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Effect of belimbing buluh *(Averrhoa bilimbi)* juice extract on oxidative stability and microbiological quality of spent chicken meat

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Abstract

This study evaluated the effects of *Averrhoa bilimbi* juice extract and storage temperature on lipid oxidation and microbial spoilage of spent chicken meat. Ten, 80 weeks old spent chickens were slaughtered, eviscerated and aged for 24 h at 4°C. Thereafter, the *Pectoralis major* muscles and right thighs were excised and marinated in either *A. bilimbi* juice extract, pure distilled water, or no marination (control) for either 4 or 9 h at room temperature or 9 or 24 h at 4°C. Lipid oxidation was monitored on the *Pectoralis major* muscles while the right thighs were assessed for Enterobacteriacea counts. Lipid oxidation was not significantly affected by the type or duration of marination. Marination showed a temperature dependent effect on Enterobacteriacea counts. At room temperature, samples that were marinated by distilled water showed significantly higher Enterobacteriacea counts than the control while those that were marinated with *A. bilimbi* juice extract showed no growth at both 4 and 9 h of marination. At chilled temperature, marination had no significant effects on the growth of Enterobacteriacea during the 9 or 24 h storage. These results indicated that *A. bilimbi* juice extract marinade has some antibacterial activities but works better when combined with refrigerated storage.

Introduction

It is well consented that the spoilage of meat and meat product occurs at different rates and extent depending on the condition of storage (Nakyinsige *et al*., 2014; Adeyemi *et al.*, 2015a; Sabow *et al.*, 2015). In Malaysia, poultry meat (whole chicken or parts) is commonly displayed at either room temperature or chilled conditions in wet markets and supermarkets, respectively. However, unlike freezing, both conditions are favorable for the proliferation of microorganisms as well as lipid oxidation, which cause meat spoilage and in turn results in major economic losses (Adeyemi *et al.,* 2015b; Sabow *et al*., 2015b). In comparison with red meat, poultry meat is more susceptible to deterioration due to its higher content of less stable unsaturated fatty acids (Adeyemi and Olorunsanya, 2012; Olorunsanya *et al.*, 2012).

Nowadays, extracts derived from herbs, fruits and plants are commonly used as marinade for meat and meat products, not only for the improvement of taste and flavor but also for the shelf life and quality control (Adeyemi *et al.*, 2011; Adeyemi *et al*., 2013). These natural extracts possess antioxidant and antimicrobial properties besides having the advantage of being readily accepted by consumers because they are considered natural and harmless (Sallama *et al*., 2004). Such extracts contain antioxidants and antimicrobial agents that may improve the oxidative stability and microbiological quality of different foodstuffs (Adeyemi and Olorunsanya, 2012; Adeyemi *et al*., 2013; Olorunsanya *et al.*, 2013).

Averrhoa bilimbi fruits, commonly known as belimbing buluh, have been reported to be rich in organic acids, phenols, vitamin C, tannins, and minerals (Kolar *et al*., 2011; Chowdhury *et al.*, 2012; Patil *et al*., 2013). *A. bilimbi* juice extract had

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previously demonstrated antibacterial effects against different strains of spoilage bacteria (Mackeen *et al.*, 1997; Nurul Huda *et al.*, 2009). It has also been reported to have antimicrobial effects in raw shrimps (Wan Norhana *et al*., 2009). However, the effects of *A. bilimbi* fruit juice marination on lipid oxidation and microbial counts of poultry meat products during storage are yet to be examined. Thus, this study was conducted to determine the effects of *A. bilimbi j*uice extract marination on the oxidative stability and microbiological quality of chicken breast meat under ambient and chilled display conditions.

