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Lipid oxidation and antimicrobial activity of cooked beef patties as influenced by leaf extracts of "Cemba" (*Albizia lebbeckoides* [DC.] Benth)

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ABSTRACT

Cemba (*Albizia lebbeckoides* [DC.] Benth.) leaf extract (CLE) was evaluated for some physical properties, antioxidant and antimicrobial activities incorporated into beef patties during cold storage. Four Formulation employed were control, butylated hydroxytoluene (BHT) 0.01, CLE 0.5, and CLE 1% (w/w). The variables measured were proximate composition, cooking parameters, pH, a_w, WHC, color, total phenolic content, antioxidant capacity, DPPH scavenging activity, TBARS value, and microbial total. The data were analyzed using ANOVA one factor for proximate and cooking parameters, and ANOVA with factorial 4x5 for pH, aw, WHC, color, total phenolic content, antioxidant capacity, DPPH scavenging activity, TBARS value, and microbial total and continued with Tukey test. The results of the study showed that the addition of the CLE did not affect the proximate composition and cooking parameters of the patties. The cooked beef patties with 1% CLE showed significantly lower ($P<0.05$) for TBARS value, pH, bacterial total (mesophilic and psychrophilic) compared to 0.5% CLE and controls. The total phenolic content, antioxidant capacity, scavenging activity of CLE 1 % were significantly higher ($P<0.05$) than 0.5% CLE and controls during the cold storage period (0, 7, 14, 21, and 28 days). Addition of both 0.5 and 1% CLE in cooked beef patty reduced bacteria total. The addition of 1% CLE had equivalent to BHT 0.01% effect in retarding lipid oxidation. In conclusion, the CLE 1% was effective to retard lipid oxidation and inhibit bacteria growth of cooked beef patties.

Keywords: *Albizia lebbeckoides*, Antimicrobial, Antioxidant, Beef patties, Leaf extract

Introduction

Patty is one of meat products that popular for some consumers. Unfortunately, this product is susceptible to oxidative and microbiological changes. Lipid oxidation and pathogenic microbiological deterioration generate low quality of meat product parameters such as color, flavor, texture, and nutritional value (Turgut *et al*., 2016); and accumulates toxic (Karre *et al*., 2013). For retarding deterioration, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butyl hydro quinone (TBHQ) (Zhang *et al*., 2016), and antimicrobial like nitrite, phosphate, potassium sorbate, propylparaben, organic acids etc. (Hawashin *et al*., 2016) are employed. However, for health reasons, producers and consumers consent to apply a natural agent possessing antioxidant and antimicrobial properties and highly accepted by consumers and producers (Jiang and Xiong, 2016).

One of the natural agent's candidates for food preservation is cemba (*Albizia lebbeckoides* [DC.] Benth) leaf. This plants grow and are cultivated by people in Enrekang District, South Sulawesi, Indonesia. Traditionally, cemba leaf is used as seasons to cuisine. Some researches showed the genus Albazia extract was potential as antioxidant and antibacterial (Shahid and Firdous, 2012) due to presence of tri-O-glycoside flavonol kaempferol, quercetin, and Albizzia-hexoside (Kokila *et al*., 2013). There have been no any reports the use of cemba leaves for food application. We studied the application of cemba leaf extract to beef patties. The research was to investigate the effect of cemba leaf extract on physicochemical, lipid oxidation, and microbiological characteristics of cooked beef patty during cold storage.

Materials and Methods

The main material were Cemba (*A. Lebbeckoides* [DC.] Benth) leaves obtained from Enrekang District, South Sulawesi; topside cut of Brahman Cross (BC) beef and fat of strip loin of BC obtained from a Slaughterhouse (Elders,

Bogor) within a 24-hour of slaughter (2-3 years old); garlic powder, black pepper powder, white pepper powder, salt, skim milk, and olive oil (local market). The chemical were methanol, sodium carbonate, sodium chloride, sodium nitrate, and sodium hydroxide (Merck, Darmstadt, Hesse, Germany). Folin-Ciocalteau reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2-thiobarbituric acid, and trichloroacetic acid (Sigma-Aldrich, USA). Gallic acid monohydrate, L-Ascorbic acid and butylated hydroxytoluene (HiMedia, Mumbai, India).

