

Effect of Grape Seed and Green Tea Extracts on Lipid Oxidation and Quality of Ground Buffalo Meat during Storage

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Abstract

A study was conducted to investigate the effect of natural plant extracts (grape seed extract, GSE and green tea extract, GTE) and synthetic antioxidant (butylatedhydroxytoluene, BHT) on lipid oxidation, metmyoglobin formation, colour and microbial load of ground buffalo meat. The experiment consisted of 4 treatment as follow; a) control (without additive), b) 0.02% BHT, c) 0.10% GSE and d) 0.04% GTE. Control and all treatment samples were held in refrigerated for 9 days. The ground meat samples were taken for measured at 3 day interval. It was found that GSE and GTE reduced lipid oxidation (as determined by TBARS number) comparable to BHT. Meat sample addition with GTE inhibited metmyoglobin formation and lower b* (yellowness) values. No statistical differences were found in the L* (lightness) and a* (redness) values between control and the plant extract treatments. The total plate count in all meat samples increased as the storing time progressed and there was no statistical difference among treatments. Results from the study suggested for the potential use of GSE and GTE as the antioxidant for ground buffalo meat under refrigerated storage.

Keywords: Grape seed extract, Green tea extract, Lipid oxidation, Buffalo meat

1. Introduction

Nowadays in Thailand, swamp buffalo has been recognized as a meat-producing animal. Such the animals are raised in a small scale farming that produces the animals at various aged. Meat from an aged buffalo is rather coarse and tough which needs to be improved before cooking. The conventional method for overcoming toughness is grounding. However, meat grinding leads to rapid oxidative rancidity (Sahoo and Anjaneyulu, 1997) and a potential source of food borne illness (Jimenez-Villarreal *et al.*, 2003), thus seriously affecting the consumer's acceptance.

In order to control the lipid oxidation in meat, many substances have been used include chemical substances such as butylatedhydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Since the use of chemical or synthetic antioxidants have become less acceptable in recent

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years, the interest in utilization of plant extracts to combat the food borne pathogens, inhibiting lipid oxidation, thus extending the shelf-life of muscle foods has increased.

Grape seed extract (GSE) and Green tea extract (GTE) are two natural plant extracts that have been widely used in various food and beverage applications. These two plant extracts (polyphenolic and proanthocyanidin rich compounds) have potential antioxidant properties in inhibiting the lipid oxidation and antimicrobial activities against food spoilage bacteria (Perumalla and Hettiarachchy, 2011). The principal antimicrobial and antioxidant activity of the plant extracts have been reviewed on the published literature somewhere (Reygaert, 2014; Nowshehri *et al.*, 2015).

It was found that the inhibition of lipid oxidation in meat were dose dependent. Addition of GTE at levels of 200–400 mg/kg into meat has demonstrated the inhibitory effects on lipid oxidation in red meat and poultry patties (Tang *et al.*, 2001; Mitsumoto *et al.*, 2005), while GSE at 0.1% ww. can be used as on effective antioxidant in both raw and cooked meat systems (Ahn *et al.*, 2002; Ahn *et al.*, 2004). The application of these two plant extracts in various kind of meat such as beef, pork, poultry and fish have been reviewed by Perumalla and Hettiarachchy (2011). However, report on the use of GSE and GTE in extending the shelf-life of ground buffalo meat is not much elucidated in the literature. Hence, the present study was taken to investigate the effect of GSE and GTE in ground buffalo meat quality at refrigerated storage conditions.

2. Materials and Methods

2.1 Chemicals

All chemicals used for analysis were AnalaR grade. Grape seed extract (94% proanthocyanidins, Manufacturer's data) was purchased from Natrol, Inc., Chatsworth, CA, USA. Green tea extract (90% Epigallocatechin gallate, Manufacturer's data) obtained from Swanson Health Products, Fargo, ND, USA. BHT was from Sigma Chemical Co., St. Louis, MO. Malondialdehyde bis (diethyl acetal) was supplied from Merck KGaA, Darmstadt, Germany.

2.2 Meat samples

The round bottom (*Semimembranosus*) from three swamp buffalo carcasses (of about 4–5 year's age) were collected separately after slaughter. Following the removal of separable fat and loose connective tissue, the cuts were packed in plastic bags and kept frozen at -14°C until use (for less than one month).

