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BV Vivekananda Reddy
Ph.D Scholar, Department of Livestock Products Technology, NTR College of Veterinary Science, Sri Venkateswara Veterinary University, Gannavaram, Andhra Pradesh, India

V Monish
Department of Livestock Products Technology, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

T Mounika
Department of Livestock Products Technology, NTR College of Veterinary Science, Sri Venkateswara Veterinary University, Gannavaram, Andhra Pradesh, India

GV Bhaskar Reddy
Department of Livestock Products Technology, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

E Naga Mallika
Department of Livestock Products Technology, NTR College of Veterinary Science, Sri Venkateswara Veterinary University, Gannavaram, Andhra Pradesh, India

Bidyut Prava Mishra
Department of Livestock Products Technology, NTR College of Veterinary Science, Sri Venkateswara Veterinary University, Gannavaram, Andhra Pradesh, India

Corresponding Author:
BV Vivekananda Reddy
Ph.D Scholar, Department of Livestock Products Technology, NTR College of Veterinary Science, Sri Venkateswara Veterinary University, Gannavaram, Andhra Pradesh, India

Oxidative and microbiological stability of ground pork incorporated with lemon grass essential oil

BV Vivekananda Reddy, V Monish, T Mounika, GV Bhaskar Reddy, E Naga Mallika and Bidyut Prava Mishra

Abstract
The study aimed to examine the impact of two antioxidants, lemon grass essential oil (a natural antioxidant) and butylated hydroxytoluene (a synthetic antioxidant), on the quality of raw ground pork stored in refrigeration. The study assessed various physico-chemical characteristics such as pH, Thiobarbituric acid reactive substances, peroxide values, microbial characteristics, as well as sensory characteristics over a period of 12 days. The inclusion of lemon grass essential oil in the ground pork resulted in reduced lipid oxidation and microbial counts compared to the control and synthetic antioxidant samples. Additionally, the sensory characteristics of the samples with lemon grass essential oil were preferred by the evaluators. These findings indicate that the use of natural antioxidants can improve the stability and safety of raw ground pork, thereby extending its shelf-life.

Keywords: Ground pork, lemon grass essential oil, butylated hydroxytoluene, lipid oxidation, sensory characteristics

Introduction
Pork is a popular food choice in Asian cuisines, enjoyed in both fresh and processed forms. It is highly valued for its fat content and appealing texture. However, compared to meats from other animals, pork has a higher concentration of unsaturated fatty acids, which makes it more susceptible to lipid oxidation during processing and storage. The oxidative alterations in minced meat are heightened compared to intact meat, primarily because of increased surface area and exposure to air during mincing and processing facilitate accelerated oxidation processes. Due to their low antioxidant content and the combination of unsaturated fatty acids, high moisture, and nutrient-rich environment, meat and meat products are prone to lipid peroxidation and microbial spoilage (Chatli *et al.*, 2015) [3]. Lipid oxidation poses a significant challenge in the meat processing industry, resulting in the deterioration of quality. This deterioration is evident through undesirable alterations in color, flavor, and nutritional value. Additionally, it leads to the buildup of harmful substances that can contribute to various human ailments, including cancer, atherosclerosis, and heart problems (Olorunsanya *et al.*, 2010) [12]. Lipid oxidation is a multifaceted process in which the unsaturated fatty acid component of membrane phospholipids undergoes oxidation, resulting in the formation of hydroperoxides. These hydroperoxides are susceptible to further oxidation or decomposition, leading to the generation of secondary oxidation products, including short-chain aldehydes, ketones, and other oxidized compounds. The presence of these compounds can have a detrimental impact on the overall quality and acceptability of meat and meat products.

In order to mitigate the adverse effects of lipid oxidation and spoilage in meat, synthetic antioxidants such as butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), tertiary butyl hydroquinone (TBHQ), and propyl gallate have been successfully employed. However, the use of these synthetic antioxidants has been limited due to their toxicity. Consequently, natural antioxidants have found wider application in the meat industry, primarily due to their greater acceptability among consumers when compared to synthetic alternatives. Various plants, vegetables, fruits, oil seeds, and herbs serve as important sources of natural antioxidants. Essential oils, derived from plants, have been widely applied in the meat industry as a safe alternatives and approved for their use in foods by the Food and Drug Administration (FDA 2015) [8], exhibiting antimicrobial and antioxidant properties (Atares *et al.* 2016) [2]. The acceptance among consumers for the use of essential oils has been growing, and more than 150 essential oils are listed as GRAS (generally recognized as safe) by the US Food and Drug Administration.

