Antioxidant Activity of Lemongrass Leaves In Chicken Burger

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Abstract: During meat/chicken cooking and storage warmed over flavor (WOF) appears within 24 hrs of refrigerated storage. In this study, the anti-oxidation and color stability effect of lemongrass leaves powder were investigated in fresh and cooked chicken burgersamples. Results indicated that lowest value of Thiobarbituric acid reactive substance (TBA-RS) in all samples treated with lemongrass (2%) concentration provides best effectiveness. Met-myoglobin formation was decreased in uncooked samples by adding lemongrass powder. The effect of lemongrass was more pronounced in cooked samples. In addition to that, lemongrass reduced lipid peroxidation and degradation of heme pigments caused by cooking and storage.

Keywords: Lemongrass, Antioxidant, chicken burger, Lipid peroxidation, Heme degradation

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I. Introduction

Chicken meat is favored by consumers, it contains low fat with high concentration of poly-unsaturated fatty acids. Oxidation of lipids is a major reason of chicken spoilage thus reduces quality of meat product (Devatkal and Naveena, 2010; Zhang et al., 2016). Stability of lipid in meats and meat products is very important factor, which has influence on quality and consumer acceptability (Mitsumoto et al., 2005). Reducing of lipid oxidation during storage of meat products can be achieved with antioxidants (Naveena et al., 2006). In recent times many investigations were carried out on natural antioxidants such as rosemary, sage, soy protein, plum and tea catechins (Nunez de Gonzalez et al., 2008). Lemongrass is a rich source of citral and bioactive compounds such as vitamin C and flavonoids, which is used in pharmaceutical industries. Flavonoids are anti-inflammatory and anti-carcinogenic agents because of their lipid anti-peroxidation effects (Marin et al., 2002). Lemongrass contains several bioactive compounds, which are useful in several health issues and are found in leaves (Olorunisola et al., 2014). Warmed over flavor (WOF) is the rapid development of oxidized flavor in refrigerated, pre-cooked and cooked meat, where rancid flavor became apparent within the first 48 hrs of refrigeration (Kulkarni, 2011). Many plants containing polyphenol compounds are effective antioxidants and can retard the development of WOF in meat products (Ahnet al., 2007).

Therefore the aim of this study was to study the antioxidant effectiveness of lemongrass leaves at various ratios on reducing peroxidation and development of warmed over flavor (WOF) in chicken burger during cold storage.

II. Material and Methods

Lemongrass leaves were purchased from local market, washed with water, dried and milled to a fine powder. Fresh chicken fillet (boneless breasts) and spices were obtained from a local market (Ismailia, Egypt). All reagents, solvents and chemicals used in this study were of analytical grade. Trichloroacetic acid (TCA); 2-thiobarbituric acid (TBA-RS); nitric acid; ferrozine [3-(2-pyridyl)-5, 6-bis (4-phenylsulfonic acid)-1, 2, 4-triazine]; α-tocopherol and ammonium acetate were obtained from Sigma-Aldrich Chemical Co. (St. Louis, Mo, USA).

A- Chicken Burger Processing

Chicken fillets were minced using a meat mincer (SAP Meat Mincer Tc22, Italy), and divided into six equal parts. Chicken burger was prepared by adding lemongrass at (0.0, 0.5, 1.0, 1.5 and 2.0%), 0.56% spices mixture (clove 4.0%; cumin 15.0%; coriander 12.0%; nutmeg 2.5%; black pepper 45.0%; all spice 10.0% and red pepper 11.5%), onion 0.3%; garlic 0.3% and 1.0% sodium chloride. A positive reference control was prepared by mixing 150 mg tocopherol acetate with 100g of minced chicken. After mixing, samples were divided into small lots (100g) to prepare burger. All samples before and after cooking in a microwave oven for 90 sec. were stored covered in petri dishes for 12 days at 4°C.
B- Analytical Methods

1- Total Phenolic Compounds (TPC) Determination:
Total phenolic compounds (TPC) were determined in ethanolic extract (one gram sample was extracted by 25ml ethanol 50%) according to the method described by Jaramillo-Flores et al., (2003) with some modifications as follows:

100µl of ethanolic extract was mixed with 900µl of Folin-Ciocalteu 10% (diluted in distilled water 1:10) and allowed to stand for 5 min. at room temperature.0.75ml of sodium bicarbonate 7% (NaHCO₃) was added to the mixture and vortexed for 30sec, then allowed to stand for 90min. at room temperature. The absorbance was measured at725nm using a spectrophotometer (6505 Uv/vis, Jenway, UK).