2.3 Meat sample preparation

For the preparation of ground buffalo meat, the cut from each buffalo was thawed and sliced with meat slicer (SirMan Suc V, Pallodio, Italy) into 6 mm thick meat. The batch of meat

slice was minced with meat grinder (Talleres Ramon-SL, Girona, Espana) using 4 mm plate to obtain ground buffalo meat. The ground buffalo meat from each carcass was then divided into four portions of 1 kg each and randomly allotted to the four treatments as followed; control (without addition), 0.02% BHT (200 mg/kg meat), 0.10% GSE (1000 mg/kg meat) and 0.04% GTE (400 mg/kg meat).

The additive treatments were freshly prepared by dissolving it in the refine safflower oil and preblended separately. Each additive solution was added to the ground buffalo meat (1.0% v/w of meat) and mixed for 1 min by bowl mixer (Kitchen Aid Inc., St. Joseph, Mich., USA) for uniform dispersion of the additive. For control, only the refine safflower oil was added to the meat. After blending, each treated ground buffalo meat was divided into 200 g and packed in plastic foam meat trays. Each tray was wrapped with polyethylene film and kept in a refrigerator at $4\pm 2^{\circ}\text{C}$ for 9 days.

Four packs (one for each treatment) were removed from the refrigerator at day 1, 3, 5, 7 and 9 for analysis. The meat samples were analysed for 2-thiobarbituric acid reactive substances (TBARS), metmyoglobin formation, instrumental colour, pH and microbial counts at each storage interval.

2.4 TBARS measurement

The 2-thiobarbituric acid (TBARS) assay was carried out according to the extraction method described by Salih *et al.* (1987) and modified by Roldan *et al.* (2014). The TBA values were calculated from standard curves and known dilutions of malonaldehyde and the results were expressed as TBARS number, mg malonadehyde (MDA) equivalentents per kg of ground meat.

2.5 Determination of metmyoglobin

Ground meat samples (5 g) were blended with 25 ml of cold 0.04 M phosphate buffer at pH 6.8. The mixtures were homogenized for 10 s at 13,000 rpm. After keeping in refrigerator for 1 h, the homogenized samples were centrifuged at 5,000 rpm for 30 min. For each sample the supernatant was then filtered through Whatman No.1 filter paper, and the absorbance was read at 700, 572 and 525 nm using a spectrophotometer (BiomateTM 3 series, Thermo Electron Corporation, Madison, WI, USA.). The percent metmyoglobin was calculated according to the formula proposed by Krzywicki (1982).

$$\% \text{ Met Mb} = (1.395 - [(A_{572}-A_{700})/(A_{525}-A_{700})]) \times 100$$

Where "A = Absorbance at lambda (nm)"

2.6 Colour analysis

Colour parameters were measured using portable colorimeter (Hunter Associates Laboratory Inc., Reston, VA, USA) with illuminant D 65 lighting conditions and the 10 standard observer angle as a reference system. Meat colour was estimate in the CIELAB space: lightness, (L^*); redness, (a^*); yellowness, (b^*). The colour was measured at both side of each sample. Before each series of measurements, the instrument was adjusted using a white ceramic tile.

2.7 pH determination

A 10 g sample was homogenized in 50 ml distilled water. The pH value of slurry was determined with a standardized combination electrode attacked to a digital pH meter (Sartorius PP-15, Itin Scale Co., Inc., Glenwood, Brooklyn, NY).

2.8 Microbiological evaluation

For determination of total aerobic microbial population in meat sample, Simplate Total Plate Count Color Indicator method (Biocontrol Systems Inc., Bellevus, WA; USA) was employed. The methodology is based on binary detection technology (BDT) which equates the presence of total aerobic microorganisms to the presence of colour change in the medium. Briefly, 50 g of meat sample was homogenized with 450 mL of 0.1% sterile peptone salt solution by stomacher. Serial 10 fold dilutions were prepared by diluting 1 mL of homogenate in 9 ml of 0.1% peptone salt solution. The sample mixture was then dispensed into a simplate device and incubates at 35°C for 24 h. The total aerobic plate count was determined by counting the wells with changed colour and referring to the simplate conversion Table (Feldsine *et al.*, 2003). The total aerobic microbial reported as log cfu/g of ground meat sample.

2.9 Statistical analysis

The data were analyzed as a Split plot design with three replicates. The whole unit factors were replicate and treatments and the sub unit factors were storage time and treatment x storage time (Myers and Well, 1995). When significance by ANOVA at $p < 0.05$, the means were separated using the Duncan's new multiple range test.

