Meat quality, colour stability, lipid and protein oxidation of broilers on diets supplemented with *Piper sarmentosum* leaves

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**ABSTRACT**

A feeding trial was conducted to investigate the effects of *Piper sarmentosum* leaves (PSL) on meat quality of broilers. The 144 commercial broiler chicks, one day old, were randomly assigned to 5 groups with three replicate cages. The five treatments were: 1) basal feed (8 chicks/cage), 2) basal feed (10 chicks/cage), 3) basal feed supplemented with 1% of PSL (10 chicks/cage), 4) basal feed supplemented with 2% of PSL (10 chicks/cage), and 5) basal feed supplemented with 3% of PSL (10 chicks/cage). The duration of dietary treatments was 42 days. The results showed that the group supplemented with 3% PSL had higher redness, yellowness, and cooking loss than the control group with 10 chickens/cage ($P < 0.05$). It was also observed that the 3% PSL treatment gave significantly lower lightness than the 0% and 1% PSL treatments with 10 chickens/cage ($P < 0.05$). Overall, the study indicated that PSL might improve chicken meat colour and protect against protein oxidation, but it has no potential in protecting against lipid oxidation in broiler filet.

**Key words:** Chicken, Lipid oxidation, Myoglobin oxidation, Protein oxidation, Wild betel

**INTRODUCTION**

Recently the search for natural plant compounds that could substitute for antibiotics in animal feed has become more intense. Wild betel (*Piper sarmentosum*) is an edible plant and widely found in tropical and subtropical countries. Its leaves and roots have been used in traditional medicine as expectorant and in the treatment of toothaches, fungoid dermatitis, asthma and pleurisy (Hafizah et al., 2010). Prior studies have demonstrated that extracts of this plant exhibit antimicrobial, antioxidant and antimutagenic activities *in vitro* (Hafizah et al., 2010; Lee et al., 2014; Boonla et al., 2014). The natural antioxidant from *Piper sarmentosum* leaves (PSL) is a superoxide scavenger, naringenin (Subramaniam et al., 2003) which is hypothesized to protect cells from oxidative stress by inhibiting production of reactive oxygen species (ROS) including hydrogen peroxide ($\text{H}_2\text{O}_2$). The results from study by Cavia-Saiz et al. (2010) confirmed that naringin and naringenin from PSL are effective in protecting against lipid oxidation and reduce DNA damage *in vitro*.

Oxidation is a major cause of quality deterioration of stored meat and meat products. It is well known that ROS and other non-radical species such as $\text{H}_2\text{O}_2$ and hydroperoxides ($\text{ROOH}$) have been recognized as potential initiators of oxidation (Morrisssey et al., 1998; Chaijan, 2008; Estévez, 2011). Lipid, pigment and protein oxidative processes in meat appear to be linked. The oxidation of one of these leads to the formation of chemical species that can exacerbate oxidation of the others (Chaijan, 2008; Faustman et al., 2010; Estévez, 2011). Oxidation of deoxymyoglobin to metmyoglobin causes brown discolouration in meat (Mancini and Hunt, 2005). Lipid oxidation leads to discolouration, drip losses, off-odour and off-flavour development, and decreases the nutritional quality and safety by the formation of secondary reaction products in foods after cooking and processing (Frankel, 1980; Morrisssey et al., 1998). Protein oxidation leads to loss of activity of muscle proteases and the functionality of myofibrillar proteins and reduced water-holding capacity, digestibility, and tenderness of meat (Estévez, 2011).

The numerous studies have shown the effects of natural plants on performance, oxidative status and oxidation in animals (Cetingul et al., 2016; Li et al., 2017; Punyatong et al., 2018). In addition, previous *in vitro* studies have concluded that *Piper sarmentosum* extracts might have potential as dietary supplements (Lee et al., 2014; Boonla et al., 2014), but their effects *in vivo* as feed additives are still largely unknown and require further study. Therefore, this research was aimed to examine the effects of *Piper sarmentosum* leaves, which possess antimicrobial and antioxidant activities, in broiler diet on meat quality and oxidation in meat.

