International Food Research Journal 22(6): 2619-2728 (2015)
Journal homepage: *http://www.ifrj.upm.edu.my*

Chicken blood promotes growth of *Listeria monocytogenes, Salmonella* Typhimurium, *Campylobacter jejuni* and *Pseudomonas aeruginosa* in minced chicken during refrigerated storage

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Article history

Received: 3 December 2014
Received in revised form: 19 May 2015
Accepted: 22 May 2015

Keywords

Microbiological quality
Refrigerated storage
Slaughtering method
Spoilage microorganisms
Foodborne pathogens
Minced chicken

Abstract

Microorganisms are typically introduced to poultry meat through cross-contamination during processing, visible matters, including residual blood can further affect levels of contamination and deterioration of the meat. This study is aimed to investigate effects of chicken blood that may be left from slaughtering process or methods typically at retail environments (fresh markets) on microbiological quality of minced chicken during refrigerated storage. Medium broth with chicken blood added at 1, 2.5 and 5 µmol/g showed no difference in the growth of *Listeria monocytogenes, Salmonella* Typhimurium, *Campylobacter jejuni* and *Pseudomonas aeruginosa*, compared with the no blood-added broth at 48 h of incubation. After 2 days of storage at 4°C, mesophilic and psychrophilic bacteria increased rapidly in both the minced chicken with and without blood. At 8 days of storage, minced chicken without blood showed lower counts of *Pseudomonas aeruginosa, Salmonella* Typhimurium, and *Campylobacter jejuni* ($P < 0.05$), except for *Listeria monocytogenes*. Comparison of microbial growth in minced chicken obtained from the Islamic slaughtering method (IM) and conventional neck cut method (CM) showed that only *Campylobacter jejuni* had higher growth in the CM-minced chicken than those found in the IM-minced chicken during 8 days of refrigerated storage. Our study suggests that chicken blood can promote bacterial growth. The conventional slaughtering process may allow for the presence of more blood residual in poultry meat or environments which could allow for higher counts of *Campylobacter jejuni* than in minced chicken from the Islamic method. Minimizing residual blood in chicken meat or partial tissues and in preparing or retailing environments is important to prevent an increase of some pathogenic and spoilage bacteria.

Introduction

Poultry meat is generally perceived as cheap and wholesome for consumption. While global poultry production is estimated to be 106 million tons in 2013, poultry consumption in Asia is about 42.5 million tons (approximately 40 percent of the world total) (FAO, 2013). Poultry products are highly perishable foods which usually deteriorate within a week of slaughtering due to some intrinsic factors, e.g., high pH, high protein and moisture contents (FAO, 2010). These factors can further facilitate growth of pathogenic and spoilage microorganisms, leading to food safety concerns. In Southeast Asia, while all exporters of poultry meat are large and medium-sized companies, small-scale business operations serve primarily domestic consumers. Small-scale business operations here (both halal and non-halal operations) do not have uniform processing. After slaughtering, poultry carcasses are commonly taken to the fresh markets or counters at the supermarkets for further processing (sectioning, slicing and packaging) for both halal and non-halal poultry meat. Some locations, live chickens are sold at the fresh markets and they can be freshly slaughtered per consumers' request. This nature of processing manner makes processing difficult to control and can often lead to public health concerns with regards to high loads of pathogenic or spoilage microorganisms (Vindigni *et al*., 2007; Minami *et al.*, 2010; Indrawattana *et al*., 2011). For example, *Campylobacter* spp. were isolated from chicken samples (200 samples from 50 markets): 15% of chicken samples from open markets and 35% of chicken samples from supermarkets (Vindigni *et al*., 2007).

Psychrophilic microorganisms have been reported to have the ability to survive processing operations and to further multiply during refrigerated storage which can typically cause spoilage of poultry meat (Hinton Jr. *et al.*, 2004; Ma *et al.*, 2014). High numbers of

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microorganism in raw meat can transform products to unsuitable and unappealing for consumption (Doulgeraki *et al.*, 2012). Contamination of poultry meat by foodborne pathogens is also a major public health concern. Several bacterial pathogens, e.g., *Campylobacter, Salmonella* spp., and *Listeria monocytogenes* have been often linked to human illness cases from consumption of contaminated poultry products (CDC, 2014b). The European Food Safety Authority (EFSA) also reported that occurrence of *Campylobacter* was high in broiler meat and this pathogen was found to be the most common cause of foodborne illnesses in 2011 (with 220,209 confirmed human cases) (EFSA, 2013). *Campylobacter* spp. and *Listeria monocytogenes* can be found in both poultry carcasses and in poultry processing plant environments (Ma *et al*., 2014; Sasaki *et al*., 2014). Both pathogenic and spoilage microorganisms can be typically introduced to poultry meat through cross-contamination during processing operations (Bolder, 2007; Foley *et al*., 2011; Ma *et al*., 2014).