Materials and Methods

Experiment sample preparation

A total of 10 corn fed spent chickens at 80 weeks old were obtained from the Poultry Unit, Faculty of Agriculture, Universiti Putra Malaysia. The birds were slaughtered at the slaughter house of the department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia according to the halal slaughtering procedure as outlined in the Malaysian Standard; MS1500: 2009 (Department of Standard Malaysia, 2009). Following the processing and dressing, the dressed carcasses were subjected to 24 h aging at 4°C. Upon completion of the aging, Pectoralis major muscle samples were then collected for lipid oxidation analysis while the right thighs were collected for microbiological quality assessment. The samples were subjected to three different marination treatments, which included marination with *A. bilimbi* juice extract, pure distilled water and without marination (control). The samples were displayed at either ambient temperature or chilled conditions.

Extraction of A. bilimbi *juice*

A. bilimbi was obtained from a local village in Kampung Melayu Subang, Selangor. The fruits were cut into small pieces and then homogenised in an Ultra-Turrax T5FU homogeniser (IKA-Labrortechnik Staufen, Germany). The crude extract was filtered using clean muslin cloth. The fresh *A. bilimbi* juice filtrate was used for marination.

Lipid oxidation measurement

Lipid oxidation was measured as 2-thiobarbituric acid reactive substances (TBARS) using QuantiChromTM TBARS Assay Kit (DTBA-100, BioAssay Systems, USA) followed the procedure previously described by Nakyinsige *et al.* (2015). Briefly, the samples were manually crushed in liquid nitrogen. Approximately, about 500 mg of samples were mixed with 5 ml ice-cold phosphate buffered saline (PBS) and homogenised for 20 s on ice using an Ultra-Turrax T5FU homogeniser (IKA-Labrortechnik Staufen, Germany). Homogenates (200 µl) were then mixed with 200 µl of ice-cold 10% trichloroacetic acid (TCA), incubated for 5 min on crushed ice and centrifuged at 21900 x g, 4°C for 5 min (Eppendorf Centrifuge, Mikro 22R Hettich, Germany). Malondialdehyde (MDA) standards were prepared by mixing 15 µl of the 1.5 mM MDA with 735 µl ultra-pure water to obtain a final concentration of 30 µM MDA. Subsequently, 300, 180, 90 and 0 µl of the 30 µM MDA were diluted with 0, 120, 210 and 300 µl of ultra-pure water to obtain the final 30, 18, 9 and 0 µM MDA standards, respectively. A 200 µl of thiobarbituric acid reagent were added to 200 µl of both samples and standards. The mixtures were incubated in a dry heating block at 100°C for 60 min. After cooling to room temperature, 100 µl of standards and samples were loaded in duplicate into wells of a clear flat- bottom 96-well plate (Greiner Bio-One, Germany) and optical density (OD) was determined at 535 nm using auto UV Xenon flash lamp microplate reader (infinite M200, Tecan, Austria). The OD of blank was subtracted from all standard and sample absorbencies and a standard curve was obtained by plotting the ΔOD_{535} against standard concentrations. TBARS (µM MDA equivalent) concentration of the samples was calculated according to Nakyinsige *et al.* (2014) from the equation: $[TBARS] = [(R_{sample} - R_{blank}) \div Slope\ (\mu M^{-1})] \times n$. Where R_{sample}, R_{blank} are the OD_{535nm} of the sample and dH_2O blank (STD4) and n is the sample dilution factor (n= 3 for deproteinated samples).

Microbiological analysis

Enumeration of Enterobacteriacea was carried out following the procedure of (Harrigan, 1998). About 5 g of meat samples were aseptically weighed and transferred to a stomacher bag containing 45 ml of 0.1% of peptone water (Merk KGaA, Germany) and homogenised using a stomacher (Inter Science, France) for 60 s. In triplicate, a 100 µl serial dilutions (1:10 diluent: peptone water) of homogenates were spread on the surface of dry Violet Red Bile Glucose Agar (Merk KGaA, Germany). The inoculated media was then incubated for 24 h at 37°C, followed by the colony count of the bacteria (AOAC, 1995).