Lemongrass, scientifically known as *Cymbopogon citratus*, is a tall perennial grass found in numerous tropical and subtropical countries. It is native to warm regions and thrives in a wide range of climates (Cheel *et al*., 2005) [5]. This aromatic herb, widely utilized in Asian cuisine, boasts a subtle citrus flavor and can be employed in both fresh and dried, powdered forms. Lemongrass is highly abundant in citral, a valuable compound utilized in perfumery, pharmaceutical industries, and the production of bioactive substances such as flavonoids and vitamin C. The presence of natural flavonoids is gaining increased recognition, not only for their antioxidant properties, but also for their potential as anti-carcinogenic and anti-inflammatory agents, owing to their beneficial effects on lipid anti-peroxidation (Marin *et al*., 2002) [11]. Lemongrass is frequently employed as a herbal remedy for various ailments such as flu, headaches, pneumonia, malaria, coughs, vascular disorders, elephantiasis, diarrhea, stomach upsets, and digestive issues. It is also utilized as a stimulant, diuretic, antispasmodic, and a mild irritant (Olorunsanya *et al.*, 2010) [12]. The oil extracted from this particular plant exhibits a striking sherry color and possesses a strong, sharp taste. Its primary component is citral, while other constituents include citronellol, limonene, geraniol, terpineol, $-myrcene, dipentene, methyl heptenone, and nerol (Simon *et al*. 1984) [14]. The aim of the present study is to evaluate the effect of lemongrass essential oil in comparison to a synthetic antioxidant, butylated hydroxytoluene (BHT) on physicochemical, microbiological and sensory characteristics of ground pork during refrigeration storage.

Materials and Methods

Preparation of minced pork samples: Lean pork was collected from slaughter of crossbred Large White Yorkshire pigs in department of Livestock products technology, college of veterinary sciences, Tirupati. The pork was cut into small pieces to facilitate easy mincing and these pieces were further subjected to thorough mincing by using a meat mincer (Sirman TC12E, Italy) through 6 mm diameter plate to obtain a uniform mix and later through 4 mm diameter plate. The meat samples were divided into four groups. The control sample (C) without any antioxidant, pork added with 0.25% lemon grass essential oil (LGO) (T_1), pork added with 0.5% lemon grass essential oil (LGO) (T_2) and pork sample with 0.01% butylated hydroxytoluene (BHT) (T_3). The samples were packed in low density polyethylene (LDPE) bags and stored at refrigerated temperature (4±1 °C) for 12 days. The same samples were analysed on 0, 3, 6, 9 and 12 days for various physico-chemical, microbiological and sensory characteristics.

pH: The pH of meat samples were determined by homogenizing 10 g of sample with 50 ml distilled water with the help of mortar and pestle for 1 min. The pH of the suspension was recorded by immersing the combined glass electrode of digital pH meter (Systronics, µ pH System, Type 361, Sr. No. 7856) which was calibrated against buffer of pH 4, 7 and 10 (Trout *et al*., 1992) [17].

Thiobarbituric acid reactive substances (2-TBARS) value: The TBARS value was determined according to the method described by (Witte *et al.* 1970) [18]. To prepare the Trichloroacetic acid (TCA) extract of the sample, 4 g of the sample was homogenized with 20 ml of pre-cooled 20% TCA solution using an ultra turrex homogenizer for 2 minutes. The mixture was allowed to extract for 10 minutes and then centrifuged at 3000 rpm for 10 minutes using a CPR-24 centrifuge (Remi Instruments, Mumbai, India). Three ml of the supernatant was combined with an equal volume of 0.1% TBA reagent. The resulting mixture was then boiled in a water bath for 30 minutes, allowed to cool, and the absorbance was measured at 532 nm using a UV-VIS spectrophotometer (Model: UV-1700 PharmaSpec, SHIMADZU, Japan). The TBARS values were calculated using a TBA standard curve and expressed in mg malonaldehyde/kg. For the blank, the same procedure was followed as described above, except that 3 ml of 20% chilled TCA solution was added instead of the TCA extract.

Free fatty acids (%): Free fatty acids per cent was determined according to method described by Koniecko (1979) [10]. Exactly 5 g of sample was blended for 2 min with 30 ml of chloroform in the presence of about 5 g anhydrous sodium sulphate. Then it was filtered through Whatman No. 1 filter paper into a 150 ml conical flask. Two to three drops of 0.2 % phenolphthalein indicator was added to the chloroform extract and titrated against 0.1 N alcoholic potassium hydroxide till a pale pink colour was obtained as end point. The quantity of potassium hydroxide consumed during the titration was recorded. Free fatty acid content was calculated and expressed as percentage as follows.