2- Determination of Antioxidant Activity

The antioxidant activity was determined by 2,2-diphenyl 1-1-picyhydrazyl (DPPH) method according to Lee et al., (2003) with some modifications as follows: 100µl of sample extract was reacted with 3.9ml of DPPH solution (60 µmol/L DPPH solution was made in ethanol 99%) for 60min in a dark place. The absorbance was measured using a spectrophotometer at 515nm against a blank of ethanol 95%. Antioxidant activity was calculated as follows:

DPPH radical-scavenging activity (%) = (Acontrol – Asample) / Acontrol X 100
Where, A is the absorbance at 515nm.

3- Total Iron

Total iron concentration was determined in wet-ashed samples as described by Stockey, 1970). Chicken burger (0.2 – 0.3g) in a test tube digested with concentrated nitric acid and hydrogen peroxide (30%) on a hot plate until white ash was formed. Then dissolve in 0.2ml HCl (1.0N), deionized water (0.85ml) and ascorbic acid (1ml, 1%) was added and the tube contents were mixed. After 20min. 1ml of ammonium acetate buffer (10%) and 1ml of ferrous color reagent (1mM)were added and well mixed. The mixture was allowed to stand at room temperature for 45min. then the absorbance was determined at 562nm using Bausch and Lamb spectrophotometer. The concentration of iron was determined from a standard curve made with standard iron solution (Sigma, St. Louis, Mo).

4- Heme Iron

Heme iron was measured according to the method of Lee et al., (1998). (2g) of cooked chicken burger was transferred into a tube of polypropylene (50ml), then 9 ml of acetone 90% acidified with HCl 2% was added. The mixture was then macerated with a glass rod and allowed to stand for 1hrs in a dark cabinet at room temperature. The extract was filtered through Whatman filter paper 42 and the absorbance was read at 640nm against the acid acetone blank.

Total pigments as acid hematin, were calculated using the formula:

Total pigments (ppm) = A460 X 680
And heme iron was calculated as follows (Clark et al., 1997):

Heme iron (ppm) = total pigment (ppm) x 8.82/100

5- Non-heme Iron

The concentration of non heme iron in samples was calculated using the formula:

Non-heme iron (ppm) = total iron – heme iron

6- Metmyoglobin

Metmyoglobin was determined according to Krzywicki (1982) as following:Minced meat (5g) was mixed with 25ml of ice-cold phosphate buffer (pH6.8, 40mM). The mixture was homogenized at 10.000rpm for 10sec using an Ultra-Turrax T50 (Jank & Kunkel Gmbh, Straufen Germany). The homogenized sample was allowed to stand for 1hr at 4°C and centrifuged at 5000rpm for 30min. at 4°C. The supernatant was filtered through whatman (1) filter paper and the absorbance was read at 700, 572 and 525nm with spectrophotometer. Percent of metmyoglobin calculated according to the formula:

% MetMb = 1.395 - (A572 – A700)/ (A525 – A700) x 100

7- TBA Reactive Substance Values (TBA-RS)

Thiobarbituric acidreactive substance values (TBA-RS) was determined as described by Pearson (1981).

8- Warmed-Over Flavor (WOF)

Sensory evaluation was carried out after 48hrs of storage of cooked samples at 4°C ±1. All chicken burgersamples were reheated in bath water to an approximate internal temperature of 70°C and served while hot.
The panel scoring system was as following: 5: no WOF, 4: slight WOF, 3: moderate WOF, 2: pronounced WOF and 1: very pronounced WOF.