Preparation of extracts

Cemba leaves were air-dried at room temperature and followed by oven-drying at 40^{0}C for an hour. The dried leaves were pulverized using a kitchen blender and sieved using a 35 mesh. The powder leaves were stored in airtight container at -25^{0}C until further use.

The powder (40 g) was macerated in distilled water (400 mL) for 24 h in enclosed Erlenmeyer flasks put on a shaker (WNB 7-45, Memmert, Germany) with constant shaking at level 8. These extracts were filtered using Whatman No.1. The filtrates were concentrated (1/20 of initial concentration) using a vacuum rotary evaporator (Heidolph Type Antrieb-W-Mikro, Germany) at 40^{0}C before freeze-drying (Snijders Scientific, LY5FNE, The Nederlands) for 48 h. Freeze-dried extracts were placed in the dark bottle and stored at -25^{0}C until further use.

Patty preparation

The meat was cleaned from subcutaneous and intramuscular fat, then meat and fat were cut into small pieces (4 x 5 cm) and ground using a meat grinder (Panasonic, Malaysia) with a hole diameter of 5 mm. The formulation treatment employed were control (CON) containing lean meat (71.8%), fat (10%), salt (1.2%), ice (10%), skim milk powder (5%), white pepper powder (0.25%), black pepper powder (0.25%), garlic powder (0.5%), and olive oil (1%); BHT containing control formulation added 0.01% BHT; CLE-0.5 containing control formulation added 0.5% CL; CLE-1 containing control formulation added 1% CLE.

All ingredients were mixed manually for 10 min. Each dough (75 g) was shaped into patty with 90 mm diameter and 11 mm thickness. The patties were cooked for 11 min in an oven (Yamato Hi-Tech Controller DK 600, Japan) at 180 ± 1^{0}C and followed with cooling at room temperature (27-29^{0}C). All patties were stored at 4^{0}C prior to microbiological count, lipid oxidation and color were evaluated on day 0, 7, 14, 21 and 28.

Proximate analysis

Approximate composition (moisture, protein, ash and fat contents) of cooked beef patties was determined using the official standard method (AOAC, 2005).

Measurement of pH

The pH value was determined according to AOAC (2005). Briefly, a 10.0 g sample of the patties was homogenized in 100 mL distilled water and then filtered. The filtrate was measured using a pH meter (SCHOTT, Instrumental).

Determination of water holding capacity

Water Holding Capacity (WHC) was measured according to Jung and Joo (2013) with slight modification. A-2.5 g of sample was added with 10 mL of water and then incubated in water bath at 30^{0}C for 30 min. The mixture was then centrifuged at 3,000 rpm for 30 min. The supernatant was pipetted before additional incubation for 10 min and removed by pipetting again. The results were expressed as a percentage of fluid release.

$$\text{Water holding capacity (\%)} = \frac{\text{weight of sample after removing supernatant}}{\text{weight of sample mixed with distilled water}} \times 100$$

Water activity (a_w) determination

The a_w value was determined using a Lab Swift a_w meter (Novasina, Switzerland) at 25 ±1^{0}C according to manufacturer's protocols. Approximately 5 g of a homogenous sample was put in a disposable cup, completely covering the bottom and filling not more than half of the cup.

Determination of cooking parameters

The cooking loss of patties were calculated as follow (Jung and Jo, 2013):

$$\text{Cooking loss (\%)} = \frac{(\text{raw weight beef patty} - \text{cooked weight beef patty})}{\text{raw weight beef patty}} \times 100$$

Determination of diameter and thickness reduction

The diameter and thickness of the cooked patties were recorded using vernier calipers (SER No. 500-197-20, Mitutoyo Corp, Japan) and calculated using the following expression (Jung and Jo, 2013).

$$\text{Reduction in diameter (\%)} = \frac{(\text{raw beef patty diameter} - \text{cooked beef patty diameter})}{\text{raw beef patty diameter}} \times 100$$

$$\text{Reduction in thickness (\%)} = \frac{(\text{raw beef patty thickness} - \text{cooked beef thickness})}{\text{raw beef patty thickness}} \times 100$$

Determination of total phenolic content (TPC)

The total phenolic content was analyzed using the Folin-Ciocalteu reagent as described by Zhang *et al.* (2016) with modification in reagent proportion and concentration. One g of the cooked patty was homogenized with 5 mL of methanol and kept overnight for extraction at room temperature. A volume of 1.5 mL methanol-extracts was added with 1.5 mL of Folin Ciocalteu reagent (3-fold diluted with distilled water) in a 10 mL volumetric flask. After 3 min, the mixture was added with 4.5 mL of 6% sodium carbonate

(Na_2CO_3) solution and reached to the volume with distilled water and followed by incubation at an ambient temperature in a dark place for 2 h. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Agilent UV-VIS 8453, USA). The TPC was determined by plotting to the standard curve of gallic acid and expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g).