Free fatty acids (% oleic acid): 0.1 X ml of 0.1 N alcoholic KOH X 0.282 / weight of sample (g) X 100.

Peroxide value: The peroxide value (POV) was determined according to the method of (Sallam *et al.*, 2004) [13]. The samples (3 g) were weighed in a 250-mL glass stopper flask. Then, it was heated for 3 min at 60 °C in a water bath to melt the fat. After that, the flask was thoroughly agitated for 3 min with 30 mL acetic acid-chloroform solution (3:2 v/v) to dissolve the fat. Filtration was performed using Whatman filter paper number 1 to remove particles from the filtrate. Following filtration, saturated potassium iodide solution (0.5 mL) was added to the filtrate, and starch solution was added as an indicator. The titration was continued against a standard solution of sodium thiosulfate. POV was calculated using the following equation and expressed as milli equivalent peroxide per kilogram of sample:

$$POV\ (meq/kg) = \{(S \times N) / W\} \times 100.$$

In the equation, "S" represents the volume of titration (ml), "N" is the normality of sodium thiosulfate solution (N=0.01), and "W" is the sample weight (g).

Microbiological analysis: Bacterial counts were determined by the pour plate method. Meat samples (10 g) were homogenized with 90 ml, 0.1% sterile peptone water. Serial 10-fold dilutions were prepared by diluting 1 ml of homogenate in 9 ml of 0.1% peptone water. Appropriate serial dilutions were duplicate plated with plate count agar for total plate counts and total psychrophilic count. The plates were incubated at 37 °C for 48 hrs for total plate count and at 4±1 °C for 14 days for total psychrophilic count. For enumeration of yeast and mold counts, about 20 ml of Potato Dextrose agar melted and maintained at 44–46 °C was poured

gently. The plates were incubated at 25 °C for 7 days. The number of colonies were multiplied with reciprocal of the dilution and expressed as $\log_{10}$ CFU/g.

Sensory analysis: The sensory attributes such as colour, flavour and off-odour of the pork samples were evaluated using five-point scale (AMSA, 2015) [1]. In the five-point scale, for colour and flavour, five corresponds to characteristics of highest quality and one corresponds to the lowest quality and considered unacceptable. For off-odour scale, 5 = not present, 4 = weak, 3 = moderate, 2 = strong, 1 = very strong. The panel consisted of six trained and experienced members, who are familiarized with the sensory characters of pork meat. The samples were placed in covered cups labeled with random three-digit numbers at room temperature prior to evaluation.

Statistical analysis: The data generated for different quality characteristics were compiled and analyzed after repeating the experiment for three times. In order to determine significant differences (p<0.05) between groups, storage periods, and their interactions for the various parameters in the trials, the data underwent statistical analysis. This included conducting a two-way analysis of variance, followed by the least significant difference test, paired t-test (Snedecor & Cochran, 1995) [15], and Duncan's multiple range test (Steel & Torris, 1981) [16] for comparing the means.

Results and Discussion

Physico-chemical characteristics: The physico-chemical properties of ground pork added with antioxidants were shown in table-1. The pH of the ground pork was not significantly varied between the treatments on day zero and on the 12th day control was having higher pH compared to lemon grass and BHT added pork. Lemon grass added samples recorded lower pH than BHT added samples and no significant difference was found between the two concentrations (0.25% and 0.5%) of lemon grass essential oil. The increase in pH values of the control samples during the storage period can be attributed to the accumulation of ammonia and the by products released from amino acid degradation. This suggests that bacteria utilized the amino acids present, contributing to the pH rise. Chauhan *et al.* (2019) [4] reported similar results of ground pork added with *Terminalia arjuna* fruit extract for a storage period of 9 days.

The levels of lipid peroxidation, an indicator of oxidative stress, were estimated using the 2-TBARS assay. The lipid oxidation of ground pork was influenced by the addition of lemon grass essential oil and the storage period. As the storage days progressed, the 2-TBARS values increased in all treatment groups. The ground pork treated with antioxidants showed significantly lower values compared to the control group. Among the different concentrations of lemon grass oil added, the lowest values were observed with a concentration of 0.5%, as compared to 0.25% LGO and BHT added products. The presence of bioactive compounds such as flavonoids and vitamin C in lemon grass may be responsible for inhibiting chain reactions during lipid oxidation. These findings are similar with a study by Ibrahim and Salem (2013) [9], which reported that lemon grass essential oil effectively reduced lipid oxidation in chicken patties.