9- Statistical Analysis
Standard Deviation (SD) and significant differences between the mean values of the estimated tests were performed using the software package Statistical 9.1 for Windows, Stat Soft, Tulsa, Oklahoma, USA, 2009. Differences were considered significant at P<0.05.

III. Results and Discussion

1- Total Phenolic Compounds (TPC)
Phenolic compounds are secondary metabolites found in fruits, legume, cereals and vegetables. Phenolic compounds content in chicken burger during refrigerated storage for 12 days are presented in Table (1). The data showed that addition of lemongrass powder to chicken burger increased TPC in the sample.

There were differences among control, sample treated with α-tocopherol as a positive control, and samples treated with different concentrations of lemongrass (0.5, 1.0, 1.5, and 2.0%).

Phenolic compounds level decreased in all chicken burger as storage time increased. After 12 days of storage, phenolic compound amounts were: 44.32, 57.21, 72.02, 88.71, 95.62, and 112.23 mg/100g for control, positive control, lemongrass 0.5, 1.0, 1.5, and 2.0% respectively. Chicken burger sample which containing lemongrass powder 2.0% had the highest level of TPC (112.23 ±0.301mg /100g).

Our results are in agreement with those reported by Devatkal et al. (2010) who reported that pomegranate rind powder significantly increased level of total phenolic compounds in chicken burger.

The results exhibited higher content of phenolic compounds due to lemongrass treatments (proportionally to the used concentration) compared to untreated samples. The highest amount of phenolic compounds were recorded to the highest concentration of lemongrass. The antioxidant activity of lemongrass is overwhelming due to phenolic compounds which could retard lipid oxidation, consequent mark-downing of oxidative rancidity and deterioration, delaying off-flavor development and upturning consumer acceptance, which could be a natural source replacing synthetic antioxidants recently restricted in application to food because of its toxicological hazards and their carcinogenic probability (Johnston et al., 2005, Engy et al., 2018).

Table 1: Changes in total phenolic compounds (mg/100g) during storage of chicken burger at 4°C± 1 (mean ± S.D).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T1</th>
<th>T2</th>
<th>Lemon grassConcentration%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage Time</td>
<td>T3</td>
<td>T4</td>
<td>T5</td>
</tr>
<tr>
<td>0</td>
<td>71.06 ±0.015</td>
<td>95.12 ±0.081</td>
<td>112.08±0.351</td>
</tr>
<tr>
<td>2</td>
<td>67.32 ±0.035</td>
<td>89.81 ±0.080</td>
<td>105.17±0.158</td>
</tr>
<tr>
<td>4</td>
<td>63.06 ±0.158</td>
<td>81.13 ±0.315</td>
<td>98.82±0.211</td>
</tr>
<tr>
<td>6</td>
<td>57.01 ±0.151</td>
<td>75.09 ±0.022</td>
<td>91.72±0.501</td>
</tr>
<tr>
<td>8</td>
<td>52.10 ±0.033</td>
<td>69.15 ±0.115</td>
<td>85.19±0.082</td>
</tr>
<tr>
<td>10</td>
<td>49.81 ±0.101</td>
<td>63.81 ±0.009</td>
<td>79.83±0.213</td>
</tr>
<tr>
<td>12</td>
<td>44.32 ±0.008</td>
<td>57.21 ±0.310</td>
<td>72.02±0.312</td>
</tr>
</tbody>
</table>

Total phenolic compounds (TPC) of lemongrass powder 49.22%
T1 = control zero lemongrass
T2 = positive control 150 mg α tocopherol

2- Antioxidant Activity (DPPH)
Antioxidant activity (DPPH) was used as free radical to evaluate antioxidant activity. Effect of storage conditions on antioxidant activity at different lemongrass concentrations and storage time intervals compared with control samples are given in Table(2). The results showed that antioxidant activity of α-tocopherol was weaker than that of lemongrass at all treatment levels. The chicken burger formulated with lemongrass at 2% had higher levels of antioxidant activity than other treatments.