In fresh markets where inappropriate sanitation and handling are often seen, chicken processing, including freshly slaughtering for both slaughtering methods (Islamic and conventional), may allow for the presence of some blood residual left in chicken meat or partial tissues and in the preparing or retailing environments. However, it is not well elucidated in terms of the differences in the amount of blood retained in chicken meat or partial tissues and in the preparing or retailing environments between the conventional and Islamic slaughtering methods. In the Islamic slaughtering, blood is forbidden to be consumed, thus soaking meat in salt or water is very important to remove blood residual (Farouk *et al*., 2014). Major difference between Islamic and conventional methods is the cutting trachea and esophagus in addition to cutting the carotid arteries and jugular veins (for the conventional method). In a previous study, chicken blood could be not only an excellent medium for the growth of bacteria due to its high nutritive value, but it could also be a major defect that could lead to undesirable short shelf-life (Alvarado *et al*., 2007). This study is aimed to investigate potential effects of chicken blood that may be left from the slaughtering process typically at retail environments (fresh markets) on microbiological quality of chicken meat, i.e., minced chicken, during refrigerated storage for up to eight days. In this study, chicken meat from the Islamic (Halal) and conventional (non-Halal) slaughtering methods, in a minced form, was also monitored for changes in the spoilage (i.e., *Pseudomonas aeruginosa*) and pathogenic (i.e., *Listeria monocytogenes, Salmonella* Typhimurium, and *Campylobacter jejuni*) bacterial counts during 8 days of refrigerated storage.

Material and Methods

Bacterial strains

Three pathogenic bacterial strains (*Listeria monocytogenes* DMST 1327, *Salmonella enterica* serovar Typhimurium DMST 562, *Campylobacter jejuni* PSU 03151) and one spoilage bacterial strain (*Pseudomonas aeruginosa* TISTR 781) were used in this study. These strains were obtained from the Department of Medicine Science, Ministry of Public Health, Nonthaburi, Thailand (DMST), Thailand Institute of Scientific and Technological Research (TISTR) and the Department of Microbiology, Faculty of Science, Prince of Songkla University (PSU). Prior to experiments, the bacterial strains were sub-cultured twice. An isolated colony of each strain was inoculated into 10 ml of Nutrient Broth (NB; Difco, BD Diagnostics, Sparks, MD, USA), followed by incubation at 37°C for 18 h for *Pseudomonas aeruginosa, Listeria monocytogenes* and *Salmonella enterica* serovar Typhimurium, and at 42°C for 42 h for *Campylobacter jejuni*. Culture (1 ml of each strain) was then separately transferred to another tube containing 10 ml of NB, and grown at the same corresponding temperatures for another 18 h or 42 h. Overnight culture from the second passage, representing approximately 10^9 CFU/ml was used in each challenge experiment detailed below.

Effect of chicken blood on the growth of selected pathogenic and spoilage bacteria in medium broth

A broiler chicken (age of 6 weeks with approx. 2 kg body weight) was obtained from a poultry farm in Songkhla, Thailand. Bleeding was performed prior to scalding and defeathering (picking) at the fresh market. Chicken blood was collected by an aseptic technique using a sterilized syringe, and sterilized heparin was used as an anticoagulant. Blood was withdrawn into a sterilized syringe (20 ml) and transferred into a sterilized centrifuge tube, previously rinsed with 150 mM NaCl solution, containing 5 ml of sodium heparin (30 U/ml) (Sigma, St. Louis, MO, USA), representing a blood/heparin ratio of 4:1 (v/v). Approximately 40 ml of chicken blood was obtained from one broiler chicken which was sufficient for further studies. Chicken blood mixed with heparin was kept at 4°C until use. A medium broth containing chicken blood ("blood broth") was prepared by adding prepared blood into Brain Heart Infusion (BHI; Oxoid, Basingstoke, Hampshire, UK) broth. Blood broths containing different final blood levels in BHI

(1, 2.5 and 5 µmol/ml) were prepared, following the procedures of haemoglobin quantification described in Richards and Hultin (2000), based on the standard curves using bovine haemoglobin standard (Sigma, St. Louis, Mo). Each blood broth was inoculated with 1 ml (10^4 CFU/ml) of the diluted overnight culture of separate pathogenic or spoilage bacteria prepared as detailed above. The blood broths inoculated with *Pseudomonas aeruginosa, Listeria monocytogenes* or *Salmonella* Typhimurium were incubated at 37°C and those inoculated with *Campylobacter jejuni* were incubated at 42°C under microaerophilic conditions (approximately 5% O_2, 10% CO_2 and 85% N_2). Controls (culture-inoculated broths without additional chicken blood) were included in the study. Bacterial growth was monitored every 6 h for 48 h. One milliliter of each blood broth was transferred into a test tube containing 9 ml of 0.85% normal saline solution (NSS), followed by a preparation of 10-fold serial dilutions. Appropriate dilutions were used for microbiological analyses as detailed below.

