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Effect of dried mao pomace (DMP) and lactic acid bacteria (LAB) on meat lipid oxidation and meat quality in goats with post-mortem aging time

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Abstract

Sixteen male goats were divided into four groups and individually penned for 80 days. Over this period, each group was fed untreated silage (CO), dried mao pomace-treated silage (DMP), lactic acid bacteria-treated silage (LAB) or DMP plus LAB silage (DMP+LAB). After being fed for 80 days, the goats were slaughtered and the *Longissimus lumborum* (LL) muscle was sampled. The goats fed with LAB-treated silage had higher carcass dressing (%) than the other diets. The TBARS values were lower in meat from all of the goats fed treated silage compared to those fed untreated silage at 5 days of aging. Higher H^* values were observed in meat from untreated silage-fed animals compared to meat from the goats receiving other treatments. We conclude that under conditions that promote oxidative stress in meat, DMP and LAB-treated silage can improve the oxidative stability of meat compared to untreated silage.

Introduction

The demand for goat meat has been increasing in recent years, which may be caused by the leaner and lower fat content of goat compared to other types of red meat (Park *et al*., 1991; Webb *et al*., 2005). Meat quality is important in animal production to meet consumer demands, which include such characteristics as meat colour, flavor and nutritional value (Liu *et al*., 1995). The lipid oxidation of meat during post-mortem aging has been reported to result in the deterioration of meat colour, odour, texture and nutritive value (Fernandez *et al*., 1997; Luciano *et al*., 2009). The reduction of lipid oxidation during meat storage can be improved by dietary supplementation of antioxidants. Recently, there has been an increased interest in applying natural antioxidants in livestock, and it has been demonstrated that meat shelf-life and quality can be improved by natural antioxidants added to animal diets (Devatkal and Naveena, 2010; Karami *et al*., 2011a; Karami *et al*., 2011b; Moyo *et al*., 2012; Falowo *et al*., 2014). By-products from the processing of plant food are one of the richest sources of natural antioxidants for which antioxidant activity has been reported (Bhushan *et al*., 2008; Iora *et al*., 2015).

Antidesma is a diverse genus of tropical plant that occurs in Southeast Asia, Australia, tropical Asia, and islands in the Pacific Oceans. The fruits are acidic, like cranberries, and less acidic and slightly sweet when fully ripe. Mao luang (*Antidesma thwaitesianum* Muell Arq.) is the most suitable variety for beverages in Thailand. Mao juice and mao wine have become increasingly popular, and waste products, such as mao pomaces from the process, are plentiful. Moreover, several researchers have revealed that mao pomace contains organic acid, amino acid and sugar (Samappito and Butkhup, 2008). Puangpronpitag *et al*. (2008) also found that mao marcs contained an abundant source of polyphenols and suggested that these polyphenolic compounds exhibited similar antioxidant potential to grape seed extract. Phenolic compounds have been successfully used to reduce lipid oxidation in meat products (Estevez *et al*., 2005; Balasundram *et al*., 2006; Karami *et al*., 2010).

The production of silage is a practical way to preserve forage under anaerobic conditions to support livestock throughout the year. Silage should contain a low enough pH value (<4.2) and high enough lactic acid content to maintain the good quality during the ensiling period (McDonald *et al*., 1991). The application of an acid additive results in an immediate drop in pH, which inhibits the growth of undesirable bacteria in the ensiling process (McDonald *et al*., 1991). However, using a strong acid is unsafe during handling and corrosive to equipment. The fermented juice of epiphytic lactic acid bacteria (LAB) has been recommended as a silage additive for tropical grass silage (Bureenok *et al*., 2007; Bureenok *et al*., 2012). Lactic acid bacteria (LAB), such as *Lactobacillus* and *Streptococcus*, have been reported to exhibit

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antioxidative activity (Said and Gilliland, 2005; Wu *et al*., 2010). Wang *et al.* (2006) also reported that the antioxidative activity of soymilk fermented with LAB was higher than that found in the unfermented soymilk and the antioxidative activity increased as the fermentation period was prolonged. However, this additive can be ineffective because of the low substrate level of the legume. As mentioned above, dried mao pomace (DMP) is acidic and contains some organic acid and sugar that may have the potential as a silage additive to inhibit the growth of undesirable bacteria and stimulate LAB growth during the ensiling process. Based on the fact that both DMP and LAB have antioxidative capacities, the aim of this study was to investigate the effect of applying DMP alone and combined with LAB on the meat quality of goats fed with Stylo legume silages.