Lemon grass essential oil had a significant impact on the peroxide values of ground pork during refrigerated storage. Throughout the storage period, ground pork treated with 0.5% lemon grass essential oil consistently exhibited lower peroxide values compared to other treatments. Regardless of the treatment applied, the peroxide values increased as the storage period progressed. The addition of lemon grass essential oil resulted in a significant reduction in lipid oxidation in ground pork compared to the control and BHT-added samples, leading to the observed lower peroxide values. These findings align with a study by Choe *et al.* (2017) [6] who investigated the effects of persimmon peel extract on raw ground pork after 10 days of storage.

Table 1: Mean ± S.E values of physico-chemical characteristics of ground pork added with lemon grass essential oil

Treatment	Storage days				
	0	3	6	9	12
pH					
Control	6.08±0.03^{eA}	6.21±0.02^{dA}	6.29±0.02^{cA}	6.41±0.^{01bA}	6.63±0.04^{aA}
T_1	6.04±0.01^{cA}	6.07±0.03^{cB}	6.16±0.04^{bB}	6.19±0.02^{bC}	6.23±0.03^{aC}
T_2	6.05±0.01^{cA}	6.07±0.03^{cB}	6.12±0.04^{bB}	6.14±0.01^{bC}	6.19±0.02^{aC}
T_3	6.07±0.02^{dA}	6.09±0.03^{dB}	6.18±0.01^{cB}	6.23±0.03^{bB}	6.29±0.02^{aB}
2-TBARS values (mg malonaldehyde/kg)					
Control	0.53±0.03^{eA}	0.69±0.07^{dA}	0.79±0.02^{cA}	0.97±0.04^{bA}	1.08±0.03^{aA}
T_1	0.49±0.07^{eA}	0.61±0.04^{dB}	0.69±0.05^{cB}	0.78±0.06^{bC}	0.84±0.04^{aB}
T_2	0.48±0.04^{dA}	0.63±0.05^{cB}	0.67±0.02^{bBC}	0.71±0.03^{bD}	0.79±0.02^{aBC}
T_3	0.52±0.11^{eA}	0.64±0.05^{dB}	0.71±0.03^{cB}	0.82±0.05^{bB}	0.87±0.03^{aB}
Peroxide value (m Eq/kg)					
Control	3.65±0.14^{eA}	3.98±0.27^{dA}	4.47±0.11^{cA}	5.23±0.31^{bA}	6.79±0.24^{aA}
T_1	3.12±0.25^{eC}	3.28±0.30^{dC}	3.79±0.33^{cC}	4.12±0.21^{bC}	4.47±0.35^{aC}
T_2	2.98±0.12^{eD}	3.15±0.38^{dC}	3.48±0.41^{cD}	3.95±0.20^{bC}	4.25±0.44^{aD}
T_3	3.42±0.15^{eB}	3.61±0.37^{dB}	4.17±0.36^{cB}	4.72±0.26^{bB}	5.67±0.38^{aB}

C = Ground pork without any antioxidant; T_1 = Ground pork with 0.25% lemon grass essential oil; T_2 = Ground pork with 0.5% lemon grass essential oil; T_3 = Ground pork with 0.01% butylated hydroxy toluene (n = 6)

$^{a–e}$ Mean ± SE values bearing with small letter superscripts on the same row are significantly different (p<0.05).

$^{A–D}$ Mean ± SE values bearing with capital letter superscripts on the same column are significantly different (p<0.05)

Microbial characteristics

Microbial counts of all samples were tabulated in table 2. The total plate counts and total psychrophilic counts of ground pork varied significantly across different treatments and storage periods. Over the course of 12 days, the control and BHT added samples exhibited significantly higher total plate counts and total psychrophilic counts compared to the samples with lemon grass essential oil added. Among the

lemon grass essential oil added samples, those with 0.5% concentration displayed significantly lower counts than those with 0.25% concentration. The active substances present in lemon grass essential oil may be responsible for the reduction in bacterial counts. These findings are similar with Zaki *et al.* (2018) [19], who observed a significant decrease in total bacterial counts and total psychrophilic counts in camel burgers with the addition of lemon grass essential oil.