Effect of chicken blood on the growth of selected spoilage and pathogenic microorganisms in minced chicken

A broiler chicken (age of 6 weeks with approx. 2 kg body weight) was obtained from a poultry farm in Songkhla, Thailand. After bleeding for 3 min by a normal neck cut, a broiler chicken was plucked in a rotary-drum picker for 30 s and eviscerated. Breast muscles were dissected from the carcasses. The chicken breast was cut into wide strips of approximately 0.5 x 0.5 cm^2 using a sterilized knife. Chicken strips were minced using a food processor at high speed for 3 min (MK- Panasonic 5087M, Selangor Darul Ehsan, Malaysia). Prepared chicken blood, as detailed above, was added to minced chicken (25 g) and mixed throughout in a sterile polyethylene bag to obtain different blood concentrations (2.5, 5, 7.5, and 10 µmol/g). Overnight culture (1 ml) of *Pseudomonas aeruginosa, Listeria monocytogenes, Salmonella* Typhimurium, and *Campylobacter jejuni* was separately added into a sterile polyethylene bag containing blood added-minced chicken (25 g) with varied blood concentrations to achieve the final inoculum level of approximately 10^4 CFU/g. Controls, culture-inoculated minced chicken without additional chicken blood, were included in this study. Samples were stored at 4°C, and the growth of microorganisms was monitored every 2 days up to 8 days.

Effect of the slaughtering methods on the growth of selected spoilage and pathogenic bacteria in minced chicken

Two broiler chickens (age of 6 weeks with approx. 2 kg body weight) were obtained from a poultry farm in Songkhla, Thailand, and processed with two slaughtering methods at the fresh market: (i) Islamic method and (ii) conventional neck cut method. After bleeding for 3 min, following each slaughtering method, broiler chickens were plucked in a rotary-drum picker for 30 s and eviscerated. Breast muscles were dissected from the carcasses using a sterilized knife, followed by mincing using a food processor as detailed above. Culture (1 ml) of *Pseudomonas aeruginosa, Listeria monocytogenes, Salmonella* Typhimurium, and *Campylobacter jejuni* was separately added into a sterile polyethylene bag containing minced chicken (25 g) obtained from each slaughtering method to achieve the final inoculum level of approximately 10^4 CFU/g. Samples were stored at 4°C, and the growth of microorganisms was monitored every 2 days up to 8 days.

Sample preparation for microbiological analysis

Microbiological analysis was performed following the methods of Cousin *et al.* (1992) with some modifications. Twenty five grams of minced chicken from each study were transferred into a stomacher bag containing 225 ml of 0.85% saline solution. Sample mixtures were homogenized by using a stomacher (400 Circulator, Seward, West Sussex, UK) for 1 min at 230 rpm. Aliquot of the homogenates (1 ml) was used to prepare 10-fold serial dilutions in 0.85% saline solution. Appropriate dilutions were used for microbial enumeration as detailed below.

Determination of mesophilic and psychrophilic bacterial counts

Mesophilic and psychrophilic bacterial counts were determined using the pour plate method according to Cousin *et al*. (1992) with some modifications. One milliliter of appropriate dilution was transferred onto a sterile petri dish, and 15 ml of waterbath-equilibrated (45°C) plate count agar (PCA; Difco, BD Diagnostics, Sparks, MD, USA) was poured and mixed with the diluted sample on the petri dish. Plates were incubated at 37°C for 2 days for mesophilic bacteria counts, and at 4°C for 7 days for psychrophilic bacteria counts.

Enumeration of Pseudomonas aeruginosa, Listeria monocytogenes, Salmonella *Typhimurium, and* Campylobacter jejuni

Aliquot of appropriate dilutions (100 µl each) was spread plated onto (i) *Pseudomonas* Isolation Agar (Difco, BD Diagnostics, Sparks, MD, USA) for *Pseudomonas aeruginosa* counts; (ii) Agar *Listeria* Ottaviani Agosti (ALOA; AES laboratories, Marcy l'Etoile, France) for *Listeria monocytogenes* counts; (iii) Xylose Lysine Deoxycholate (XLD; Difco, BD Diagnostics, Sparks, MD, USA) agar for *Salmonella* counts. Plates were incubated at 37°C 24 h. *Pseudomonas aeruginosa* colonies were green to blue-green with pigment that diffused into the medium (Mead and Adams, 1977). *Listeria monocytogenes* produced colonies with blue–green surrounding halo on ALOA (FDA, 2011). *Salmonella* colonies were pink with or without black centers on XLD agar (FDA, 2014). *Campylobacter jejuni* was determined on Blood free *Campylobacter* selective agar base (mCCDA; Oxoid, Basingstoke, Hampshire, UK). Spread plate was prepared by spreading 100 µl of appropriate dilutions onto mCCDA, followed by incubation at 42°C for 48 h in a microaerophilic environment as detailed above. Colonies with grayish-white or creamy grey in color and moist in appearance indicated the presence of *Campylobacter jejuni* (FDA, 2013).

Statistical analysis

Each study was performed in three independent replicates with three different sets of samples collected. Data were converted to $\log_{10}$ CFU numbers for analysis. Values were expressed as means + SE. Data were subjected to analysis using ANOVA and the mean comparison was carried out using Duncan's multiple range test (DMRT) (Bewick *et al*., 2004). Differences were considered significant at $P < 0.05$. Analysis in this study was performed using the Statistical Package for Social Science package (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA).