Materials and Methods

Animals and management

Sixteen male goats crossbred Boer x Saanen goats (mean initial body weight ±SD (13.03 ± 1.64 kg)) were used in a completely randomized design. Dried mao pomace was obtained after juice extraction from a fruit juice factory (88.32% DM, 6.98% CP, 49.45% NDF, 32.57% ADF and 7.19% ash on a dry matter basis; total phenolic content (110 mg gallic acid/g DM; pH 3.7)). The fermented juice of lactic acid bacteria (LAB) were prepared from fresh Stylo legumes using the method of Bureenok *et al.* (2012). The fermented juice of LAB was applied at 5.58 $\log_{10}$ CFU g^{-1} of fresh matter. Silages were untreated (CO) or treated with 10% DMP (DMP), 1% LAB (LAB), or 10% DMP plus 1% LAB (DMP+LAB). All silage treatments were supplied to the animals ad libitum. The animals were fed a concentrated base diet intake at 1.5% of body weight, and clean drinking water was provided. The chemical composition of the experimental diet is shown in Table 1. All procedures were approved by the Ethical Principles for the Use of Animals for Scientific Purposes of the National Research Council of Thailand.

Slaughter procedure and post mortem aging

At the end of the 80-day fattening period, the goats were slaughtered after electrical stunning at the experimental slaughter unit. After slaughtering, non-carcass components (head, skin, feet, trachea and lungs, liver, heart, spleen, pancreas, kidney, gastro-intestinal tract, diaphragm and testicles) were removed, and then the hot carcasses were weighed. The dressing percentage was calculated using the slaughter weight. The carcasses were then split along the vertebral column into left and right halves. The left sides of the carcasses were individually placed in polystyrene trays and stored at 4°C in the chamber room for post mortem aging.

Chemical analysis

Feed samples were sampled, and the samples were analysed for dry matter (DM) and ash according to the standard methods of AOAC (1995). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were determined by the method of Van Soest *et al.* (1991) and were expressed inclusive of residual ash. Nitrogen (N) was analysed using the Kjeldahl method, and crude protein (CP) was calculated as N × 6.25. The silage pH was determined with a pH meter (Lab 860, Schott). Lactic acid and volatile fatty acid; VFA (i.e., acetic, propionic and butyric acids) content was measured by HPLC (Aminex® HPX-87H, 300 mm × 7.8 mm i.d; column temperature, 40°C flow rate, 0.60 ml/min, Shimadzu Co., Ltd., Kyoto, Japan). The total phenolic content was determined according to the method described by Waterhouse (2005). The results are expressed in gallic acid equivalents (mg gallic acid/ g DM).

Meat quality analysis

A meat sample from the Longissimus lumborum (LL) muscle was ground to a homogeneous consistency using a bowl chopper. The freeze-dried sample was then blended with a 2-mm screen to produce a fine powder. One subsample was analysed for DM, CP, EE and ash concentrations as described by AOAC (1995).

After slaughter, a sample cutting for meat quality was made from the LL muscle and stored at 4°C for 24 h. The instrumental meat quality characteristics investigated in the current study were the carcass pH, meat colour (L^*, a^*, and b^*), drip loss (%), cooking loss (%), shear force (N), and thiobarbituric acid reactive substances (TBARS). The carcass pH was measured at 24 h post-slaughter using a digital pH meter (SI analytics GmbH). The meat colour was measured on the cut surface of 2.5-cm-thick samples from a fat-free area. The colour stability of the raw meat was measured 24 h after storage at 4°C. The colour was evaluated using a MiniScan EZ (Hunter Associates Laboratory, Inc. Reston, VA) and reported using the complete International Commission on Illumination (CIE) system colour profile of L^* (lightness), a^* (redness) and b^* (yellowness).