A substantial decrease was observed in control samples compared to treated samples with lemongrass. Increase in lemongrass concentrations tends to improve the stability of antioxidant activity. Higher effects were observed with higher concentrations, and lower activity was associated with lower concentrations of lemongrass. Thus, the high level of antioxidant activity in lemongrass was attributed to the high level of phenolic compounds of lemongrass powder.

DPPH free radicals are widely known and used repeatedly to monitor the scavenging abilities of many natural additives and extracts (Amarowicz et al., 2004). The antioxidant activity mechanism of natural herbs might be explained by 2 major functions: (1) Scavenging activity, (2) Hydrogen donating ability (Hyam and Ferial, 2004).
Polyphenolic compound extracts are superior donors of electron and proton and have immediate stable radicals because of delocalization phenomenon of electron and lacking of contravention by oxygen (O₂) (Djenane et al., 2005). In our study lemongrass treatment exhibited a higher scavenging activity on DPPH radicals than those of both control samples.

Our results are in agreement with results obtained by Tiwari et al. (2010) who reported that leaves extract of lemongrass shows antioxidant property by DPPH scavenging test. According to Hasim et al. (2015) lemongrass leaves extract have bioactivity compounds, such as Tannins, flavonoids and phenols as an antioxidant.

### Table 2: Changes in antioxidant activity (DPPH%) during storage treatments at 4°C±1 (mean ± S.D)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T1</th>
<th>T2</th>
<th>T3 (0.5)</th>
<th>T4 (1.0)</th>
<th>T5 (1.5)</th>
<th>T6 (2.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td>32.15±0.114</td>
<td>38.11±0.029</td>
<td>51.22±0.009</td>
<td>73.08±0.115</td>
</tr>
<tr>
<td>2</td>
<td>13.01±0.021</td>
<td>29.11±0.031</td>
<td>36.21±0.017</td>
<td>50.03±0.101</td>
<td>71.61±0.202</td>
<td>88.13±0.002</td>
</tr>
<tr>
<td>4</td>
<td>10.92±0.081</td>
<td>28.05±0.326</td>
<td>34.52±0.212</td>
<td>48.91±0.305</td>
<td>69.02±0.119</td>
<td>86.72±0.131</td>
</tr>
<tr>
<td>6</td>
<td>10.01±0.009</td>
<td>26.91±0.118</td>
<td>33.81±0.003</td>
<td>47.55±0.331</td>
<td>67.32±0.502</td>
<td>84.88±0.001</td>
</tr>
<tr>
<td>8</td>
<td>09.52±0.124</td>
<td>25.00±0.017</td>
<td>33.10±0.115</td>
<td>46.72±0.148</td>
<td>64.82±0.111</td>
<td>82.09±0.121</td>
</tr>
<tr>
<td>10</td>
<td>08.87±0.119</td>
<td>23.12±0.052</td>
<td>31.92±0.118</td>
<td>45.19±0.006</td>
<td>60.19±0.101</td>
<td>79.83±0.102</td>
</tr>
<tr>
<td>12</td>
<td>07.35±0.225</td>
<td>21.08±0.006</td>
<td>30.02±0.109</td>
<td>43.45±0.209</td>
<td>58.21±0.331</td>
<td>77.65±0.119</td>
</tr>
</tbody>
</table>

*Antioxidant activity (DPPH) of lemongrass powder (88.92%)
T1 = control zero lemongrass
T2 = positive control 150 mg α tocopherol

### 3- Metmyoglobin

Mean values of metmyoglobin in chicken burger samples during storage were shown in Table (3). Differences observed between both control samples and treated samples during storage time. The formations of metmyoglobin were time-dependent when burger samples were kept for 12 days at 4°C.

Sample treated with lemongrass showed lower metmyoglobin formation after 12 days of storage. Treated samples with higher concentrations of lemon grass were more effective for inhibiting of metmyoglobin formation than those with α-tocopherol and control. The antioxidation activity of lemongrass plays a sizable role in maximizing reduction of metmyoglobin formation. The levels were: 95.3, 62.8, 73.2, 63.1, 55.2, and 47.2% for control, positive control, lemongrass 0.5%, lemongrass 1.0%, lemongrass 1.5%, and lemongrass 2.0% respectively.