Determination of antioxidant activity

The antioxidant activity of the CLE was assessed with 2,2-diphenyl-1-picrylhydrazyl (DPPH) method by Tohidi *et al*. (2017) with modification in temperature of incubation The patty methanolic-extracts (0.2 mL) and 1.8 mL of 0.06 mM methanol DPPH solution was mixed in a test tube and shaken gently. The mixture was incubated at 37°C for 40 min. The absorbance of the sample was measured at 517 nm using UV-Vis spectrophotometer versus methanol as a blank solvent. L-Ascorbic acid concentration range of 0-50 µg/mL was used as a standard. The inhibition percentage of radicals was calculated using the following formula:

$$\%\ \text{inhibitori of DPPH} = \frac{(\text{Acontrol} - \text{Asample})}{\text{Acontrols}} \times 100$$

where $A_{control}$ is the absorbance of 0.06 mM methanolic DPPH solution and A_{sample} is the absorbance of sample. The antioxidant capacity was determined by plotting scavenging percentage to linear regression equation of L-Ascorbic acid standard.

Determination of thiobarbituric acid reactive substances (TBARS)

Lipid oxidation was measured by 2-thiobarbituric acid reactive substances (TBARS) assay as described by Soltanizadeh and Ghiasi-Esfahani (2015) with modification in sample preparation. For extraction, 5 g patties was prepared by mortar and mixed with 10 mL TCA 20% for 3 min and 10 mL of distilled water followed by filtration (Whatman No 1). Three mL of supernatant was mixed equal volume of freshly prepared 0.01 M thiobarbituric acid in test tube and was further vortexed for 30 s. The sample was incubated in a boiling water 100^0C for 1 h, and then cooled under running water for 10 min. The absorbance was determined at 532 nm against a blank containing 3 mL of distiled water and 3 mL TBA reagent. Malondialdehyde standard curves were prepared by using 1,1,3,3-tetra-ethoxypropane. The TBARS values was calculated from the standard curve and was expressed as milligrams malondialdehyde (MDA) per kg of beef patties.

Microbiological evaluation

The microbiological characteristics of the cooked beef patty were evaluated after 0, 7, 14, 21 and 28 days of storage according to the methodology described by Maqsood *et al.* (2015) with modification in the sample:solvent proportion. Briefly, 25.0 g of the sample was homogenized with 225 mL of 20% sterile buffer peptone water (20.0 g of buffer peptone in 1.0 L of distilled water) and was adequately blended (homogenizing). After 5 min, ten-fold serial dilutions were prepared by diluting 1.0 mL of homogenate in 9 mL of 0.1% peptone water for analyzing microbial counts. Mesophilic bacterial count (MBC) and psychrophilic bacterial count (PBC) were determined by plate count agar (PCA) with incubation at 37^0C for 2 days and 7^0C for 7 days, respectively. *S. aureus* was determined on Baird Parker agar medium, *Salmonella* was determined on *Xylose Lysine Deoxycholate agar*, *E. coli* was determined on Eosin Methylene Blue Agar after 48 h incubation at 37^0C. Plates containing 25-250 colonies were selected and counted, and the average number of CFU/g was calculated. Microbial colonies were counted and expressed as log10 CFU (colony forming units)/g beef patties (per g of patties (log CFU/g).

Color measurements

The effect of CLE on color properties (L*, a* and b*) of the cooked beef patty was evaluated using a Chroma Meter (CR-310; Konica Minolta Co., Osaka, Japan). Before measuring, the apparatus was standardized against a white plate (Y = 92.89 x = 0.3150, and y = 0.3210).