Drip loss was determined using the method described by Honikel (1998). Briefly, the meat samples were weighed and then suspended in a plastic bag without any contact with the bag. After a 24-h

Table 1. Chemical composition in experimental diets (n=4)

Item	Concentrate	CO	DMP	LAB	DMP+LAB
DM (%)	89.51	34.50	40.00	36.50	38.50
CP (%DM)	16.05	10.74	11.60	12.75	10.89
NDF (%DM)	25.75	68.43	64.96	66.51	74.12
ADF (%DM)	45.65	45.15	40.41	41.46	53.22
Ash (%DM)	7.02	11.25	14.18	13.3	17.46
Total phenolic content (mg gallic acid/g DM)	-	4.41	7.25	4.15	7.88
Silage profiles					
pH	-	4.41	3.95	4.25	4.02
Lactic acid (%DM)	-	0.69	1.15	3.87	2.78
Acetic acid (%DM)	-	0.99	1.89	2.37	3.61
Propionic acid (%DM)	-	0.13	0.11	0.16	0.67
Butyric acid (%DM)	-	0.63	0.20	0.43	0.46

CO = untreated silage, DMP = dried mao pomace, LAB = lactic acid bacteria, DMP+LAB = dried mao pomace plus lactic acid bacteria.

storage period at 4°C, the samples were gently dried with paper towels and reweighed. The drip loss (%) was estimated by the ratio of the weight loss (initial weight - final weight) to the initial sample weight. For cooking loss, the meat samples were put in a tightly sealed plastic bag and cooked in the water bath at 80°C for 10 min. Then, the samples were cooled and removed from the bag, blotted with filter paper, and weighed to determine the loss as a percentage of the initial weight. After that, the cooked meat samples were cut parallel to the muscle fibre direction with a cross section of 1.0×1.0 cm for the shear force analysis using a texture analyzer (Lloyd Instrument Ltd. Segensworth Fareham, England) equipped with a Warner Bratzler (WB) shear force apparatus. The Warner Bratzler shear force values were reported as the mean of all of the core values of the samples.

Meat lipid oxidation

The TBARS assay was performed as described by Buege and Aust (1978). In brief, the samples (10 g) were homogenized with 30 mL of distilled water for 2 min. The homogenate (2 mL) was then mixed with 4 mL of thiobarbituric acid/trichloroacetic reagent and 100 µL of 10% butylated hydroxyanisole in a glass test tube. The mixture was incubated for 15 min in a 100°C water bath, allowed to cool in the cold water and centrifuged at 4000 rpm for 10 min. The absorbance of the supernatant was then read against a blank containing the same reagents at 531 nm using a spectrophotometer. The TBARS values were calculated from a standard curve of malondialdehyde (MDA) and expressed as mg MDA/kg sample.

Post mortem aging

The meat colour was measured on day 1, 3, and 5 of aging. Drip loss (%), cooking loss (%), shear force (N), and thiobarbituric acid reactive substances (TBARS) were determined on day 1 and 5 of aging the sample using the method described above. The hue angle (H^*) and chroma (C^*) were calculated as $\tan^{-1}(b^*/a^*)$ and $(a^{*2}+b^{*2})^{1/2}$, respectively (Hunter and Harold, 1987).

Statistical analysis

The data of the carcass characteristics, meat quality and colour stability of the meat were analysed using the GLM procedures of SAS (Statistical Analysis System, SAS Institute Inc., Cary, N.C). Significantly different means were elucidated using Duncan's new multiple range test. To assess the effects of diets and post mortem aging periods and their interactions, the data were analysed using two-way analysis of variance (ANOVA) followed by Duncan's new multiple range test. A difference was considered significant at $p < 0.05$.

Results and Discussion

Carcass yield and chemical composition

The carcass characteristics and meat chemical compositions are presented in Table 2. The carcass dressing percentage was higher in goats fed with LAB-treated silages compared with the other diets ($p < 0.05$). In contrast, the liver, heart, lung, spleen and kidney were not affected ($p > 0.05$) by the feeding treatment diets. The moisture, protein and ether extract content in meat samples did not differ ($p > 0.05$) among treatments; however, a higher ash content ($p < 0.05$) was observed when feeding the animals silage treated with DMP plus LAB. Many earlier research studies reported that the supplementation of phenolic content (Krehbiel *et al*., 2003; Ahmed *et al*., 2014; Kotsampasi *et al*., 2014; Mlambo and Mapiye, 2015) and LAB (Krehbiel *et al*., 2003; Ahmed *et al*., 2014) did not affect the dressing percentage, which is consistent with our findings.