<table>
<thead>
<tr>
<th>Storage Time</th>
<th>T1</th>
<th>T2</th>
<th>T3 (0.5)</th>
<th>T4 (1.0)</th>
<th>T5 (1.5)</th>
<th>T6 (2.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>24.1±0.121</td>
<td>24.3±0.008</td>
<td>24.2±0.005</td>
<td>24.1±0.008</td>
<td>24.2±0.005</td>
<td>23.9±0.001</td>
</tr>
<tr>
<td>2</td>
<td>39.1±0.111</td>
<td>31.6±0.113</td>
<td>39.1±0.302</td>
<td>35.6±0.108</td>
<td>33.6±0.012</td>
<td>30.8±0.019</td>
</tr>
<tr>
<td>4</td>
<td>47.3±0.250</td>
<td>33.5±0.102</td>
<td>43.3±0.208</td>
<td>40.1±0.007</td>
<td>38.1±0.126</td>
<td>34.6±0.005</td>
</tr>
<tr>
<td>6</td>
<td>77.2±0.007</td>
<td>45.2±0.115</td>
<td>50.8±0.211</td>
<td>45.6±0.005</td>
<td>43.2±0.009</td>
<td>39.1±0.021</td>
</tr>
<tr>
<td>8</td>
<td>82.1±0.301</td>
<td>51.7±0.111</td>
<td>62.1±0.028</td>
<td>51.2±0.116</td>
<td>48.6±0.001</td>
<td>43.6±0.128</td>
</tr>
<tr>
<td>10</td>
<td>95.3±0.270</td>
<td>62.8±0.004</td>
<td>73.2±0.006</td>
<td>63.1±0.208</td>
<td>55.2±0.221</td>
<td>47.2±0.009</td>
</tr>
</tbody>
</table>

T1 = control zero lemongrass
T2 = positive control 150 mg α-tocopherol

Marin et al. (2002) found that lemongrass is a rich source of flavonoids as antioxidant agent. The antioxidation activity of lemongrass was due to the high levels of phenolic compounds (Kanatt et al., 2014). The data exhibited the efficiency of lemongrass antioxidation towards metmyoglobin formation.

Renere et al., (1992) mentioned that the myoglobin autoxidation susceptibility is the major contributing factor in meat and its products stability, but could not identify the exact oxidation process substances of oxymyoglobin to metmyoglobin. Other researchers concluded the oxygen initiation ability on lipid peroxidation, helping formation of peroxydants which are capable to react with oxymyoglobin resulting of metmyoglobin formation (Acton et al., 1993).
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4- TBA-RS Values

Antioxidant extracted from some plants can be used as alternatives to synthetic antioxidants due to high effect on inhibition of lipid oxidation (Aguirrezábal et al., 2000). The extent of lipid oxidation in uncooked chicken burger samples were recorded in Table (4).

The lipid oxidation levels increased in all burger samples as storage time increased. Antioxidant treated samples showed more lipid stability and differences appeared among treated chicken burger samples according to concentrations. All variables (storage time and lemongrass concentration) were venerable contributors to TBA-RS values.

On the 12th day of cold storage, TBA-RS values for uncooked samples were 1.92, 1.54, 1.49, 0.81, 0.77, and 0.53 for control, positive control, lemongrass 0.5%, 1.0%, 1.5%, and 2.0% respectively. The results demonstrated lemongrass effectiveness in reducing lipid oxidation rapidity of chicken burger throughout storage. Chicken burger with antioxidant treatment showed lower TBA-RS values than those of control samples. 2% lemongrass was the most effective addition for retarding lipid oxidation after 12 days of cold storage.