**MATERIALS AND METHODS**

**Animals and experimental design:** In the experiments, totally 144 commercial broiler chicks at 1 day of age were randomly assigned to 5 treatment groups, each treatment with three replicate cages (about 50:50 mixtures of males and females).
females). Feed and water were supplied for consumption ad libitum. Basal starter (23% protein) (1 to 3 week) and finisher (20% protein) (4 to 6 week) diets were formulated according to the nutrient requirement recommendations of NRC (1994) for broiler chickens. The duration of dietary treatments was 42 days. The treatment groups were: 1) CONTROL. Basal feed supplemented with 0% *Piper sarmentosum* leaves (PSL) powder (8 animals/cage), 2) CONTROL. Basal feed supplemented with 0% PSL powder (10 animals/cage), 3) Basal feed supplemented with 1% PSL powder (10 animals/cage), 4) Basal feed supplemented with 2% PSL powder (10 animals/cage) and 5) Basal feed supplemented with 3% PSL powder (10 animals/cage).

**Preparation of plant powder:** The leaves of *Piper sarmentosum* were collected from Surat Thani province, in southern peninsular Thailand. The plants were washed thoroughly with distilled water and dried in a hot air oven at 40°C for 12hr. The dried samples were ground into powder.

**Meat samples:** At the end of the rearing period (42 days or 6 weeks), 4 birds (2 females and 2 males) with body weights near the group average from each replicate were slaughtered and cooled according to common practices, i.e. stunning, bleeding, scalding, defeathering, eviscerating and cooling. Then breast muscle (*Pectoralis major*) samples were collected for determining the meat quality. For the simulated retail display, meat samples were wrapped in oxygen permeable foil and displayed at 4°C under fluorescent light for 24hr per day for 0, 2 and 4 days.

**Analysis of pH and water-holding capacity:** The meat pH was measured at 1 and 24hr post-mortem in the breast muscle samples. The water-holding capacity of chicken meat was determined in terms of the drip loss, the thawing loss and the cooking loss, according to Uytterhaegen et al. (1994) at 24hr post-mortem.

**Colour measurements:** Chicken meat colour was determined at in terms of the CIE-Lab coordinates *L*, *a* and *b* with a Hunterlab Miniscan colour meter (D65 light source, 10° standard observer, 45°/0° geometry, 1-inch light surface, white standard) following standard procedures at 24hr post-mortem. Hue angle [Tan(1/2(b*/a*))] and chroma (a*+b*) were calculated according to Hunter and Harold (1987). The percentage of metmyoglobin (%MetMb) was determined using reflectance values at the wavelengths 520, 530, 570, 580 and 700 nm according to the formulas of Krzywicki (1979) as modified by Lindahl et al. (2001).

**Lipid oxidation:** Lipid oxidation was determined in samples on days 0, 2 and 4 of display. The lipid oxidation was assessed spectrophotometrically by the thiobarbituric acid reactive substances (TBARS) method based on Tarladgis et al. (1960), and is expressed as μg malondialdehyde (MDA) per g meat.

**Protein oxidation:** Protein oxidation was assessed by determining the carbonyl content of the samples according to the method of Ganhão et al. (2010). Protein carbonyls were measured following their covalent reaction with 2,4-dinitrophenylhydrazine (DNPH). This reaction leads to the formation of a stable 2,4-dinitrophenylhydrazone product, which is quantified spectrophotometrically at 370 nm, using a molar absorption coefficient of 21.0 (mM.cm). The total carbonyl content is expressed as nmol DNPH incorporated/ mg protein by using the formula according to Jongberg et al. (2011).

**Statistical analysis:** Lipid oxidation, %MetMb, and protein oxidation were analyzed using fixed effects models, the influencing factors being treatment, duration of display, and their interactions. Other data were analyzed for fixed effects of the treatment. Post-hoc tests were performed at a significance level of *P* < 0.05 using Duncan’s new multiple range test. The analyses were done using SPSS version 16.0 for Windows.

**RESULTS AND DISCUSSION**

**The pH and water-holding capacity:** To accurately assess the meat quality, we compared the pH and water-holding capacity (drip loss, thawing loss and cooking loss) of chicken breast fillets between the treatments. Although it was found that the 3% PSL treatment gave higher cooking loss than the 0% and 1% PSL treatments (*P* < 0.05), no significant differences between the various treatments were observed in drip loss, thawing loss or total water loss (*P* > 0.05). No significant differences in pH at 1 or 24hr post-mortem were observed (*P* > 0.05) (Table 1). The pH of meat, which is a measure of glycolysis, can be affected by several factors including stress ante-mortem, fasting, chilling temperature and animal factors (McGeethin et al., 2001). This study found no difference in pH 1 and pH 24hr among all groups, this might resulting from there is no effect of those factors on meat glycolysis. However, Warriss (2010) states that %drip loss of meat is related with ultimate pH (pH 24hr), meat with higher ultimate pH will be less in %drip loss or more water-holding capacity.