3. Results and discussion

3.1 TBARS number and metmyoglobin formation

Table 1 shows the TBARS number of ground buffalo meats during storage under refrigerated condition. The average TBARS number at day 1 was found to be 0.27 which is comparable to the previous report in fresh buffalo meat (0.31) (Sahoo and Anjaneyulu, 1997). During storage, TBARS number in meat sample increased gradually and was found to be significantly different from the beginning on day 9. Other researchers also reported the

accumulation of TBARS number in raw ground meat during refrigerated storage (Verma and Sahoo, 2000; Naveena *et al.*, 2011). In the present study, the meat samples treated with BHT, GSE and GTE had significantly lower TBARS number than the control ($p < 0.05$). However, there was no significant difference in TBARS number among these additive treatments.

Since the TBARS number shows the oxidative changes of the meat lipid. This indicated that the addition of GSE or GTE with ground buffalo meat inhibited the lipid oxidation comparable to the BHT. GSE and GTE have been reported as the natural antioxidant compound against lipid oxidation in various meat products (Perumalla and Hettiarachchy, 2011). Our result confirmed this activity.

Metmyoglobin formation in ground buffalo meat is shown in Table 1. Metmyoglobin concentration increased in all meat samples with increasing storage time. A similar trend was observed by the previous researches (Sahoo and Anjaneyulu, 1997; Das *et al.*, 2006; Naveena *et al.*, 2011). However, the high concentration of metmyoglobin at day 1 of storage (66%) in this study was noticed. The metmyoglobin concentration of fresh ground buffalo meat has been reported to be varied from 25% (Naveena *et al.*, 2011) to 56% (Das *et al.*, 2006). The high concentration of metmyoglobin found in this study (66%) could be due to the storage of frozen meat before grounding. Ice crystal formation due to the freezing storage causes structural damage in muscle cell. Freezing could disrupt the sarcoplasmic reticulum and cellular components that were normally kept apart, and the contents were allowed to mix. This could lead to the formation of free radical species that accelerated oxidation (Young and West, 2001) which could link to metmyoglobin formation.

It was found that the meat samples incorporated with 0.04% GTE showed the lowest metmyoglobin concentration ($p < 0.05$), while the other additive treatments (0.02% BHT and 0.10% GSE) did not show any significant difference from the control. Retardation in the formation of metmyoglobin in fresh mutton due to the effect of GTE was also reported by Kumudavally *et al.* (2008). This could be due to the ability of tea catechins to bind the iron component of myoglobin that would help in delaying the metmyoglobin formation by reacting with free radicals. In our experiment, the addition of BHT and GSE did not produce a significant reduction in metmyoglobin even though these substances could inhibit the TBARS number. Whereas Sahoo and Anjaneyulu (1997) reported the significant positive correlation between % metmyoglobin and TBARS number in ground buffalo meat. We were unable to clarify whether such a contradiction occurred in our material but the unprotective effect of GSE on myoglobin oxidation evidenced the different mechanism of antioxidant properties between GSE and GTE.

Table 1 Effect of treatment on TBARS number and metmyoglobin of ground buffalo meat during refrigerated storage

Treatments (n=3)	Storage period (days)					Treatment mean±SD (n=15)
	1	3	5	7	9	
TBARS number (mg malonaldehyde / kg meat)						
Control	0.348	0.421	0.524	0.514	0.771	0.515 ^a ±0.221
0.02% BHT	0.221	0.217	0.216	0.230	0.215	0.220 ^b ±0.048
0.10% GSE	0.279	0.235	0.267	0.278	0.360	0.284 ^b ±0.284
0.04% GTE	0.228	0.256	0.217	0.252	0.240	0.239 ^b ±0.050
Day mean±SD	0.269 ^a ±0.095	0.282 ^a ±0.125	0.319 ^{ab} ±0.158	0.306 ^{ab} ±0.171	0.396 ^b ±0.246	
Metmyoglobin (%)						
Control	65.2	69.4	80.1	92.1	95.7	80.5 ^a ±13.6
0.02% BHT	66.1	68.6	71.8	85.5	92.9	77.0 ^a ±13.5
0.10% GSE	69.7	74.1	78.5	82.8	93.7	79.7 ^a ±10.8
0.04% GTE	63.9	65.9	66.5	72.9	77.6	69.3 ^b ± 8.7
Day mean±SD	66.2 ^a ±7.1	69.5 ^b ±7.7	74.2 ^c ±9.4	83.3 ^d ±10.5	90.0 ^e ±9.5	

Note: ^{a,b} Means in a row or column with different superscripts are different ($p < 0.05$),

SD: Standard Deviation.