Consumer acceptance for chicken and meat products quality affected by lipid oxidation and its products (off-odors, off-flavors). For preventing oxidation of lipids we applied lemongrass as an alternative antioxidant both inexpensive and of natural origin. The content of lipids lowered in all samples during refrigeration storage due to lipid autoxidation and can contributed to triglycerides level changes (Sampaio et al., 2012). As results expressed that all four concentrations of lemongrass could be beneficially to reduce lipid oxidation velocity in chicken burger during storage. The hydrogen atom (H) of phenolic (-OH) group of phenolic compounds hinder expansion of free radicals autooxidation chain by forming more stabilized free radical for minimal oxidation process (Falowo et al., 2014).

Warmed over flavor (WOF) is the rapid development of oxidized flavor in cooked meat products rancid flavor caused by lipid oxidation in meat products leading to deterioration in cooked meat products quality that have been chill-stored. Warmed over flavor can be qualified by measurement of TBA-RS (Kulkarni, 2011). Lipid oxidation as expressed by TBA-RS number is increased with cooking and with increasing time after cooking (Table 4). Lee and Hendricks (1995) reported that, when meat cooked, iron is liberated and interacts with phospholipids to catalyze lipid oxidation. Both α-tocopherol and lemongrass decreased TBA-RS values formation and the effect was dose-dependent. There was negative relationship between heme-iron content (Table 5) and TBA-RS values number (Table 4) of cooked burger. TBA-RS values of control sample was 2.92 ±0.221 mg/kg after 12 days of cold storage and dropped to 0.89 ±0.001 mg/kg, when sample treated with lemongrass (2%). The antioxidant effect of lemongrass may be due to phenolic compounds in lemongrass powder. Kanatt et al. (2014) mentioned that irradiated meat containing lemongrass extract had lower TBA-RS values. de Moraes Barros et al. (2012) reported that natural antioxidant improved lipid oxidation of fat products and meals.

Table 4: Effect of lemongrass powder on TBA-RS values (mg malonaldehyde/kg) in chicken burger during storage at 4°C ±1 (mean ± S.D)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lemon grass Concentration %</th>
<th>T1</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T3 (0.5)</td>
<td>T4 (1.0)</td>
<td>T5 (1.5)</td>
</tr>
<tr>
<td>0</td>
<td>0.15 ±0.012</td>
<td>0.15 ±0.112</td>
<td>0.14 ±0.013</td>
</tr>
<tr>
<td>1</td>
<td>0.22 ±0.008</td>
<td>0.00 ±0.00</td>
<td>0.00 ±0.00</td>
</tr>
<tr>
<td>2</td>
<td>0.31 ±0.111</td>
<td>0.21 ±0.113</td>
<td>0.20 ±0.008</td>
</tr>
<tr>
<td>3</td>
<td>0.32 ±0.101</td>
<td>0.30 ±0.061</td>
<td>0.28 ±0.001</td>
</tr>
<tr>
<td>4</td>
<td>0.33 ±0.028</td>
<td>0.35 ±0.221</td>
<td>0.28 ±0.001</td>
</tr>
<tr>
<td>5</td>
<td>0.31 ±0.011</td>
<td>0.30 ±0.006</td>
<td>0.28 ±0.001</td>
</tr>
<tr>
<td>6</td>
<td>0.31 ±0.009</td>
<td>0.21 ±0.016</td>
<td>0.24 ±0.021</td>
</tr>
<tr>
<td>7</td>
<td>0.32 ±0.025</td>
<td>0.22 ±0.022</td>
<td>0.24 ±0.021</td>
</tr>
<tr>
<td>8</td>
<td>0.51 ±0.031</td>
<td>0.35 ±0.108</td>
<td>0.31 ±0.001</td>
</tr>
<tr>
<td>9</td>
<td>0.53 ±0.023</td>
<td>0.28 ±0.001</td>
<td>0.25 ±0.011</td>
</tr>
<tr>
<td>10</td>
<td>0.51 ±0.011</td>
<td>0.28 ±0.001</td>
<td>0.25 ±0.011</td>
</tr>
<tr>
<td>11</td>
<td>0.52 ±0.025</td>
<td>0.38 ±0.061</td>
<td>0.31 ±0.011</td>
</tr>
<tr>
<td>12</td>
<td>0.53 ±0.023</td>
<td>0.38 ±0.061</td>
<td>0.31 ±0.011</td>
</tr>
<tr>
<td>% Fat</td>
<td>12.02 ±0.001</td>
<td>12.08 ±0.150</td>
<td>12.10 ±0.116</td>
</tr>
</tbody>
</table>