**Colour and colour stability:** Table 1 clearly demonstrates that the meat redness (*a*) and yellowness (*b*) for the 3% PSL group were higher than with 0 and 1% PSL treatments, with 10 birds/cage (*P* < 0.05). It is also observed that the 3% PSL treatment gave significantly lower lightness (*L*) than the 0 and 1% PSL treatments with 10 birds/cage (*P* < 0.05). Higher chroma was observed in the 3% PSL group relative to the other groups, and lower hue was observed in the 2% PSL group and in the control with 8 animals/cage (*P* < 0.05). These instrumental colour readings clearly suggest beneficial effects of 2 and 3% PSL in improving the colour of broiler meat.

The discolouration in meat is often attributed to the amount of surface area covered by metmyoglobin
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Table 1: Effects of supplementation with *Piper sarmentosum* leaves (PSL) on breast meat quality and colour of broilers (mean ± SD; n=12).

<table>
<thead>
<tr>
<th>Item</th>
<th>0%</th>
<th>0%</th>
<th>1%</th>
<th>2%</th>
<th>3%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 birds/cage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>6.00 ± 0.22</td>
<td>6.15 ± 0.23</td>
<td>5.97 ± 0.02</td>
<td>5.94 ± 0.14</td>
<td>6.01 ± 0.05</td>
<td>0.735</td>
</tr>
<tr>
<td>pH&lt;sub&gt;24h&lt;/sub&gt;</td>
<td>7.95 ± 2.71</td>
<td>5.72 ± 0.09</td>
<td>5.71 ± 0.14</td>
<td>5.79 ± 0.12</td>
<td>5.78 ± 0.13</td>
<td>0.843</td>
</tr>
<tr>
<td>%Drip loss (DL)</td>
<td>5.22 ± 1.10</td>
<td>4.35 ± 0.45</td>
<td>4.21 ± 0.66</td>
<td>4.6 ± 0.94</td>
<td>4.1 ± 2.05</td>
<td>0.163</td>
</tr>
<tr>
<td>%Thawing loss (TL)</td>
<td>10.73 ± 2.67</td>
<td>9.72 ± 0.09</td>
<td>9.71 ± 0.14</td>
<td>9.79 ± 0.12</td>
<td>9.78 ± 0.13</td>
<td>0.134</td>
</tr>
<tr>
<td>%Cooking loss (CL)</td>
<td>27.58 ± 2.63</td>
<td>25.72 ± 0.09</td>
<td>25.63 ± 0.45</td>
<td>25.52 ± 0.66</td>
<td>25.41 ± 0.94</td>
<td>0.118</td>
</tr>
<tr>
<td>L*</td>
<td>59.72 ± 3.15</td>
<td>61.16 ± 1.92</td>
<td>60.41 ± 1.68</td>
<td>58.47 ± 2.69</td>
<td>58.18 ± 2.43</td>
<td>0.018</td>
</tr>
<tr>
<td>a*</td>
<td>6.81 ± 0.99</td>
<td>5.63 ± 1.09</td>
<td>6.06 ± 0.61</td>
<td>6.94 ± 0.93</td>
<td>7.04 ± 1.30</td>
<td>0.003</td>
</tr>
<tr>
<td>b*</td>
<td>21.73 ± 1.57</td>
<td>21.59 ± 1.10</td>
<td>23.17 ± 1.37</td>
<td>22.41 ± 0.94</td>
<td>25.43 ± 1.83</td>
<td>0.000</td>
</tr>
<tr>
<td>Chroma</td>
<td>22.79 ± 1.55</td>
<td>22.32 ± 1.29</td>
<td>23.96 ± 1.34</td>
<td>23.48 ± 0.92</td>
<td>26.41 ± 1.92</td>
<td>0.000</td>
</tr>
<tr>
<td>Hue</td>
<td>1.27 ± 0.04</td>
<td>1.32 ± 0.03</td>
<td>1.32 ± 0.03</td>
<td>1.27 ± 0.04</td>
<td>1.30 ± 0.04</td>
<td>0.004</td>
</tr>
</tbody>
</table>

**Within a row, mean values with different superscripts differ significantly at P < 0.05.**

**Mean values within same time of display with a different superscript are significantly different at P < 0.05.**

**Lipid oxidation:** The lipid oxidation in meat allows investigating the contribution of plant leaves to the oxidative stability of meat during storage (Fig 3). There were effects (MetMb) (Mancini and Hunt, 2005). The %MetMb can be estimated from light reflectance at specific wavelengths (Krzywicki, 1979), and from the rate of decrease in $a^*$ or red colour. A higher $a^*$ value indicates more oxymoglobin on the meat surface, and a greater decrease in $a^*$ with time corresponds to greater formation of metmyoglobin.