pH

The pH values for LL muscle decreased after slaughter (Table 3). After slaughter, the pH value of goat meat fed the DMP-treated silage was lower than

Table 2. The effect of Stylo silages treated with dried mao pomace and fermented juice of epiphytic lactic acid bacteria (LAB) on carcass and chemical compositions of meat goats

	CO	DMP	LAB	DMP+LAB	SEM	P value
Slaughter weight (kg)	15.50	15.55	15.50	13.63	0.56	0.5619
Carcass weight (kg)	6.40 [ab]	6.44 [ab]	7.15 [a]	5.25 [b]	0.67	0.0606
Carcass dressing (%)	41.35 [b]	41.34 [b]	46.16 [a]	38.75 [b]	0.56	0.0040
Liver (kg/100 kgBW)	1.93	1.90	1.50	1.89	0.07	0.1879
Heart (kg/100 kgBW)	0.82	0.80	0.74	0.85	0.04	0.8329
Lung (kg/100 kgBW)	1.12	1.27	1.26	1.15	0.07	0.8344
Kidney (kg/100 kgBW)	0.45	0.45	0.45	0.45	0.03	0.9865
Chemical composition (%)						
Dry matter (%)	21.53	21.98	22.98	20.64	0.25	0.1925
CP (%DM)	14.35	14.89	14.5	14.82	0.11	0.3195
Ether Extract (%DM)	6.20	6.11	6.03	5.48	0.24	0.7187
Ash (%DM)	6.85 [b]	6.40 [b]	6.70 [b]	7.97 [a]	0.16	0.0220

[a,b] Within a row, different superscripts indicate the difference between dietary treatments ($p<0.05$).
CO = untreated silage, DMP = dried mao pomace, LAB = lactic acid bacteria, DMP+LAB = dried mao pomace plus lactic acid bacteria.
SEM = Standard error of the mean.

those of the other treatments. The pH value at 24 h post mortem was higher in meat goats fed with DMP plus LAB treated silage compared with those in goats fed the other diets ($p < 0.05$). The phenolic compound in DMP may inhibit the activity of LAB (Rodiriguez *et al*., 2009). Gram positive bacteria are less resistant to polyphenols due to cell wall structure (Puupponen-Pimiä *et al*., 2005). Also, level of phenolic compound might have an influence on LAB growth (Marsilio and Lanza, 1998). The ultimate pH is determined 24 h post-slaughter, and good quality meat has a pH of 5.4-5.7 (Hannula and Puolanne, 2004). In this study, the ultimate pH of goat meat fed with DMP silages was in the same range. In addition, the ultimate pH in this study was not different from the other research (Swan *et al*., 1998; Husain *et al.*, 2000; Simela *et al.*, 2004).

Drip and cooking loss of the LL muscle

The drip and cook loss percentage did not significantly differ between treated silage at 24 h. However, the treatment showed a decrease in the drip and cook loss with the storage period. Goat meat is less juicy than lamb, which is due to the lower fat content in goat meat (Lee *et a*l., 2008). The cooking losses from goat meat are of interest, because the remaining water in the cooked product is the major meat quality attributes in term of juiciness (Forrest *et al*., 1975). Usually, cooking loss of product are often close to or over 35% (Babiker *et al*., 1990; Swan *et al*., 1998; Dhanda *et al.*, 1999). In agreement with this report, the cooking loss levels in goat meat at 24 h in the current study ranged from 31.95 to 35.30 and decreased to 24.08-28.98 by day 5 of aging. However, the drip and cooking loss were not affected by the treatments.