3.2 Instrumental colour

Meat colour in the CIELAB space (L^* , a^* , b^*) throughout the storage time are shown in Table 2. There were no significant changes in L^* (lightness) value due to the treatment effect and storage time. However, there was a significant decrease of a^* (redness) value at day 5 of storage ($p < 0.05$). Similar observation was made by Naveena *et al.* (2006) who reported the reduction of a^* value in buffalo meat steak during the 12 day retail display. The red colour of the meat is mainly due to myoglobin content of meat. The reduction of red colour indicated the loss of myoglobin contents in storage meat. Myoglobin is highly susceptible to oxidation and microbial spoilage (Kumudavally *et al.*, 2008). Thus, the losses of myoglobin through oxidation reduce the redness of meat colour. In this experiment, the most effective antioxidant in terms of meat redness was BHT. It was found that the BHT treatment exhibited significant higher Hunter (a^*) value than control and the other ($p < 0.05$). It was also reported by McCarthy *et al.* (2001) that raw pork patties added with BHT obtained highest a^* values. The effective at delaying myoglobin loss in storage meat by BHT could be related to the anti-oxidant property. However, in our study, the addition of natural antioxidant (GSE and GTE) did not affect to the redness value even though both inhibited TBARS number. Such phenomenon could not be elucidated in this study. On the other hand, when b^* (yellowness) values was considered,

it was found that the b^* values increased as the storage period progress (Table 3). The result indicated that the ground meat sample turned brown during storage. Metmyoglobin is responsible for the undesirable brown colour of fresh meat. (Sahoo and Anjaneyulu, 1997). It has been found that the ground buffalo meat added with GTE resulted in lower b^* value than the control and the other treatments ($p < 0.05$). This could be due to the lesser extent in %metmyoglobin found in GTE treatment sample. Thus, the evidence suggested the GTE retarded the brown colour development in ground buffalo meat by inhibiting metmyoglobin formation.

Table 2 Effect of treatment on instrumental colour of ground buffalo meat during refrigerated storage

Treatments (n=3)	Storage period (days)					Treatment mean \pm SD (n=15)
	1	3	5	7	9	
L*						
Control	39.33	39.72	39.44	40.00	41.62	40.02 \pm 3.06
0.02% BHT	39.13	39.09	39.25	38.85	41.51	39.57 \pm 2.60
0.10% GSE	38.32	39.65	39.88	39.07	39.16	39.22 \pm 2.42
0.04% GTE	37.74	38.18	38.79	39.27	39.53	38.70 \pm 2.81
Day mean \pm SD	38.63 \pm 2.59	39.16 \pm 2.87	39.34 \pm 3.02	39.30 \pm 2.43	40.46 \pm 2.71	
a*						
Control	11.54	11.62	10.09	9.63	8.27	10.23 ^a \pm 1.53
0.02% BHT	12.15	12.34	11.34	11.05	8.68	11.12 ^b \pm 1.51
0.10% GSE	12.16	11.42	10.16	9.37	8.49	10.33 ^a \pm 1.51
0.04% GTE	11.13	10.76	10.48	9.59	8.48	10.09 ^a \pm 1.23
Day mean \pm SD	11.75 ^a \pm 0.88	11.54 ^a \pm 1.15	10.52 ^b \pm 0.84	9.91 ^b \pm 0.91	8.48 ^c \pm 0.48	
b*						
Control	14.15	14.02	14.40	14.35	14.50	14.28 ^{ab} \pm 0.75
0.02% BHT	14.46	14.52	15.07	15.11	15.33	14.89 ^a \pm 0.75
0.10% GSE	13.24	14.00	14.08	15.00	13.87	14.03 ^b \pm 0.87
0.04% GTE	13.14	13.25	13.54	13.70	13.86	13.50 ^c \pm 0.97
Day mean \pm SD	13.75 ^a \pm 0.85	13.94 ^{ab} \pm 0.81	14.27 ^{bc} \pm 0.98	14.54 ^c \pm 1.02	14.39 ^{bc} \pm 0.93	

Note: ^{a,b,c} Means in a row or column with different superscripts are different ($p < 0.05$),

SD: Standard Deviation.