In this study, the treatments did influence $a^*$ while the display time did not. Moreover, no interaction between treatment and display time was observed. At day 4 of display, $a^*$ value for the 3% PSL group was higher than for the other treatments ($P < 0.05$) (Fig 1). The %MetMb formation at days 2 and 4 of display (Fig 2) was lower ($P < 0.05$) in the 2% PSL group than in the 1% PSL group, but not different from the other treatments ($P > 0.05$). However, at day 0 of display, there were no significant differences between the groups. This indicates that oxymyoglobin in meat from 1% PSL group quickly turned to MetMb, producing an undesirable brown colour during storage. The 2% PSL treatment gave less MetMb formation than the 1% PSL treatment, and the rate of decrease in $a^*$ value for the 3% PSL treatment was the lowest during cold storage, suggesting that 2 or 3% PSL in the diet might inhibit myoglobin oxidation and improve colour stability in chicken breast fillets under light in aerobic conditions during refrigerated storage.

**Lipid oxidation:** The lipid oxidation in meat allows investigating the contribution of plant leaves to the oxidative stability of meat during storage (Fig 3). There were effects
of storage time, level of PSL, and their combination, on lipid oxidation \( (P < 0.05) \). The results showed that the secondary product from lipid oxidation, malondialdehyde (MDA), assessed spectrophotometrically by the thiobarbituric acid reactive substances (TBARS) method, increased by 4 days of cold storage \( (P < 0.05) \). In line with our previous study the TBARS values significantly increased by 10 days of storage in beef muscles (Pastsart et al., 2013). After 4 days of display, the TBARS values in meat from 1% PSL group were higher than with control and 3% PSL treatments \( (P < 0.05) \). This might suggest that the supplementation of PSL up to 3% has no potential to protect against lipid oxidation in broiler fillet. However, the peroxide value that indicates primary products from lipid oxidation could also be assessed on investigating the effects of a diet on oxidative stability of meat during storage. In addition, higher concentrations of PSL in diets, up to 5%, could also be considered in further studies.

The key factors affecting lipid oxidation in meat are total fat content, fatty acid composition and iron (Fe) (Min et al., 2008). Fe is the transitional metal in myoglobin pigment and has been suggested to play important roles as a catalyst and an initiator of lipid oxidation in vivo and in vitro via the Fenton reaction. This produces hydroxyl radicals (•OH) and ferrylmyoglobin from interactions of H\(_2\)O\(_2\) and metmyoglobin. The free radicals and ferrylmyoglobin can abstract a hydrogen atom from a polyunsaturated fatty acid, which initiates lipid oxidation. It is well known that chicken meat has lower myoglobin and fat contents than pork or beef (Min et al., 2008), so chicken meat has the least lipid oxidation among these. In accordance with Rhee et al. (2018), TBA values and heme iron content of frozen raw samples were higher for beef and pork than for chicken.

**Protein oxidation:** Determination of the protein carbonyl content by the DNPH method is the most common procedure to assess protein oxidation in meat and meat products (Estévez, 2011). Protein oxidation is initiated by several reactive oxygen species and is affected by the same factors that also influence lipid oxidation. In the present study, the protein carbonyl content significantly increased with time of display \( (P < 0.05) \) (Fig. 4), in line with several prior studies that have reported increasing carbonyl content during chilled storage in beef (Rowe et al., 2004; Lindahl et al., 2010), pork (Lund et al., 2007) and turkey (Mercier et al., 1998). On day 4 of display, it was observed that protein oxidation was lowest in the 2% PSL treatment group \( (P < 0.05) \). This confirms that 2% PSL in the diet might inhibit protein oxidation occurring after 4 days of cold storage.

**CONCLUSION**

Supplementation of broiler diets with up to 3% of *Piper sarmentosum* leaves had remarkable potential to improve poultry meat quality, specifically chicken breast fillets, in terms of yellowness and redness, while paleness decreased, colour stability improved and protect against protein oxidation. However, the supplementation with *Piper sarmentosum* leaves had no positive effects on meat lipid oxidation, in broilers subjected to a 6-week dietary treatment.

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