Warner-Bratzler shear force value

The shear force values of meat after 24 h post mortem and 5 days of aging were not influenced by dietary treatment. This is in agreement with earlier researches, which reported that shear force values of meat after 24 h post mortem was not influenced by dietary antioxidant treatment (Karami *et al.*, 2011a; Karami *et al.*, 2011b). After 5 days of aging, the shear force values were decreased from 69.73-94.83 to 36.97-42.17 N, but the value was not significantly different between treatments. Bickerstaffe et al. (2001) noted that the shear force values of cooked meat samples accurately reflect the consumer perception of tenderness, and meat samples classified as "very tender" by consumers had a mean shear force value of 50 N. Kannan *et al.* (2002) reported that a significant improvement in chevon tenderness occurred within the first 4 days of refrigerated storage and was not improved thereafter. After 5 days of aging, the Warner Bratzler shear force results in the current study were similar with the results reported by Karami *et al*. (2011b) for goat meat after 14 days of aging.

Lipid oxidation in the meat

The lipid oxidative stability values can be found in Table 3. At 1 day of aging, goat meat fed the DMP-treated silage had a significantly ($p < 0.05$) lower TBARS score (mg malonaldehyde/kg meat product) than other silage, which would imply that the addition of DMP could slow the onset of oxidative rancidity. After 5 days of aging, the TBARS values did not significantly differ among the various silage

Table 3. Effect of Stylo silages treated with dried mao pomace and fermented juice of epiphytic lactic acid bacteria (LAB) on meat quality characteristics of goat meat at 1 h, 24 h and 5 days of aging

	CO		DMP		LAB		DMP+LAB		SEM	P value
At 1h										
pH	6.57	ab	6.37	b	6.54	ab	6.62	a	0.04	0.1440
At 24h										
pH	5.8	b	5.76	b	5.81	b	6.00	a	0.03	0.0434
Drip loss (%)	12.08		12.65		7.68		6.60		0.94	0.0949
Cook loss (%)	35.30		32.20		31.95		35.30		0.66	0.1732
Shear force (N)	89.9		94.83		74.70		69.73		4.43	0.2445
TBARS	1.04	b	0.55	b	1.76	a	1.78	a	0.08	0.0009
At 5 d aging										
Total weight loss (%)	35.07		39.02		22.09		27.79		3.55	0.3773
Drip loss (%)	3.80		4.75		6.40		5.65		0.78	0.6778
Cook loss (%)	28.98		24.08		24.50		26.33		1.38	0.5965
Shear force (N)	41.58		36.97		42.17		38.97		2.36	0.9344
TBARS	3.38	a	1.00	b	1.89	b	1.84	b	0.21	0.0224

[a,b] Within a row, the different superscripts indicate the difference between dietary treatments ($p<0.05$).
CO = untreated silage, DMP = dried mao pomace, LAB = lactic acid bacteria, DMP+LAB = dried mao pomace plus lactic acid bacteria.
SEM = Standard error of the mean.
TBARS = thiobarbituric acid reactive substance value (mg DMA/kg meat).

treatments. The goat meat fed the untreated silage had significantly ($p < 0.05$) higher TBARS values than the treated silage groups.

There was a tendency for the aging treatment to affect the TBARS value, with the 5 days of aging treatment resulting in more oxidation than the 24 h treatment. These values agree with the results of other studies that oxidation increases with aging time in chevon cuts (Kannan *et al*., 2001; Emami *et al*., 2015). In this study, the TBARS assays showed that the addition of DMP slows the lipid oxidation process. This is in agreement with the results of several other researchers who also found that lipid oxidation was inhibited by feeding the natural antioxidant diet (which contained phenolic acid) to the animals (Kotsampasi *et al*., 2014; Emami *et al*., 2015; Zhong *et al*., 2015). At 24 h, all of the LAB-treated silage resulted in significantly higher TBARS values of goat meat compared with the untreated and DMP-treated silages. However, after 5 days of aging, the TBARS value of the goat meat from goats fed with this silage did not differ from the DMP-treated silage. Many researchers have reported that the fermented juice of the LAB additive contained a mixed culture of LAB species (Yahaya *et al*., 2004; Hiraoka *et al*., 2006). Virtanen *et al.* (2007) reported that milk fermented with mixed cultures of LAB resulted in higher radical scavenging activity than milk fermented with single bacterial strains. Ahmed *et al*. (2014), who studied the carcass characteristics and meat quality of beef steers fed fermented liquid whey inoculated with mixed lactic acid bacteria, reported that inoculated silage can improve the lipid oxidation of meat.