T1 = control zero lemongrass
T2 = positive control 150 mg α tocopherol
I = uncooked chicken burger
H = cooked chicken burger

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5- Heme and Non-Heme Iron:

WOF caused by lipid oxidation of meat products. The type of off-flavor is associated with the autoxidation of unsaturated fatty acids and iron, in one form or another is an imported catalyst.

Heme iron contents decreased progressively with treated samples (Table 6) compared with control samples which decreased more rapidly. The effectiveness of the antioxidant in preserving the heme iron values was in the following order: 2.0 > 1.0 > 1.5 > 0.5 % lemon grass > α-tocopherol > control.

Heme iron contents after 12 days of cold storage at 4°C were: 7.5, 9.9, 10.3, 12.2, 13.9, and 14.7 mg/kg for control, α-tocopherol, 0.5, 1.0, 1.5, and 2.0% lemongrass respectively, corresponds to 50.33, 57.22, 59.53, 70.52, 80.34, and 84.97 % of total heme iron content.

Meat tissues contain considerably quantities of iron linked to proteins (hemoprotein). The decreased amount of heme iron with storage time extension by means of free iron releasing from heme resulted in less retaining of heme iron and decreasing the heme pigment with increasing time of storage contributed to iron content drooping. (Ozerand Sarıçoğan, 2010). Purchaset al., (2003) mentioned that the meat drip at the mean time of storage has containable amounts of iron and predominately dissolvable heme iron. After cooking ionic iron % releasing from protein bound iron increased by heat induction (Mei et al., 1994). During storage period, values of heme iron content decreases and non-heme iron increased which may be due to heme breakdown resulting from the release of non-heme content. Lipid peroxidation of muscle is activated by this released iron during cold storage (Hafiz et al., 2015).

At the time Byrne (2001) reported that iron was released from heme pigments during cooking and leading to increase in non-heme iron which was responsible for lipid peroxidation.

Iron plays an important role as a peroxidant for lipid oxidation among transition metals. Via Fenton reaction the ferrous breaking lipid peroxide and hydrogen into free radicals which accelerate lipid oxidation (Oztrück et al., 2007). In this study, lemongrass exhibited higher ion-chelating effect of ferrous than that in inoperative control (α-tocopherol) via chelating and/or deactivating transitioned metals which could promote the reaction of Fenton and decomposition of hydroperoxides.

Table 5: Effect of lemongrass on heme and non heme iron contents in cooked chicken burger samples during cold storage at 4°C ± 1 (mean ± S.D)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T1</th>
<th>T2</th>
<th>Lemon Grass Concentration %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage Time</td>
<td></td>
<td></td>
<td>T3</td>
</tr>
<tr>
<td>1</td>
<td>14.9±0.012</td>
<td>17.4±0.009</td>
<td>17.3±0.021</td>
</tr>
<tr>
<td>0</td>
<td>6.3±0.001</td>
<td>4.2±0.001</td>
<td>3.9±0.012</td>
</tr>
<tr>
<td>2</td>
<td>8.0±0.110</td>
<td>4.7±0.113</td>
<td>4.6±0.003</td>
</tr>
<tr>
<td>4</td>
<td>9.6±0.003</td>
<td>6.3±0.101</td>
<td>5.9±0.003</td>
</tr>
<tr>
<td>6</td>
<td>11.5±0.111</td>
<td>7.4±0.28</td>
<td>7.1±0.116</td>
</tr>
<tr>
<td>8</td>
<td>12.3±0.022</td>
<td>9.6±0.116</td>
<td>9.2±0.313</td>
</tr>
<tr>
<td>10</td>
<td>13.1±0.131</td>
<td>10.4±0.131</td>
<td>9.3±0.215</td>
</tr>
<tr>
<td>12</td>
<td>13.7±0.008</td>
<td>11.3±0.009</td>
<td>10.9±0.009</td>
</tr>
</tbody>
</table>