Statistical analysis

Mean values for various parameters were calculated and compared by analysis of variance using the general linear models (GLM) procedure of SAS (SAS version 9.0). The data were analyzed using ANOVA one factor for proximate and cooking parameters, and ANOVA with factorial 4x5 (formulation and storage time as main factors) for pH, aw, WHC, color, total phenolic content, antioxidant capacity, DPPH scavenging activity, TBARS value, and microbial. Microbiological data were transformed into logarithms of the number of colony forming units (CFU/g) and subjected to analysis of variance. Differences between group means were determined using Tukey differences and were reported as significant at the $P<0.05$ level.

Result and Discussion

Chemical composition

The moisture, ash, protein and fat content of the cooked beef patty added BHT and different concentration of CLE in this study is presented in Table 1. The proximate composition of all formulation was not significantly different. It indicated that addition of CLE up to 1% in the patty exerted the similar characteristics of the proximate composition. This result confirmed other studies that plant extract did not affect the proximate composition of the chicken patties (Babatunde and Adewumi, 2015) and raw ground pork (Choe *et al.,* 2011).

Cooking loss, diameter and thickness reduction

The physical properties of the patties are presented in Table 1. The cooking loss, diameter, and thickness reduction were not affected by the formulation. The Cooking parameters value ranged 19.40-20.03% (cooking loss), 5.72-5.82% (diameter reduction), and 4.87-5.01% (thickness reduction). These results were in line with Ganhao *et al.* (2013) reporting the addition of plant extract and quercetin did not affect the patties cooking loss.

pH value, water activity (A_w) and water holding capacity (WHC)

The pH values of cooked beef patties ranged from 5.41 to 5.68 (Table 2). The pH values were significantly affected by the interaction between formulation and storage periods ($P<0.05$). The lower pH value of the CLE was most probably affected by the initial CLE pH value 3.61 as in Jung and Joo (2013) using roselle extract on pork patties, Wenjiao *et al.* (2014) using tea polyphenol on pork sausage, and Devatkal *et al.* (2010) using kinnow rind and pomegranate rind on goat meat patties. In contrast, the pH value of control and BHT patties tended to decrease during storage. Soltanizadeh and Ghiasi-Esfahani (2015) reported that the reduced pH during storage was attributed to the microbial that breaks down glycogen.

The a_w value showed there is no interaction between formulation and storage (Table 2), but affected by formulation and the storage. The a_w value decreased within 7 days of storage and followed with no significantly

Table 1. The Chemical composition, cooking losses and diameter and thickness of cooked beef patty

Variables	Formula				Average
	CON	BHT	CLE-0.5	CLE-1	
Composition					
Moisture content (%)^{ns}	65.53±0.55	65.36±0.45	65.26±0.39	64.49±0.14	65.16±0.46
Ash content (%)^{ns}	2.75±0.11	2.86±0.14	2.61±0.10	2.50±0.18	2.68±0.16
Protein content (%)^{ns}	18.67±0.31	18.70±0.11	18.24±0.27	18.43±0.11	18.51±0.21
Fat content (%)^{ns}	8.15±0.30	8.25±0.13	8.33±0.12	8.21±0.21	8.24±0.08
Losses					
Cooking loss (%)^{ns}	20.03±1.17	19.40±1.53	19.73±1.87	19.69±1.34	19.71±0.25
Diameter reduction (%)^{ns}	5.82±0.57	5.72±0.87	5.84±0.81	5.78±0.33	5.79±0.05
Thickness Reduction (%)^{ns}	4.87±0.90	4.91±1.07	4.91±0.92	5.01±0.62	4.93±0.06

ns = Not significant
CON : control, BHT = butylated hydroxytoluene, CLE = cemba leaf extract.