Results and Discussion

Growth of Pseudomonas aeruginosa, Listeria monocytogenes, Salmonella *Typhimurium, and* Campylobacter jejuni *in medium broth with additional chicken blood*

Counts of each of the four tested microorganisms (*Pseudomonas aeruginosa, Listeria monocytogenes, Salmonella* Typhimurium, and *Campylobacter jejuni*) in the blood broth (BHI broth with additional chicken blood) increased with increasing incubation times *($P < 0.05$)* (Figure 1). Broth containing the highest chicken blood concentration (5 µmol/ml) showed increased bacterial counts throughout 48 h of the incubation period, from 3.4 to 8.9 log CFU/g for *Pseudomonas aeruginosa*, from 4.3 to 8.8 log CFU/g for *Listeria monocytogenes,* from 3.4 to 10.6 log CFU/g for *Salmonella* Typhimurium, and from 3.9 to 9.9 log CFU/g for *Campylobacter jejuni.* Growth of *Pseudomonas aeruginosa, Listeria monocytogenes, Salmonella* Typhimurium, and *Campylobacter jejuni* in the blood broth at 2.5 and 5 µmol/ml increased rapidly within 12 h of the incubation period. Counts of pathogenic bacteria tested, *Listeria monocytogenes, Salmonella* Typhimurium, and *Campylobacter jejuni,* were higher in the blood broth at 2.5 and 5 µmol/ml, from 6–42 h of incubation as compared with those in the blood broth at 1 µmol/ml and the controls. Overall, although results indicated that additional chicken blood could allow for more growth within 12 h of incubation, growth of these microorganisms was not different from those observed in medium broth without blood at 48 h of incubation.

Growth of mesophilic bacteria count (MBC) and psychrophilic bacteria count (PBC) in minced chicken with additional chicken blood at different concentrations

Numbers of MBC and PBC in minced chicken with and without chicken blood added at different concentrations increased during 8 days of refrigerated storage (Figure 2). After 2 days of storage, MBC and PBC of minced chicken with high blood concentrations (7.5 and 10 µmol/g) increased at a higher rate, compared to those with lower blood concentrations (2.5 and 5 µmol/g) and the controls (no blood added). Especially, at day 4 of storage, MBC in minced chicken with 7.5 and 10 µmol blood/g exceeded 7 $\log_{10}$ CFU/g, the upper limit for APC (aerobic plate count) in processed chicken recommended by International Commission on Microbiological Specifications for Foods (ICMSF, 1986). Spoilage of chicken generally occurs when the mesophilic bacteria count reaches 7–8 $\log_{10}$ CFU/g. Spoilage is usually detected around 4–10 days, depending on slaughtering techniques, the types and numbers of bacteria initially present and their growth rates [reviewed in (Doulgeraki *et al*., 2012)]. In a study by Smaoui *et al.* (2012), although chicken meat was treated with some growth inhibitors, the increase in the storage time yielded significant proliferations of APC ($P < 0.05$), and certain sign (unpleasant odor) of spoilage started to appear around day 5 and 6 of storage. Our data showed that PBC at days 0–2 was slightly lower than MBC, and that PBC rapidly increased after 2 days of storage, suggesting that psychrophilic bacteria gradually grew during the refrigerated storage and became the dominant bacteria with increasing storage time. Overall, after 8 days of