Table 3 Effect of treatment on pH and total plate counts of ground buffalo meat during refrigerated storage

Treatments (n=3)	Storage period (days)					Treatment mean±SD (n=15)
	1	3	5	7	9	
pH						
Control	5.43	5.56	5.73	5.66	5.66	5.61 ±0.15
0.02% BHT	5.60	5.73	5.73	5.66	5.66	5.66 ±0.14
0.10% GSE	5.63	5.73	5.70	5.63	5.60	5.66 ±0.12
0.04% GTE	5.63	5.73	5.73	5.70	5.56	5.67 ±0.13
Day mean±SD	5.57 ^a ±0.11	5.69 ^{ab} ±0.12	5.73 ^b ±0.10	5.66 ^{ab} ±0.11	5.60 ^a ±0.17	
Total plate counts (log CFU/g)						
Control	5.33	5.45	5.71	5.86	5.93	5.65 ±0.59
0.02% BHT	5.11	5.19	5.67	5.95	6.02	5.59 ±0.74
0.10% GSE	5.20	5.29	5.87	5.94	5.93	5.65 ±0.73
0.04% GTE	5.41	5.60	5.76	6.45	6.61	5.97 ±0.74
Day mean±SD	5.26 ^a ±0.69	5.38 ^a ±0.73	5.75 ^{ab} ±0.45	6.05 ^b ±0.55	6.12 ^b ±0.68	

Note: ^{a,b} Means in a row with different superscripts are different ($p < 0.05$),

SD: Standard Deviation.

3.3 pH and microbial counts

pH and total plate counts of ground buffalo meat samples are shown in Table 3. There was no difference in pH between the control and treated samples. However, during storage the pH of ground meat samples significantly increased ($p < 0.05$) from 5.57 at initial to 5.73 on day 5, thereafter the pH decreased to 5.60 on day 9. Sahoo and Anjaneyulu (1997) also reported the similar pattern of pH changes in ground buffalo meat storage in refrigerator. The phenomenon of pH changes in meat during storage may be attributed to microbial metabolite. Gill (1983) stated that bacteria on exhaustion of stored glucose and ammonia accumulates is a product of amino acid degradation then which leads to pH rises. On the other hand, the decrease in pH during storage may have been owing to the growth of gram positive bacteria, especially lactic acid bacteria (Jay and Shelef, 1978). This could be true in our study, since the total plate count markedly increased on day 5 of storage and thereafter (Table 3). The lowering of pH value followed day 5 of storage could be due to the growth of lactic acid producing bacteria. Anyway, the meat samples were well below the level of incipient spoilage ($\log 7.0 \text{ cfu g}^{-1}$) (Hyytiäinen *et al.*, 1975) at the end of the storage period.

In the present study, no differences in microbial counts were observed between control and the treated samples (Table 3). This indicated that the use of GSE or GTE did not produce

the inhibitory effects on microbial growth. Ahn *et al.* (2004) reported that both the extracts (GTE and GSE) had antibacterial effect in *in vitro* conditions. Antimicrobial properties and their mode of actions have been studied extensively (reviewed by Taylor *et al.*, 2005; Nowshehri *et al.*, 2015). However, antimicrobial effect of plant extracts depends on pH and solubility of the extract in the model systems (Hao, 1998). In addition, Perumala and Hettiarachchy (2011) stated that the natural plant extracts that have potential antimicrobial components may be inactive in food system due to their interactions with lipids and proteins present in the food which may ultimately reduce the antimicrobial activity of these compounds. Thus, these reasons could provide possible explanations to the ineffective result in inhibiting the microbial growth of GSE and GTE reported herein.

4. Conclusion

The results of this study show that the addition of plant extracts (GSE or GTE) to ground buffalo meat could prevent lipid oxidation comparable to BHT. The benefit of the addition of GTE was the retardation of meat brown colour during storage. This could be due to the inhibition effect of GTE on metmyoglobin formation. When the microbial of storage meats were evaluated, the antibacterial effects of GSE and GTE were not observed. This indicated that the GSE and GTE on the concentration used could not prevent microbial load in ground buffalo meat. Thus, regarding the shelf life extension of ground buffalo meat, these two natural plant extracts may require antibacterial agent (such as nisin) that is blended together for obtain high antioxidant and inhibitory effect on microorganism.

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