Table 2. The pH value, water activity (a_w) and water holding capacity (WHC) of cooked beef patties of antioxidant source and storage time

Formula	Storage time (days)					Average
	0	7	14	21	28	
pH value*						
CON	5.67±0.05^{a}	5.56±0.01^{ab}	5.49±0.01^{de}	5.46±0.03^{efg}	5.43±0.01^{efg}	
BHT	5.65±0.01^{ab}	5.61±0.01^{abc}	5.60±0.03^{bc}	5.45±0.04^{cd}	5.45±0.03^{efg}	
CLE-0.5	5.49±0.01^{de}	5.48±0.01^{de}	5.48±0.04^{edf}	5.46±0.01^{efg}	5.45±0.22^{efg}	
CLE-1	5.41±0.01^{g}	5.42±0.01^{fg}	5.42±0.03^{efg}	5.42±0.02^{efg}	5.41±0.01^{g}	
Aw *						
CON	0.854±0.009	0.853±0.006	0.852±0.008	0.858±0.004	0.859±0.002	0.855±0.003a
BHT	0.855±0.06	0.852±0.006	0.850±0.002	0.849±0.004	0.852±0.004	0.852±0.002ab
CLE-0.5	0.853±0.005	0.846±0.004	0.845±0.006	0.846±0.004	0.842±0.002	0.846±0.004c
CLE-1	0.851±0.007	0.847±0.006	0.847±0.004	0.848±0.004	0.842±0.002	0.847±0.003cb
Average	0.853±0.002a	0.850±0.005b	0.848±0.003ab	0.851±0.005ab	0.849±0.002ab	
WHC (%)^{ns}						
CON	29.36±1.80	28.71±0.62	28.04±0.72	27.60±0.61	27.80±1.90	28.30±0.73
BHT	28.49±0.86	28.34±1.23	28.29±0.92	27.39±0.67	27.60±0.53	28.02±0.49
CLE-0.5	28.30±1.02	28.43±1.15	28.04±0.81	27.72±1.09	27.65±0.62	28.03±0.34
CLE-1	27.92±0.75	27.65±1.04	27.60±0.61	27.49±1.15	27.57±1.16	27.62±0.16
Average	28.50±0.63	28.28±0.45	27.99±0.29	27.53±0.15	27.65±0.10	

$^{a-g}$ Different superscripts at the same column or row are indicated significantly different ($P<0.05$)
* = Significantly different in $P<0.05$; ns = Not significant
CON = control, BHT = butylated hydroxytoluene, CLE = cemba leaf extract.

different value until the end of 28 days storage. The addition of CLE lowered the a_w value compared with the control, while the control was similar to BHT.

The water holding capacity of the cooked beef patties are presented in Table 2. The average of WHC ranged from 27.39 to 29.36%. The result showed that there were no statistically different among formulation and storage period. Similarly, the teak leaf extract addition did not significantly affect beef sausages WHC (Arief *et al*., 2014).

Color change

The patties color were expressed in hunter system include L* (lightness), a* (redness), and b* (yellowness) as shown in Table 3. The lightness (L) values were not affected by formula, but it decreased during storage (P<0.05). The redness (a) values were not different among formulation and storage time. The yellowness (b) value of the patties were affected by the formulation and storage period, but no interaction between formulation and storage period. The yellowness was decreased until 7 days storage and stable after 7 days of storage. The heating process had destroyed meat pigment so that extract or BHT could not influence the red of patties and reduce the extract effect on the patties color. This was due to Maillard reaction effect (Yildiz-Turp and Serdaroglu, 2010) at heating 180^0C.

Total phenolic content and antioxidant activity

The result indicated that patties containing CLE had antioxidant properties as presented in Table 4. The phenolic content was significantly affected by the interaction between the formulation and storage period (P<0.05). The BHT and CLE contained higher phenolic than control at each storage period which was attributed to the phenolic content of CLE and BHT. Some studies indicate CLE contains phenolic compound (Acharyya *et al.,* 2012). During storage, the phenolic content of the patties was decreased and assumed were used up for antioxidant activities.

The data in Table 4 show us the antioxidant activity were in line with the phenolic content. However, there was no interaction between formulation and storage period. All sample had an antioxidant capacity (59.26-102.85 mg VCE/100 g sample) and excellent ability in radical scavenging activity (16.36-35.68%) which the control was lower than BHT and CLE. Many studies indicated phenolic compounds act as an antioxidant (Wagh *et al*., 2015). This is due to their redox properties, which plays an important role in absorbing and neutralizing free radicals, quenching singlet oxygen or decomposing peroxides (Liang *et al*., 2010).