Colour stability

The colour of the LL muscle was lighter ($p < 0.05$) for goats fed DMP-treated silage and its combinations than for goats fed the LAB-treated silage (Table 4). The red colour *(a^*)* values and Chroma index *(C^*)* were not significantly different among treatments. The yellow colour *(b^*)* was lower ($p < 0.05$) for goats fed the LAB-treated silage than the control but was not different among treatments. This is in agreement with the results of Rojas and Brewer (2008), who reported that the b^* value of beef patties decreased when containing natural antioxidants. The red colour were decreased ($p < 0.05$) at 5 days compared to 1 and 3 days of the post mortem aging period. No differences were found on the lightness of the meat. Generally, the lightness is not considered an appropriate index of meat discolouration (Sapp *et al*., 1999; Dunne *et al*., 2005). The loss of redness *(a^*)* and changes in yellowness *(b^*)* over a period of storage have been used to describe meat browning (Mancini and Hunt, 2005; Morrissey *et al*., 1994). However, the hue angle *(H^*)* value, which is calculated from the a^* and b^* values, is used to indicate the more realistic perspective of meat browning than a single colour (Luciano *et al*., 2011). The H^* value was higher ($p < 0.05$) for the control than the other treatment. Emami *et al.* (2015) also reported that meat from goat kids fed phenol-rich pomegranate seed pulp showed a lower H^* value compared to the meat from goats fed a control diet. The decrease in the a^* and C^* values and the increase in the H^* value are used to demonstrate the meat discolouration due to their positive relation with metmyoglobin concentration in the meat (Luciano *et al.*, 2011). This study found

Table 4. Effect of the silage additive and storage time on colour stability of raw LL muscle at 1, 3 and 5 days of aging

	Diets				Storage time				P value		
Item	CO	DMP	LAB	DMP+LAB	1	3	5	SEM	Diet	Time	Diet × Time
L*	42.59 ^{ab}	44.92 ^{a}	40.71 ^{b}	45.7 ^{a}	44.66	43.08	42.7	0.52	0.0078	0.2877	0.7850
a*	14.61	15.28	14.69	14.84	15.99 ^{x}	15.53 ^{x}	13.04 ^{y}	0.23	0.6866	<0.0001	0.6454
b*	11.08 ^{a}	10.27 ^{ab}	9.21 ^{b}	9.52 ^{b}	9.93 ^{xy}	10.89 ^{x}	9.24 ^{y}	0.21	0.0127	0.0086	0.6127
H*	37.24 ^{a}	33.97 ^{b}	32.48 ^{b}	32.23 ^{b}	32.46 ^{y}	34.27 ^{xy}	35.21 ^{x}	0.41	0.0004	0.0308	0.9243
C*	18.34	18.44	17.35	17.66	18.47 ^{x}	19.37 ^{x}	16.00 ^{y}	0.28	0.4835	<0.0001	0.6013

$^{a, b, c}$ Within a row, the different superscripts indicate the difference between silage treatments ($p<0.05$).
$^{x, y, z}$ Within a row, the different superscripts indicate the difference between storage time ($p<0.05$).
The values are least- square means.
CO = untreated silage, DMP = dried mao pomace, LAB = lactic acid bacteria, DMP+LAB = dried mao pomace plus lactic acid bacteria.
H^* = hue angle (calculated as $\tan^{-1}(b^*/a^*)$, C^* = chroma calculated $(a^{*2}+b^{*2})^{1/2}$.
SEM = Pooled standard error of the mean.

that the H^* values were increased and the C^* values were decreased significantly when the storage time was increased. This is in agreement with many earlier studies reported that the H^* values were increased and the C^* index decreased with the storage time of goat meat (Karami *et al*., 2011a; Karami *et al*., 2011b).

Conclusion

Stylo silage treated with LAB improved the carcass dressing of growing goats. The combinations of DMP and LAB had no effects on goat meat quality. In addition, Stylo silage treated with DMP improved the lipid oxidation of goat meat with no adverse effects on carcass characteristics and it can improve lipid stability by reducing lipid oxidation. The colour stability was higher in meat from goats fed with the treated when compared with untreated silage. In this regard, DMP can provide an inexpensive alternative feed ingredient for feeding goats while reducing the environmental impact of waste disposal in the mao juice industry.

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