The presence of lemon grass essential oil and the duration of storage had a notable impact on the yeast and mold counts. Ground pork infused with lemon grass essential oil exhibited lower yeast and mold counts in comparison to the control samples and those treated with BHT. Although the samples containing 0.5% lemon grass essential oil displayed slightly higher values than those with 0.25%, no significant difference was observed between the two concentrations. The reduced yeast and mold counts in the lemon grass-treated samples can be attributed to the flavonoids present in the extract. These findings are in consistent with a previous study by Ibrahim and Salem (2013) [9], where they observed similar outcomes in chicken patties that were supplemented with lemon grass extract.

Table 2: Mean ± S.E values of microbial counts of ground pork added with lemon grass essential oil

Treatment	Storage days				
	0	3	6	9	12
Total plate count					
Control	4.52 ± 0.14^{eA}	4.74 ± 0.05^{dA}	4.97 ± 0.09^{cA}	5.12 ± 0.27^{bA}	5.33 ± 0.17^{aA}
T_1	4.21 ± 0.07^{dC}	4.39 ± 0.33^{cB}	4.62 ± 0.11^{bB}	4.69 ± 0.25^{bB}	4.87 ± 0.19^{aB}
T_2	3.98 ± 0.34^{dD}	4.25 ± 0.12^{cC}	4.48 ± 0.09^{bC}	4.57 ± 0.18^{bC}	4.76 ± 0.37^{aC}
T_3	4.43 ± 0.36^{eB}	4.78 ± 0.26^{dA}	4.95 ± 0.41^{cA}	5.15 ± 0.11^{bA}	5.27 ± 0.17^{aA}
Total psychrophilic count					
Control	2.81 ± 0.05^{eB}	2.95 ± 0.08^{dB}	3.27 ± 0.03^{cA}	3.95 ± 0.12^{bA}	4.59 ± 0.11^{aA}
T_1	2.86 ± 0.02^{dA}	2.64 ± 0.12^{eD}	3.24 ± 0.09^{cAB}	3.58 ± 0.12^{bC}	4.33 ± 0.03^{aB}
T_2	2.45 ± 0.11^{eD}	2.97 ± 0.14^{dC}	3.11 ± 0.13^{cC}	3.37 ± 0.08^{bD}	4.19 ± 0.09^{aC}
T_3	2.70 ± 0.16^{eC}	3.62 ± 0.07^{dA}	3.33 ± 0.04^{cA}	3.85 ± 0.15^{bB}	4.39 ± 0.18^{aB}
Yeast and mold count					
Control	1.59 ± 0.03^{eB}	1.72 ± 0.02^{dA}	1.96 ± 0.07^{cA}	2.35 ± 0.04^{bA}	2.67 ± 0.09^{aA}
T_1	1.53 ± 0.05^{dBC}	1.59 ± 0.03^{dB}	1.71 ± 0.08^{cB}	1.98 ± 0.03^{bB}	2.22 ± 0.02^{Cc}
T_2	1.48 ± 0.11^{dC}	1.53 ± 0.04^{dB}	1.67 ± 0.10^{cB}	1.81 ± 0.06^{bC}	2.17 ± 0.09^{aC}
T_3	1.63 ± 0.04^{eA}	1.71 ± 0.03^{dA}	1.92 ± 0.07^{cA}	2.41 ± 0.06^{bA}	2.56 ± 0.05^{aB}

C = Ground pork without any antioxidant; T_1 = Ground pork with 0.25% lemon grass essential oil; T_2 = Ground pork with 0.5% lemon grass essential oil; T_3 = Ground pork with 0.01% butylated hydroxy toluene (n = 6)

$^{a–e}$ Mean ± SE values bearing with small letter superscripts on the same row are significantly different ($p<0.05$).

$^{A–D}$ Mean ± SE values bearing with capital letter superscripts on the same column are significantly different ($p<0.05$)

Sensory characteristics

The presence of lemon grass essential oil and the duration of storage had a significant impact on the sensory characteristics, as shown in Table 3. As the storage period advanced, regardless of the treatments applied, the color and flavor scores declined. Comparing the various treatments, samples with added lemon grass essential oil consistently achieved superior scores for color and flavor than the control samples. Increasing the concentration of lemon grass essential oil led to better color and flavor scores throughout the entire storage period. Samples with added BHT showed scores that were in-between the control samples and the lemon grass essential oil added samples. The improved colour scores in treated samples could be due to the action of antioxidants preventing the oxidation of oxymyoglobin. The colour and flavour scores were similar to the results of Chauhan *et al.* (2019) [4] in ground pork added with *Terminalia arjuna* fruit extract. Zaki *et al.* (2018) [19] observed improved colour and aroma scores in camel burgers added with lemon grass oil. No off-odour was initially detected on day 0 in any of the treatments. However, as the pork aged, the off-odour gradually intensified, and it was notably more pronounced in the control sample compared to the treated samples. These results were in congruence with Das *et al.* (2011) [7], in which reduced off-odour intensities were observed in ground goat meat added with *Murraya koenigii* powder during refrigerated storage.