TBARS value

Table 4 shows TBARS values of control, BHT, CLE-0.5, and CLE-1. There was an interaction between formulation and storage period. At day 0, the TBARS values were similar among formulation, but storage time resulted in high TBARS' value. BHT and CLE significantly (P<0.05) reduced the TBARS values as compared to control. The respective formulation significantly influenced TBARS values and impacted the change over time, as evidenced by significantly interactions (P<0.05) between

Table 3. The L*, a* and b* values of cooked beef patties of antioxidant source and storage time

Treatment	Storage time (days)					Average
	0	7	14	21	28	
L * (Lightness)						
CON	49.64±0.79	48.41±1.15	47.73±1.35	46.00±3.74	46.36±1.06	47.63±1.49
BHT	50.78±1.03	48.15±1.44	48.75±0.39	48.71±0.71	48.62±2.00	49.00±1.02
CLE-0.5	48.55±2.18	48.36±0.90	48.36±0.49	49.52±0.69	48.02±2.20	48.56±0.57
CLE-1	49.84±0.94	48.08±1.09	48.08±1.09	47.29±1.38	48.41±0.58	48.34±0.94
Average	49.71±0.92^{a}	48.25±0.16^{ab}	48.23±0.43^{ab}	47.88±1.55^{b}	47.86±1.03^{b}	
a* (- = green ; + = redness)						
CON	4.82±0.80	4.74±0.46	4.84±0.83	5.10±0.55	4.83±0.29	4.86±0.14
BHT	4.72±0.80	4.86±0.43	4.80±0.79	5.03±0.79	4.70±0.72	4.82±0.13
CLE-0.5	4.83±0.52	4.76±0.60	4.83±0.43	5.04±0.13	4.81±0.50	4.85±0.11
CLE-1	4. 84±0.63	4.79±0.73	4.93±0.36	4.90±0.47	4.83±0.44	4.85±0.10
Average	4.80±0.05	4.79±0.05	4.85±0.07	5.02±0.08	4.79±0.06	
b* (- = blue ; + = yellownes)						
CON	4.61±0.44	4.53±0.46	4.41±0.70	3.93±0.17	3.67±0.42	4.23±0.41^{b}
BHT	4.68±0.73	4.57±0.59	4.49±0.54	4.56±0.48	4.60±0.45	4.58±0.07^{ab}
CLE-0.5	5.03±0.16	4.88±0.33	4.77±0.43	4.55±0.21	4.27±0.00	4.70±0.30^{a}
CLE-1	5.17±0.07	4.99±0.13	4.72±0.36	4.51±0.41	4.52±0.33	4.78±0.29^{a}
Average	4.87±0.27^{a}	4.74±0.23^{ab}	4.60±0.17ab	4.39±0.31^{ab}	4.26±0.42^{b}	

$^{a, b,c}$ Different superscripts at the same column or row are indicated significantly different (P<0.05)
* = Significantly different in P<0,05; ns = Not significant
CON = control, BHT = butylated hydroxytoluene, CLE = cemba leaf extract.

Table 4. The effect of the addition of antioxidant source and storage time on oxidative characteristic of cooked beef patties during storage at 4^0C

