 °C conventional freezer, whereas fast freezing was conducted in a liquid nitrogen chamber (-70 °C).

^CStorage period: storage periods in a 2 °C cooler (0 and 3 days) after thawing.

^DSEM: standard error of the mean.

^ENS: non-significance.

a,b Different letters within each main effect are significantly different ($P < 0.05$).

Fig. 1. Temperature decline of chicken breasts during slow and fast freezing procedures.
The slow freezing was conducted in a -30 °C conventional freezer, while fast freezing was performed in -70 °C liquid nitrogen chamber. Red solid and black dotted lines indicate temperature decline of breasts assigned to fast and slow freezing, respectively.

(a) Probiotic supplementation effect

(b) Freezing rate $\times$ storage period effects

Fig. 2. Effect of probiotic supplementation, freezing rate, and storage period after thawing on peroxide value (POV) of chicken breast muscles. a,b Different letters are significantly different ($P < 0.05$).

Fig. 3. Effect of probiotic feeding, freezing rate, and storage period after thawing on 2-thiobarbituric reactive substances (TBARS) value of chicken breast muscles. a-e Different letters are significantly different ($P < 0.05$).