Table 3: Mean ± S.E values of sensory attributes of ground pork added with lemon grass essential oil

Treatment	Storage days				
	0	3	6	9	12
Colour					
Control	4.49 ± 0.13^{aB}	3.47 ± 0.09^{bC}	2.84 ± 0.05^{cC}	2.29 ± 0.11^{dC}	1.67 ± 0.09^{eC}
T_1	4.58 ± 0.08^{aA}	4.17 ± 0.09^{bA}	3.86 ± 0.08^{cA}	3.28 ± 0.13^{dB}	2.47 ± 0.15^{eA}
T_2	4.51 ± 0.06^{aB}	4.15 ± 0.11^{bA}	3.79 ± 0.07^{cB}	3.35 ± 0.12^{dA}	2.43 ± 0.05^{eA}
T_3	4.56 ± 0.11^{aA}	4.03 ± 0.09^{bB}	3.77 ± 0.20^{cB}	3.26 ± 0.09^{dB}	2.39 ± 0.16^{eB}
Flavour					
Control	4.57 ± 0.07^{aB}	3.73 ± 0.05^{bC}	3.07 ± 0.12^{cC}	2.57 ± 0.06^{dC}	1.93 ± 0.15^{eC}
T_1	4.63 ± 0.04^{aA}	4.29 ± 0.08^{bA}	3.84 ± 0.05^{cA}	3.05 ± 0.08^{dA}	2.84 ± 0.07^{Ea}
T_2	4.65 ± 0.05^{aA}	4.31 ± 0.08^{bA}	3.81 ± 0.14^{cA}	3.09 ± 0.11^{dA}	2.89 ± 0.07^{eA}
T_3	4.53 ± 0.06^{aB}	4.05 ± 0.09^{bB}	3.73 ± 0.15^{cB}	2.93 ± 0.08^{dB}	2.52 ± 0.13^{eB}
Off-odour					
Control	5^{Aa}	4.18 ± 0.12^{bD}	3.51 ± 0.04^{cD}	2.39 ± 0.23^{dD}	1.98 ± 0.10^{eD}
T_1	5^{aA}	4.51 ± 0.22^{bB}	3.98 ± 0.06^{cB}	3.32 ± 0.09^{dB}	2.57 ± 0.13^{eB}
T_2	5^{aA}	4.62 ± 0.05^{bA}	4.29 ± 0.14^{cA}	3.75 ± 0.08^{dA}	2.94 ± 0.06^{eA}

T_3	5^{aA}	4.37 ± 0.15^{bC}	3.77 ± 0.20^{cC}	3.18 ± 0.04^{dC}	2.25 ± 0.09^{eC}

C = Ground pork without any antioxidant; T_1 = Ground pork with 0.25% lemon grass essential oil; T_2 = Ground pork with 0.5% lemon grass essential oil; T_3 = Ground pork with 0.01% butylated hydroxy toluene (n = 6)

[a–e] Mean ± SE values bearing with small letter superscripts on the same row are significantly different ($p<0.05$).

[A–D] Mean ± SE values bearing with capital letter superscripts on the same column are significantly different ($p<0.05$)

Conclusion

The findings of this study clearly showed that the inclusion of lemon grass essential oil improved the quality of raw ground pork by harnessing its antioxidant and antimicrobial properties. Lemon grass essential oil effectively reduced lipid oxidation in comparison to both the control group and the group treated with BHT. The active compounds present in lemon grass led to reduced microbial counts in the pork. Moreover, the samples supplemented with 0.5% lemon grass essential oil maintained better sensory characteristics for up to 12 days. The ground pork enriched with 0.5% lemon grass essential oil exhibited superior physico-chemical, microbial, and sensory qualities compared to the samples treated with 0.25% lemon grass essential oil. In conclusion, in order to meet consumer demands for healthier and safer food, the utilization of natural antioxidants could be a viable approach to preserve meat during storage.

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