Formula	Storage days					Average
	0	7	14	21	24	
			Total phenolics (mg Gallic acid 100 g^{-1} sample)*			
CON	15.96±0.35i	14.78±0.32ij	13.46±0.29jk	12.78±0.24kl	11.46±0.28l	
BHT	29.25±0.56ab	28.41±0.62abc	7.05±0.58cde	26.49±0.89edf	25.33±0.38f	
CLE-0.5	26.95±0.30cde	25.86±0.69ef	23.39±0.46g	22.51±0.32g	20.75±0.92h	
CLE-1	29.77±0.11a	8.67±0.69ab	27.95±0.22cde	26.83±0.10def	25.84±0.56ef	
			Scavenging activity (%)*			
CON	22.41±0.98	23.85±0.53	21.91±0.69	20.38±0.89	16.58±0.98	21.03±2.78d
BHT	34.42±0.88	35.77±0.88	34.05±0.27	31.89±0.51	27.96±0.55	32.82±3.05b
CLE-0.5	29.16±0.79	29.66±1.26	27.92±0.58	26.45±0.51	24.38±0.48	27.51±2.15c
CLE-1	35.68±0.83	36.56±0.70	35.68±0.91	33.44±0.43	31.39±0.77	34.55±2.11a
Average	30.42±5.23b	31.46±5.15a	29.89±5.44b	28.04±5.13c	25.08±5.50d	
			Antioxidant capacity (mg EVC/100 g sample)*			
CON	70.90±2.20	74.43±1.09	70.41±1.20	66.30±2.00	59.26±2.28	68.38±5.90d
BHT	99.90± 0.12	101.99±1.27	99.08±2.33	99.08±2.33	94.10±2.74	98.83±2.90b
CLE-0.5	86.95±3.13	89.96±2.94	84.11±2.29	82.31±1.19	76.75±2.74	83.99±4.95c
CLE-1	102.85±3.14	103.84±3.41	102.84±3.12	97.66±1.73	93.86±1.44	100.21±4.30a
Average	90.15±14.57b	92.62 ± 13.39a	89.11±14.86b	86.34 ± 1.81c	80.99± 16.60d	
			Thiobarbituric acid reactive substances (TBARS) in mg malonaldehyde/kg samples*			
CON	0.28±0.01kl	0.39±0.02ef	0.43±0.02cd	0.56±0.01b	0.69±0.02a	
BHT	0.24±0.01l	0.27±0.01jkl	0.30±0.01hij	0.33±0.01gh	0.38±0.01ef	
CLE-0.5	0.26±0.01kl	0.32±0.01hi	0.36±0.01de	0.40±0.02de	0.45±0.02c	
CLE-1	0.23±0.01l	0.26±0.01kl	0.29±0.00ijk	0.32±0.01hi	0.36±0.01fg	

[a-l] Different superscripts at the same column or row indicated significantly different (P<0.05).
* Significant different ((P<0.05).
CON = control, BHT = butylated hydroxytoluene, CLE = cemba leaf extract, GAE = Gallic acid equivalent, VCE = Vitamin C equivalent.

formulation and time. The control had the highest TBARS values by the end of storage (day 28), and patties CLE-1 and BHT was no difference.

TBARS values of the 28-day storage period ranged 0.23-0.69 mg/kg patties. This value is under detection threshold which 2 mg MDA/kg sample was considered as a limit threshold for the acceptability of oxidized beef (Campo *et al.,* 2006). Reduction of TBARS values may be attributed to polyphenols and antioxidant effects of *A. lebbeckoides*. These results agree with those reported by Hawashin *et al.* (2016) and Soltanizadeh and Ghiasi-Esfahani (2015) for natural antioxidants applied to cooked beef. The main active constituent responsible for antioxidant activities is polyphenol, flavonoids, phenolic diterpenes and tannin (Zhang *et al.,* 2010).

Microbial change

The effect of CLE and BHT on bacterial growth of cooked beef patties were represented by mesophilic bacterial count (MBC) and psychrophilic bacterial count (PBC) as depicted in Figure 1. There was an interaction between the formulation and storage period. MBC and PBC increased (P<0.05) during storage time. At day 0, there were no MBC and PBC detected. This was most probably caused by the cooking preventing microbial growth. Prolonged storage time resulted in high microbial total. Figure 1 shows us that MBC and PBC of patties containing CLE was significantly lower than BHT and the control from the day-7 through day-28. CLE was more effective in retarding the mesophilic than psychrophilic. Patties containing CLE-1 exhibited a delayed growth of MBC about 0.81 log10 CFU/g and PBC about 0.47 log10 CFU/g, thus extending shelf life up to 28 days during storage at 4 °C. The phenolic compounds in the CLE (Table 4) are known as antimicrobial (Shahid and Firdous, 2012). Bobby and Wesely (2012) found that the *A. lebbeckoides* leaves have a potential to inhibit Gram positive and Gram negative bacteria.

Phenolic compounds play an important role to inhibit the microbial growth through multiple modes of action such as break down the cell wall, disrupt the cytoplasmic membrane, cause leakage of cellular components, alter fatty acid and phospholipid constituents, influence the synthesis of DNA and RNA and destroy protein translocation (Shan *et al.,* 2007).