- heme iron in chicken meat 18.1 mg/kg
- Non heme iron in chicken meat 3.1 mg/kg
- Total iron in chicken meat 21.2 mg/kg
- T1 = control zero lemongrass
- T2 = positive control 150 mg α-tocopherol

6- Warmed-Over Flavor (WOF) Evaluation:

Quantities of free iron were released during cooking and storage of meat products. Free iron and negative charged phospholipids caused specific oxidation that generated WOF during cold storage (Graf and Panter, 1991). Data shown in Table (4) demonstrates the high effect of lemongrass on TBA-RS formation in cooked chicken burger. WOF in precooked samples is caused by the degradation products of lipid hydroperoxides such as aldehydes, malondialdehyde, heptanal and hexanal.

To evaluate lemongrass on WOF development in cooked samples kept under possible consumer home refrigerator abuse conditions, samples were stored uncovered on a plate at 4°C ±1. The evaluation showed that TBA-RS values raised from 0.22 after 2 days to 2.92 after 12 days of cold storage, for control sample and from 0.22 to 0.89 mg MDA/kg for the test sample containing 2% lemongrass powder. Thus our results indicated that...
lemongrass leaves powder may be useful as additive to delay onset of WOF in precooked chicken burger (Table 4, 6).

**Table 6: Sensory scores in cooked chicken burger during storage at 4°C.**

<table>
<thead>
<tr>
<th>Lemongrass (%)</th>
<th>T1</th>
<th>T2</th>
<th>T3 (0.5)</th>
<th>T4 (1.0)</th>
<th>T5 (1.5)</th>
<th>T6 (2.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.37a</td>
<td>4.60a</td>
<td>4.63a</td>
<td>4.61a</td>
<td>4.63a</td>
<td>4.64a</td>
</tr>
<tr>
<td>2</td>
<td>3.49b</td>
<td>3.87b</td>
<td>3.97b</td>
<td>4.31a</td>
<td>4.41a</td>
<td>4.42a</td>
</tr>
<tr>
<td>4</td>
<td>2.50a</td>
<td>3.61b</td>
<td>3.69b</td>
<td>4.02b</td>
<td>4.11a</td>
<td>4.25b</td>
</tr>
<tr>
<td>8</td>
<td>1.93a</td>
<td>3.44b</td>
<td>3.50c</td>
<td>3.94b</td>
<td>4.05c</td>
<td>4.01d</td>
</tr>
<tr>
<td>10</td>
<td>1.33b</td>
<td>2.68c</td>
<td>3.16c</td>
<td>3.71c</td>
<td>3.72a</td>
<td>3.90a</td>
</tr>
</tbody>
</table>

-Mean in the same row with different superscripts are significantly different at p≤ 0.05
T1 = control zero lemongrass
T2 = positive control 150 mg α tocopherol

**IV. Conclusion**

This study examined the interaction effects of two factors: addition of antioxidant factor, and storage time/condition factor. The current results exhibited the effectiveness of lemongrass as a noteworthy food bioactive compounds source in food products/industry as a natural additive with antioxidant activity retarding lipid oxidation, improving the overall organoleptic characteristics for more consumer satisfaction and extending shelf-life of raw/cooked chicken burger during cold storage at 4°C for 12 days. Lemongrass treatment could interact positively to reduce the harmful influence of storage time factor and lipid oxidation promoters as compared with control samples’ shelf-life and total acceptability. The highest effect was achieved by 2% lemongrass followed by lower concentrations in descending order due to its highly valuable effectiveness of the phenolic compounds antioxidant activity. Lemongrass in food technology challenges for replacing the commercial and synthetic antioxidants (of adverse health effectsuations) with equivalent or even better natural antioxidants, supplying chicken and meat products with good alternatives for functional healthy food products.

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**References**


Antioxidant Activity of Lemongrass Leaves in Chicken Burger