Statistical analysis

Data were analyzed using the GLM procedure of Statistical Analysis System package (SAS) Version 9.1 software (Statistical Analysis System, SAS Institute Inc., Cary, NC, USA) and statistical significance was set at $p<0.05$. Duncan's multiple

Table 1. Differences in lipid oxidation (TBARS activity as mg MDA/kg meat) of spent chicken *Pectoralis major* muscles subjected to different types and duration of marination at room temperature

Time	Marination type			Effects		
	Control	dH_2O	*A. bilimbi*	Marination type	Time	Marination type x Time
4	0.053 ± 0.002^{b}	0.050 ± 0.001^{b}	0.052 ± 0.002^{b}	NS	S	NS
9	0.072 ± 0.008^{a}	0.064 ± 0.007^{a}	0.068 ± 0.007^{a}	NS	S	NS

dH_2O - distilled water; S: significant; NS: non-significant
* ^{ab} means within a column with different superscripts are significantly different within each marination type at $p<0.05$.

Table 2. Differences in lipid oxidation (TBARS activity as mg MDA/kg meat) of spent chicken *Pectoralis major* muscles subjected to different types and duration of marination at chilled temperature

Time	Marination type			Effects		
	Control	dH_2O	*A. bilimbi*	Marination type	Time	Marination type x Time
9	0.060 ± 0.005	0.057 ± 0.005	0.046 ± 0.001	NS	NS	NS
24	0.074 ± 0.008	0.061 ± 0.005	0.062 ± 0.005	NS	NS	NS

dH_2O - distilled water; NS: non-significant

range test was used to separate significantly different means.

Results

The results for the effect of marination on lipid oxidation in spent chicken *Pectoralis major* muscle at ambient temperature and chilled storage are shown in Table 1 and 2, respectively. Accordingly, lipid oxidation was not significantly affected by the type or duration of marination. However, at both ambient temperature and chilled storage, samples that were marinated with *A. bilimb*i juice extract had numerically lowered the MDA values compared to control samples and those that were marinated with dH_2O.

Treatments have a significant effect on the Enterobacteriacea counts (Tables 3 and 4). At room temperature, samples marinated with A. bilimbi juice extract showed no growth at both 4 and 9 h of marination. The Enterobacteriacea count increased with the length of marination time. Both control and those that were marinated with distilled water for 9 h had significantly higher counts than those that were stored for 4 h.

At chilled temperature, both treatment and marination time had no significant effects on the growth of Enterobacteriacea during the 9 or 24 h storage (Table 4). Although they are not statistically different, samples that were marinated with dH_2O showed the highest microbial counts, followed by the control group and lastly those marinated with A. bilimbi juice extract. The Enterobacteriacea counts increased as the time for marination increased.

Discussion

Effect of A. bilimbi *juice extract marination on lipid oxidation of spent chicken meat*

Lipid oxidation constitutes a major cause of non-microbial meat spoilage and therefore, delaying the onset of this autocatalytic process is important to extend the shelf life of meat (Nakyinsige *et al*., 2015; Sabow *et al*., 2016). Oxidative processes in meat leads to the degradation of lipids, which in turn, contributes to the deterioration in flavour, texture and colour of displayed meat products (Fernández-López *et al*., 2005; Adeyemi *et al*., 2015a; Adeyemi *et al*., 2015b). Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are effective in controlling lipid oxidation in meat (Adeyemi and Olorunsanya, 2012; Adeyemi *et al*., 2013). However, synthetic antioxidants are expensive and may be scarce especially in developing or underdeveloped countries (Adeyemi *et al*., 2011; Adeyemi and Olorunsanya, 2012). In addition, synthetic antioxidants are quite perilous and their toxicity is a problem of disquiet as they are suspected

Table 3. Differences in microbiological quality (Enterobacteriacea colony count as log cfu) of spent chicken thigh muscles subjected to different types and duration of marination at room temperature

Time	Marination type			Effects		
	Control	dH_2O	*A. bilimbi*	Marination type	Time	Marination type x Time
4	$1.6 \times 10^6 \pm 0.2^{ax}$	$0.3 \times 10^6 \pm 0.1^{ax}$	0^{ay}	S	S	NS
9	$8.0 \times 10^6 \pm 0.1^{bx}$	$6.0 \times 10^6 \pm 2.0^{bx}$	0^{by}	S	S	NS

dH_2O - distilled water; S: significant; NS: non-significant

[ab] means within a column with different superscripts are significantly different within each marination type at $p<0.05$.