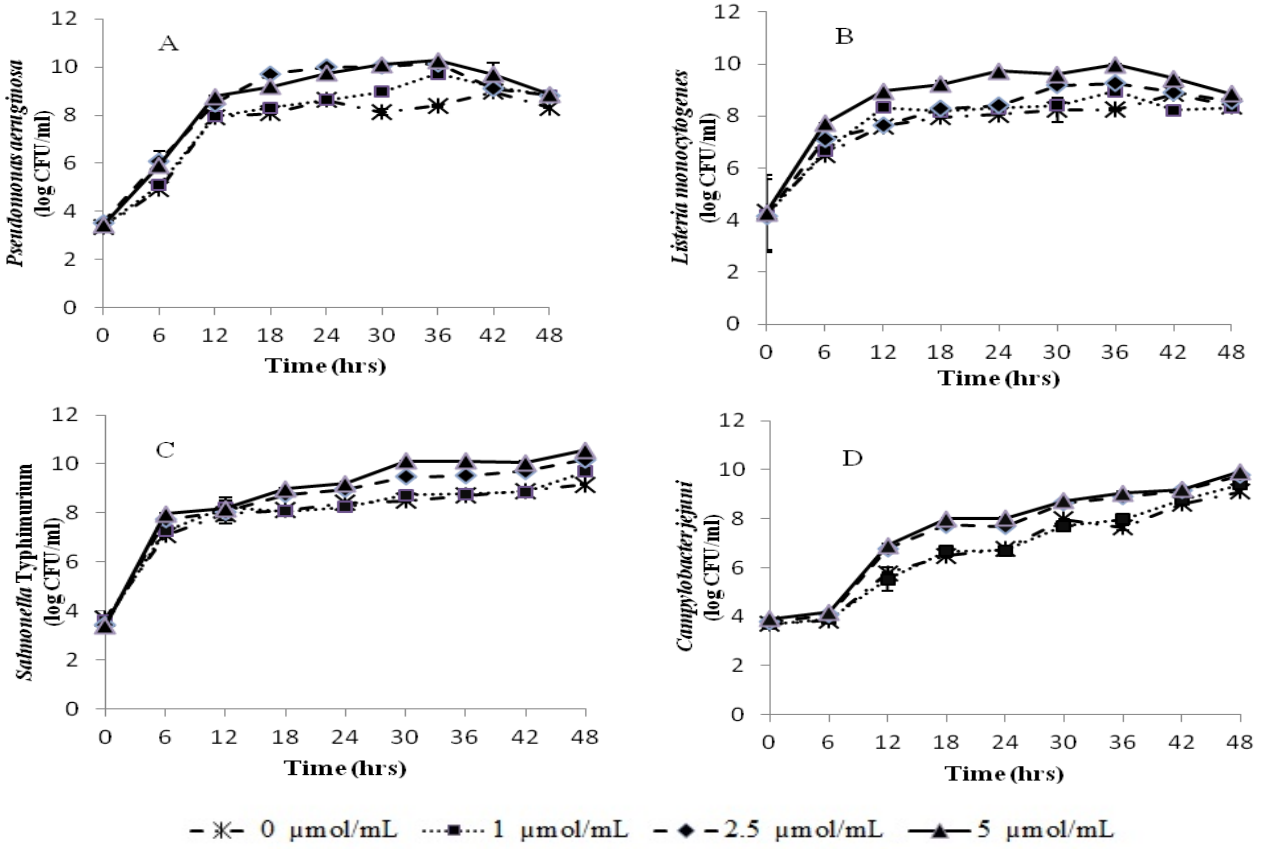

Figure 1. Effect of chicken blood added into BHI broth at different concentrations on the growth of *Pseudomonas aeruginosa*, (A); *Listeria monocytogenes*, (B); *Salmonella* Typhimurium, (C); and *Campylobacter jejuni*, (D) over 48 h of incubation period. Cross mark represents control (no chicken blood added). For different chicken blood concentrations: dark square represents 1 µmol/mL, dark diamond shape represents 2.5 µmol/mL, and dark triangle represents 5 µmol/mL

storage at 4°C, our results indicated that numbers of psychrophilic bacteria count could increase from about 3 to 8 $\log_{10}$ CFU/g in minced chicken with no chicken blood added and from about 3 to 9 $\log_{10}$ CFU/g in minced chicken with chicken blood added. Only 1 $\log_{10}$ CFU/g different was observed in these two types of minced chicken tested, thus suggesting that chicken blood did not have much influence on the rapid growth of psychrophilic bacteria in minced chicken in our study. A previous study has shown that there was a significant increase in the bacterial population during refrigerated storage of commercial poultry carcasses (Hinton Jr. *et al*., 2004). While various bacterial genera could be recovered from carcasses undergone processing and from carcasses stored at refrigerated temperatures, including *Aeromonas, Pseudomona* and *Chromobacterium, Pseudomonas* spp. became predominant after 7 days of storage at 4°C (Hinton Jr. *et al.*, 2004).

Growth of Pseudomonas aeruginosa, Listeria monocytogenes, Salmonella *Typhimurium, and* Campylobacter jejuni *in minced chicken with additional chicken blood at different concentrations*

At 8 days of storage, minced chicken without blood showed lower counts of *Pseudomonas aeruginosa, Salmonella* Typhimurium, and *Campylobacter jejuni* ($P < 0.05$), except for *Listeria monocytogenes* (Figure 3). For *Listeria monocytogenes* and *Campylobacter jejuni,* there were no marked differences in the counts in minced chicken with blood added at 7.5 and 10 µmol/g. *Campylobacter jejuni* in minced chicken without blood remained unchanged in the counts (about 3.5 $\log_{10}$ CFU/g) throughout 8 days of refrigerated storage. However, an increase of this pathogen was observed in minced chicken with chicken blood added at different concentrations. *Campylobacter jejuni* are typically difficult to grow in the laboratory culture and the optimal temperature for growth is about 37–42°C (CDC, 2014a). However, in the medium broth with chicken blood added and stored at refrigerated temperature in our study, *Campylobacter jejuni* showed the ability to survive and increase the numbers. While some study reported that *Campylobacter jejuni* could become viable but non-culturable in response to various stressors, including low temperatures (Magajna and Schraft, 2014), a study by Lazaro *et al.* (1999) also reported the ability to survive of this pathogen for as long as 7 months in phosphate-buffered saline at 4°C while