Our study also indicated that *E. coli*, *S.aureus* and *Salmonella sp*. (as pathogenic bacteria) were not detected in all prepared beef patties containing 0%, BHT, CLE- 0.5 and CLE-1. This may be due to the strict hygienic practices

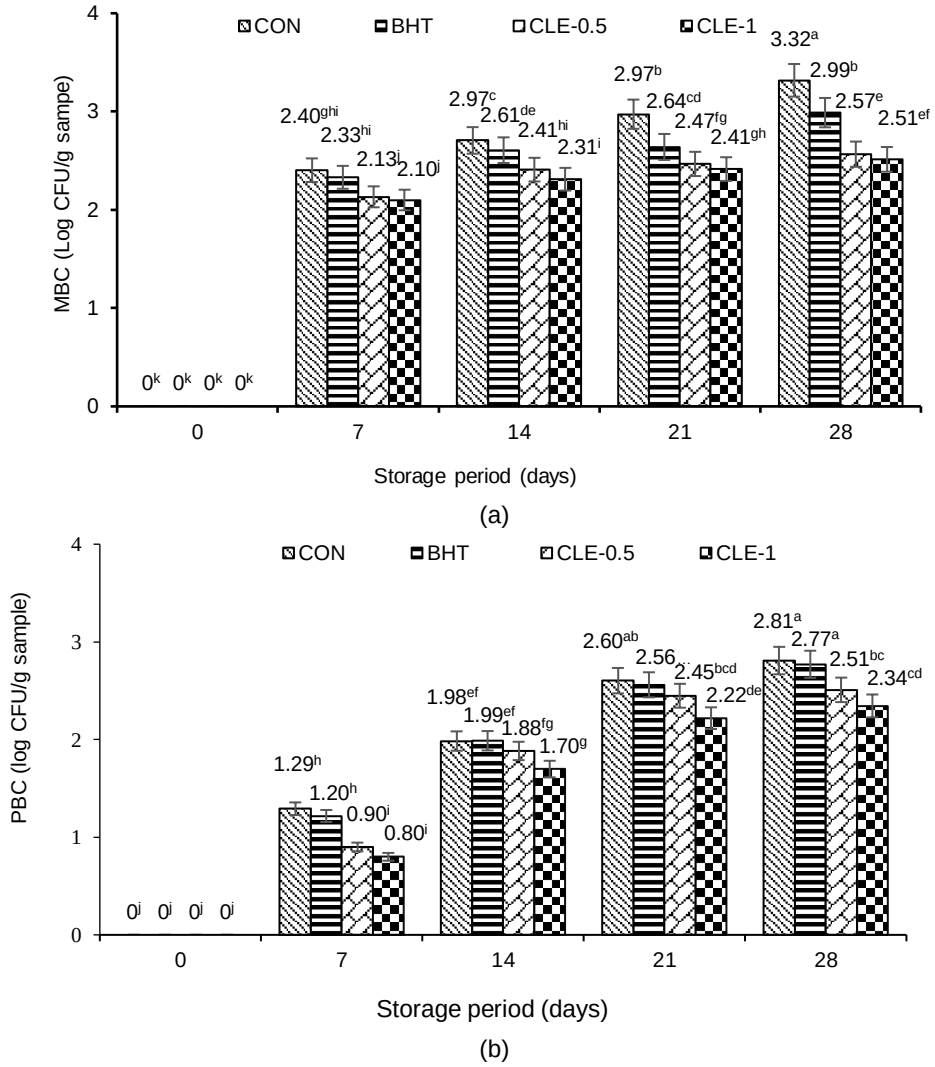

Figure 1. The effect of different concentrations of CLE and BHT on (a) mesosphilic bacterial count (MBC) and (b) psychrophilic bacterial count (PBC) in cooked beef patties stored at 4^0C.
[a-k] Different letters on the bar within the same storage time denote the significant differences (P<0.05). Values are expressed as the mean ± SD (n = 3).

followed during the production process at PT Elders Slaughters as a source of beef and in the manufacturing of the patties by heating with 180^0C.

Conclusions

There was an interaction effect between formulation and storage period on pH value, total phenolic content, TBA and a microbial total of the patties. The formulation and storage period affected the b* (yellowness) value, a_w, scavenging percentage, and antioxidant capacity, but did not affect the a* (redness) and WHC value. The L* (lightness) was only affected by the storage. The proximate and cooking parameter were not different among formulation. The supplementation of cemba leaf extract 1% reached similar to BHT 0.01% in the oxidation inhibition and could retard the microbial total of the cooked beef patties.

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