[xy] means within a row with different superscripts are significantly different within each marination type at $p<0.05$.

to be carcinogenic (Patil *et al*., 2013), which increased the search for natural antioxidants. Therefore, this experiment was conducted to determine the effects of the *A. bilimbi* juice extract marinade on lipid oxidation in spent chicken meat. The lipid oxidation in spent chicken Pectoralis major muscle was not significantly affected by the different treatments or the duration of marination. However, at both ambient temperature and chilled storage, samples that were marinated with *A. bilimbi* juice extract had numerically lowered the MDA values compared to control samples and those that were marinated with dH_2O. It should be noted that lipid oxidation is an autocatalytic reaction whose rate increases as the reaction proceeds (Fernández *et al*., 1997). The process begin immediately after slaughtering and its magnitude depends on the balance between pro-oxidants and antioxidants that present in the system (Adeyemi *et al.*, 2015a; Sabow *et al.*, 2016). The MDA values in this study were very low (between 0.046 ± 0.001 and 0.074 ± 0.008 mg MDA/kg meat) compared to the threshold for the detection of off-odours and off-taste for humans, which according to Insausti *et al.* (2001) is TBARS values equal to or greater than 5 mg MDA/kg meat. According to Renerre *et al.* (1999), after the death of an animal, the inherent antioxidant defense system of its muscle will remain active for a few days. This explains why the MDA values in this experiment were very low and not significantly different.

The numerically lower MDA in samples marinated with *A. bilimbi* juice extract can be attributed to the additional amount of antioxidants contributed by the *A. bilimbi* juice extract marinade. *A. bilimbi* fruit contains a wide range of polyphenolic compounds, which include alkaloids, tanins, saponins, flavonoids, cardiac glycosides, glycosides, phytosterols, triterpenes and phenols. The juice extract of *A. bilimbi* has a potential antioxidant capacity. For instance, its ascorbic acid content when ripe has been reported by Kolar *et al*. (2011) to be 60.95 mg/100 g. The extract of *A. bilimbi* fruits have been reported to display remarkable total antioxidant capacity of 417.093 ± 6.577 mg/g ascorbic acid equivalent (AAE), total phenol of 106.16 ± 2.818 mg/g gallic acid equivalent (GAE) and total flavonoid contents of 276.73 ± 25.25 mg/g quercetin equivalent (QE) (Chowdhury *et al.*, 2012). Aqueous fruit extracts of *Averrhoa bilimbi* Linn. were also found to contain a noticeable amount of total phenols (Patil *et al.,* 2013). Phenols and polyphenolic compounds, such as flavonoids possess significant antioxidant activities, which play a major role in controlling the oxidation process (Adeyemi and Olorunsanya, 2012; Adeyemi *et al*., 2013).

Effect of A. bilimbi *juice extract marination on microbiological quality of spent chicken meat*

Bacterial spoilage is the main factor that determines food quality loss and shelf life reduction, thus making its prevention highly relevant to food processors (Nakyinsige *et al.*, 2015; Sabow *et al*., 2015; Sabow *et al*., 2016). Although synthetic or chemical additives have been widely used in the meat industry to inhibit microbial growth, the current trend is to decrease their usage due to the growing health concern among consumers (Fernández-López *et al.*, 2005). This has made it relevant to the search for natural antimicrobial agents of plant origin. Experiments were therefore conducted to investigate the antibacterial activity of the *A. bilimbi* juice extract marinade on Enterobacteriacea growth in spent chicken meat. Enterobacteriacea is large family of gram-negative bacteria such as *Salmonella, Escherichia coli, Yersinia pestis, Klebsiella* spp, *Shigella* spp, *Proteus* spp, *Enterobacter* spp, *Serratia* spp and *Citrobacter* spp. As shown in Table 3, treatments have significant effects on the Enterobacteriacea counts, with the samples marinated with *A. bilimbi* juice extract showing no growth at both 4 and 9 h of marination at room temperature.