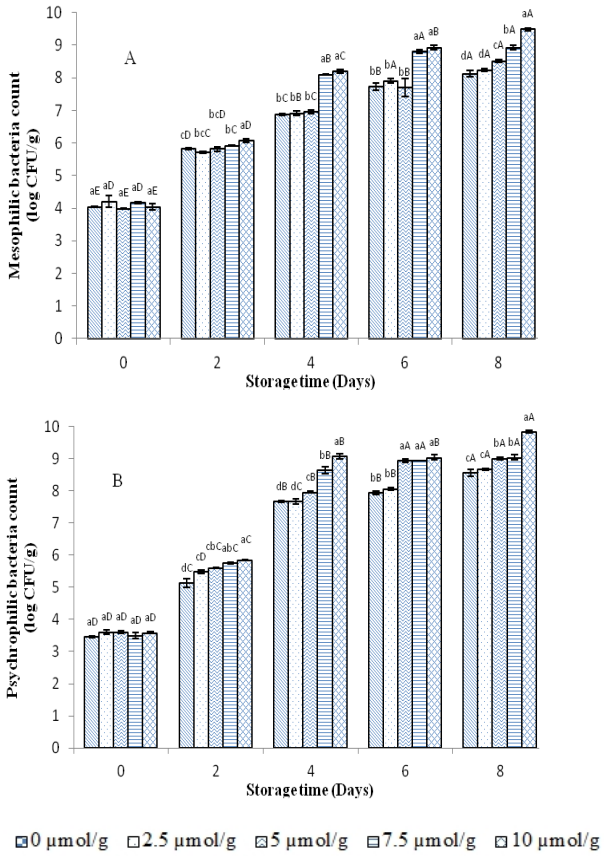

Figure 2. Effect of chicken blood at different concentrations on the growth of mesophilic bacteria, (A); and psychrophilic bacteria, (B) in minced chicken during 8 days of refrigerated storage. Dissimilar lowercase letters on the bars indicate the significant differences within the same storage time ($P < 0.05$). Dissimilar uppercase letters on the bars indicate the significant differences within the same treatment ($P < 0.05$). Bar patterns from left to right: diagonal line pattern represents control (no chicken blood added). For different chicken blood concentrations: dot pattern represents 2.5 µmol/g, zigzag line pattern represents 5 µmol/g, horizontal line pattern represents 7.5 µmol/g, and cross line pattern represents 10 µmol/g

maintaining respiratory and some cellular integrity. Some amount of blood present in the minced chicken samples may have an effect on facilitating the recovery of cells that could have been stressed from low temperature during storage. These cells may be able to utilize some amino acids and organic acids for energy for recovery and growth (Velayudhan *et al*., 2004; Guccione *et al*., 2008; Hofreuter *et al*., 2008). However, further studies are needed for evaluating the effects of chicken blood on the recovery of some strains of *Campylobacter jejuni* that are undergone chill or low-temperature stresses.

Chicken meat is considered a food that supports growth of pathogens and spoilage microorganisms, numbers of these microorganisms could reach at higher levels in the presence of some residual blood. In many fresh markets in Southeast Asia, chicken meat that is prepared and sold can have inconsistent amount of residual blood present due to some uncontrollable processing in many steps, including evisceration and washing. This can lead to a food safety concern as growth of these pathogens and spoilage microorganism may be moderately promoted while meat products are stored for up to 4–8 days. Especially, some pathogens have the ability to survive and grow at refrigeration conditions (e.g., *Listeria monocytogenes*) (CDC, 2013). A previous study showed that *Salmonella* Typhimurium and *Salmonella* Heidelberg could survive in chicken slurry and medium broth at low temperatures, i.e., 4–10°C (Morey and Singh, 2012).

In fresh markets where chickens can be inconsistently slaughtered or poultry meat can be uncontrollable processed, it is therefore important to minimize residual blood content in chicken meat during the slaughtering process. Higher counts of the spoilage microorganism *Pseudomonas aeruginosa* in minced chicken with chicken blood added that was observed in this study can increase the likelihood of chicken meat becoming deteriorated more quickly. A previous study by Alvarado *et al*. (2007) showed that blood components in breast meat, as a result from the CO_2 slaughtering and no bleeding, could promote microbial growth as observed by higher total aerobic plate counts and the greatest increase in APC during 5 days of storage than those meat samples from other treatments that allowed for bleeding after slaughtering. Occurrence of microorganisms in raw meat, especially at high numbers, can cause further changes, yielding the products that are unappealing and unsuitable for human consumption (Fung, 2010).

Effect of the slaughtering methods on the growth of Pseudomonas aeruginosa, Listeria monocytogenes, Salmonella *Typhimurium, and* Campylobacter jejuni *in minced chicken*

Comparison of microbial growth in minced chicken obtained from the Islamic slaughtering method (IM) and conventional neck cut method (CM) in this study showed that only *Campylobacter jejuni* showed higher growth in the CM-minced chicken than those found in the IM-minced chicken during 8 days of refrigerated storage (Figure 4). As reported in the earlier section, some blood residual may facilitate the recovery of some *Campylobacter jejuni* cells from refrigerated conditions. Similarly, some difference in the blood residual in minced chicken from each slaughtering method, which may be slightly higher in the CM-minced chicken, could allow for a fair increase of *Campylobacter jejuni* (about 1 $\log_{10}$ CFU/g). While various studies reported