Table 4. Differences in microbiological quality (Enterobacteriacea colony count as log cfu) of spent chicken thigh muscles subjected to different types and duration of marination at chilled temperature

Time	Marination type			Effects		
	Control	dH_2O	*A. bilimbi*	Marination type	Time	Marination type x Time
9	$0.015 \times 10^6 \pm 0.015$	$0.025 \times 10^6 \pm 0.015$	0	NS	NS	NS
24	$0.02 \times 10^6 \pm 0.02$	$0.065 \times 10^6 \pm 0.065$	$0.025 \times 10^6 \pm 0.005$	NS	NS	NS

dH_2O - distilled water; NS: non-significant

Similar to this finding, Wan Norhana *et al*. (2009) reported that washing and rubbing raw shrimps with bilimbi juice significantly reduced aerobic plate counts and *L. monocytogenes* Scott A compared to distilled water. Bilimbi has been previously reported to demonstrate an antibacterial effect against Pseudomonas aeruginosa (Mackeen *et al*., 1997). Furthermore, *Averrhoa bilimbi* fruit extracts was active against *Aeromonas hydrophila, Escherichia coli, Klebsiella pneumoniae, Saccharimyces cerevisiae, Staphylococcus aureus, Streptococcus agalactiae* and *Bacillus subtilis* (Nurul Huda *et al.*, 2009).

The antimicrobial activity of bilimbi can be attributed to its highly acidic pH. The pH of bilimbi extracts ranged from pH 0.9–1.5 (De Lima *et al*., 2001). Bilimbi fruit has a high level of oxalic (8.57–10.32 g/100 g) and ascorbic acid (32.23–60.95 mg/100 g) (De Lima *et al*., 2001). These organic acids are known to exhibit antimicrobial activity particularly in their undissociated form since they are able to effectively penetrate the cell membrane of bacteria in this form (Wan Norhana *et al*., 2009). The ability of *A. bilimbi* juice extract marinade to suppress the growth of Enterobacteriacea shows its potential as a viable antimicrobial agent in meat. Enterobacteriacea are the gram-negative bacteria and they are known to have greater resistance to many antibacterial compounds than gram positive bacteria (Shan *et al.*, 2007) probably due to the protective effect of their extra layers of outer membrane and periplasmic space (Zaika, 1988).

During storage at room temperature, the Enterobacteriacea counts increased with the length of marination time as the samples that were stored for 9 h exhibited significantly higher counts than those that were stored for 4 h for both control and those marinated with distilled water samples. The bacteria counts for these 2 groups ($8.0 \times 10^6 \pm 0.1$ and $6.0 \times 10^6 \pm 2.0$ cfu/g for control samples and those marinated with distilled water, respectively) exceeded the threshold for the spoilage of raw meat, which is 10^6–10^7 cfu/cm^2 or g according to International Commission for Microbial Specifications in Food (ICMSF) (ICMSF, 1986). At chilled temperature, both treatment and marination time did not have any significant effects on the growth of Enterobacteriacea during storage (Table 4). Numerically, the Enterobacteriacea counts increased with the length of marination time and at both 9 and 24 h of storage, samples that were marinated with dH_2O showed the highest microbial counts, followed by the control group and lastly, those that were marinated with *A. bilimbi* juice extract. At 24 h of storage at chilled conditions the Enterobacteriacea counts ranged from $0.02 \times 10^6 \pm 0.02$ to $0.065 \times 10^6 \pm 0.065$ cfu/g, which is very low.

Conclusion

The results of the present experiment showed that *A. bilimbi* juice extract marinade has some antibacterial activity but works better when combined with refrigerated storage. However, further work to extend these results to a larger number of species should be conducted before broader conclusion can be drawn.

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