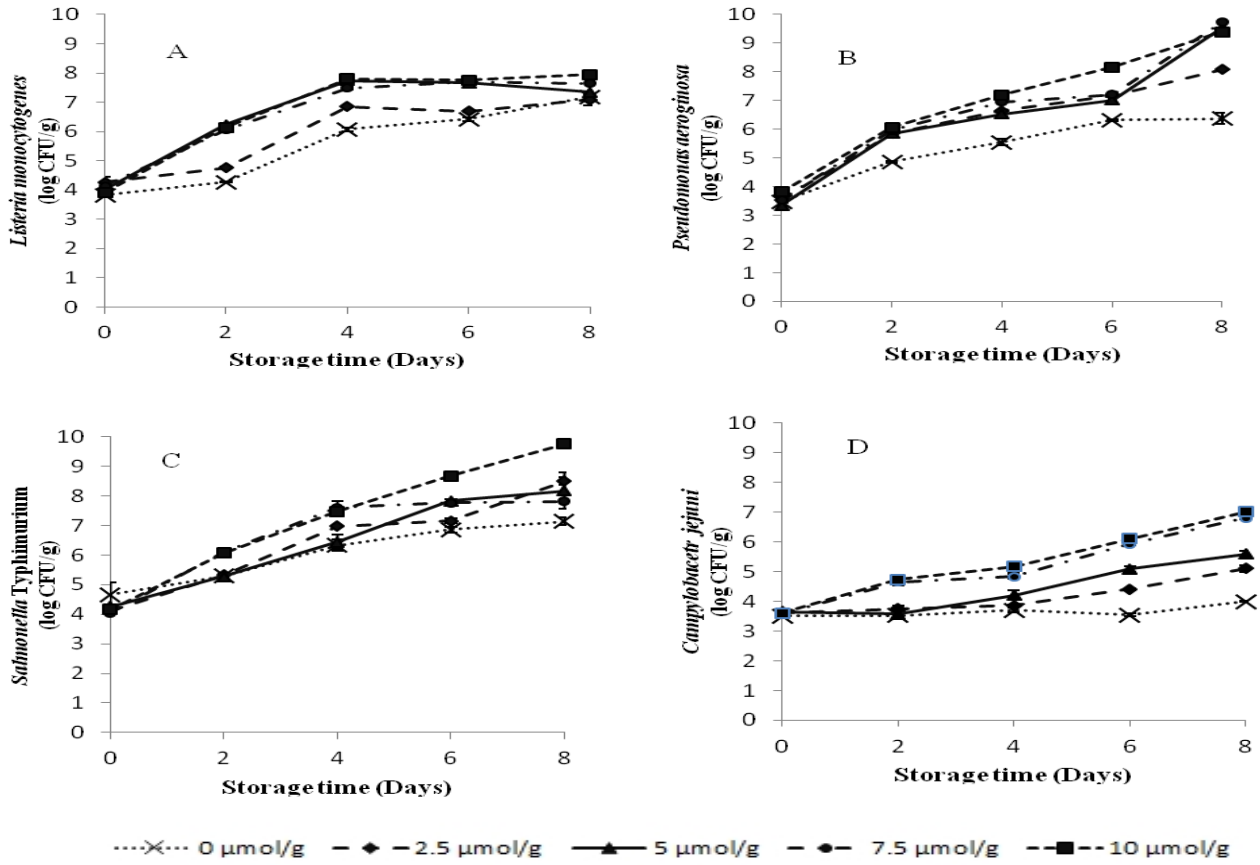

Figure 3. Effect of chicken blood at different concentrations on the growth of *Pseudomonas aeruginosa,* (A); *Listeria monocytogenes*, (B); *Salmonella* Typhimurium, (C); and *Campylobacter jejuni,* (D) in minced chicken during 8 days of refrigerated storage. Cross mark represents control (no chicken blood added). For different chicken blood concentrations: dark diamond shape represents 2.5 µmol/g, dark triangle represents 5 µmol/g. dark circle represents 7.5 µmol/g, and dark square represents 10 µmol/g

unpredictable ability of *Campylobacter jejuni* to survive stress conditions [reviewed in (Bronowski *et al*., 2014)], amino acid sources could allow for recovery and growth (Rasmussen *et al.*, 2013). However, further studies are needed for evaluating the ability of *Campylobacter jejuni* to be recovered from chill or low-temperature stresses in the presence of chicken blood which may be the potential amino acid sources. Results obtained in this study indicate that the conventional slaughtering process in fresh markets could allow for the presence of more blood residual in poultry meat or environments which may suggest the effect of blood residual on higher growth of *Campylobacter jejuni* in CM-minced chicken. While cutting that includes trachea and esophagus in addition to cutting the carotid arteries and jugular veins is the major difference between Islamic and conventional methods, the conventional method may result to more blood retained after slaughtering.

Counts of *Pseudomonas aeruginosa, Listeria monocytogenes,* and *Salmonella* Typhimurium were not different in minced chicken from both slaughtering methods throughout 8 days of storage. Pseudomonas aeruginosa counts in both CM- and IM-minced chicken increased rapidly from 3.5 to 9.7 and 3.3 to 9.5 $\log_{10}$ CFU/g, respectively. *Pseudomonas* spp., have been identified as the predominant microorganism responsible for spoilage of the chilled meat under different packaging conditions and different storage temperatures (Li *et al*., 2006), while *Listeria monocytogenes, Salmonella* Typhimurium and *Campylobacter jejuni* constituted as the natural pathogens often found in chicken meat (Berrang *et al*., 2010; Foley *et al*., 2011; Ma *et al*., 2014).

Conclusions

In fresh markets, chicken processing (i.e., sectioning, slicing and packaging), including freshly slaughtering for both slaughtering methods (Islamic and conventional), may allow for some blood residual left in chicken meat or partial tissues and in preparing or retailing environments. Minced chicken that was added with chicken blood showed higher growth of both pathogenic and spoilage bacteria (*Pseudomonas aeruginosa, Listeria monocytogenes, Salmonella*

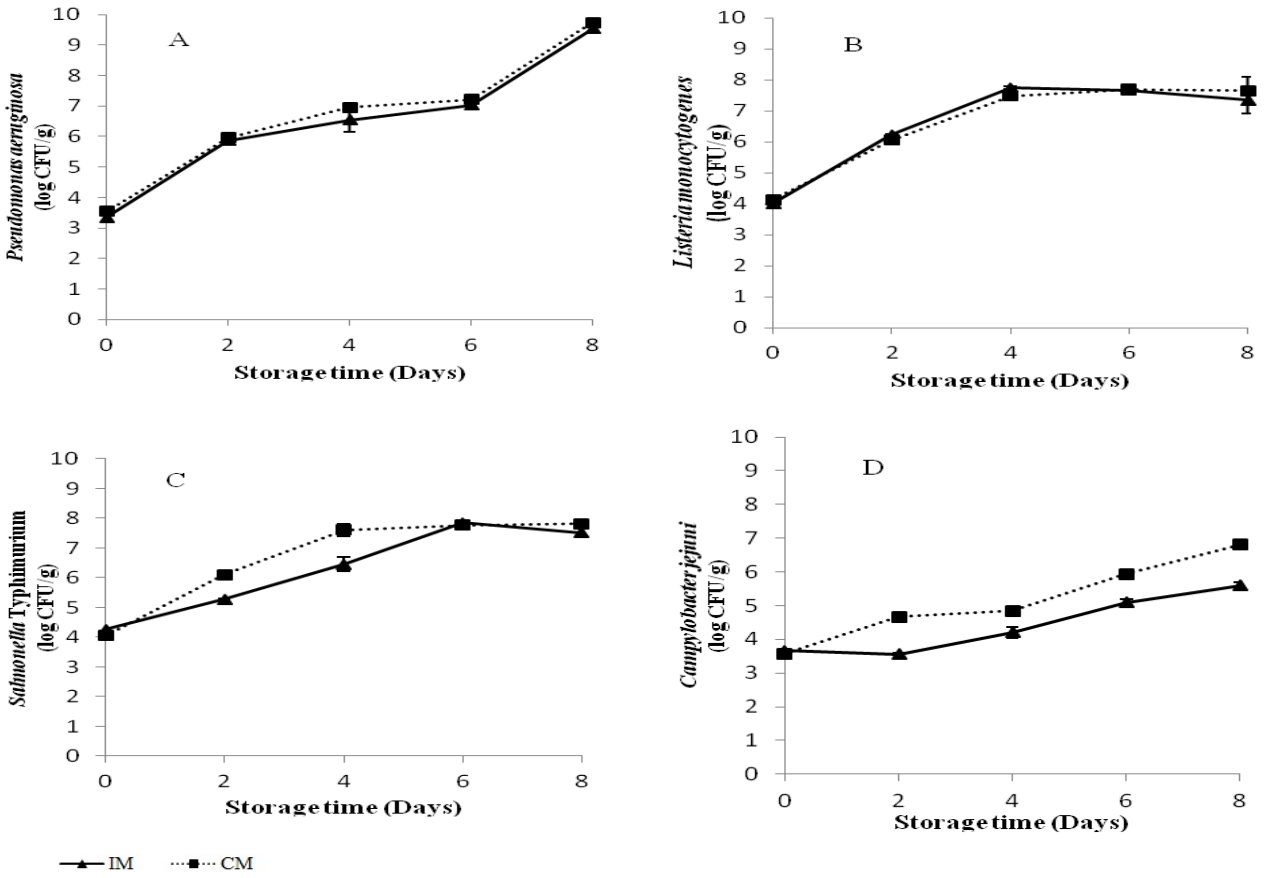

Figure 4. Effect of the slaughtering methods on the growth of *Pseudomonas aeruginosa*, (A); *Listeria monocytogenes,* (B); *Salmonella* Typhimurium, (C); and *Campylobacter jejuni*, (D) in mined chicken during 8 days of refrigerated storage. Dark triangle indicates Islamic method (IM).Dark square indicates Conventional neck cut method (CM)

Typhimurium, and *Campylobacter jejuni*). From this study, there could be some difference in the blood residual in minced chicken from each slaughtering method. The conventional slaughtering process in fresh markets may allow for the presence of more blood residual in poultry meat or environments which could allow for higher counts of *Campylobacter jejuni* than those found in minced chicken from the Islamic method. Minimizing residual blood after bleeding in chicken meat or partial tissues and in preparing or retailing environments is important to prevent an increase of some pathogenic and spoilage bacteria.

Acknowledgements

This study was supported by the Graduate School of Prince of Songkla University and the Halal Institute of Prince of Songkla University.

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