The effect of using Chamomile extract for the preservation of chicken breast meat on oxidative parameters and microbial profile of chicken meat

Mervat S. Hassanin, Mamdouh A. Abdel-Moneim* and Ghadir A. El-Chaghaby

Regional Center for Food and Feed, Agricultural Research Center, Giza, Egypt.

*Corresponding author. E-mail: abbas_mamdouh@yahoo.com

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Chicken meat is an important protein source in food and its preservation is of major concern. In the present study, chamomile leave extract was prepared by maceration method using ethanol as solvent. The extract was found to contain appreciate amounts of phenols (43.46 mgGA/g) and flavonoids (25.37 mgQE/g) which both contribute to its high antioxidant activity (202 mgAAE/g). Chamomile leave extract was effective on inhibiting the growth of three Gram positive bacteria and three Gram negative bacteria. The extract was applied as preservative for chicken breast meat under refrigerated storage. Three levels of the extract were applied (100, 200 and 400ppm) to chicken breast meat. The results showed that the tested extract was effective in scavenging the free radical DPPH. The use of Chamomile leave extract suppressed the lipid oxidation of raw chicken meat as indicated by the TBARS analysis. The extract showed bactericidal effect against food spoilage bacteria. The results of the present study indicates that chicken breast meat could be preserved using chamomile leave extract as natural preservative up to fifteen days of refrigerated storage. The economics behind using such natural preservative is also a major advantage as it has limited cost of production and decreases food loss by extending its shelf life.

Key words: Chamomile leave, chicken breast meat, lipid oxidation, bacterial count, antioxidant, antibacterial.

INTRODUCTION

Chicken meat is an important source of protein in human diets. It is the second most consumed meat in the world (Neto et al., 2015). Preservation of Chicken meat is usually limited due to lipid oxidation and microbial growth that may lead to meat spoilage. Lipid oxidation is considered the cause of quality damages of meat and meat products (Racanici et al., 2008). Microbial growth relies on the condition of the carcasses at the time of slaughter, the type of packaging and storage conditions (Scotia, 2011). The shelf life of poultry meat is decreased even under refrigerated conditions as a result of oxidation products (Tavárez et al., 2011). On the other hand, lipid oxidation and microbial growth during storage can be reduced by applying antioxidant and antimicrobial agents to the meat products, leading to a retardation of spoilage, extension of shelf-life and maintenance of quality and safety (Radha krishnan et al., 2015a).

Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been widely used in food preservation; however these synthetic preservatives have showed
carcinogenic effects in living organisms (El Abed et al., 2014). Currently, there is an increasing trend towards limiting the negative aspects of synthetic chemical preservatives by employing natural techniques in food preservation (Padam et al., 2012).

Plant extracts have been studied as natural antioxidants and antimicrobials that can be used as natural preservative to inhibit the growth of food-borne and spoiling microbes and to extend the shelf-life of foods. Chamomile (Matricaria chamomilla L.) is one of the important medicinal herbs that have been used in herbal remedies for thousands of years, known in ancient Egypt (Singh et al., 2011).

Several studies have reported the bioactivity of chamomile flowers but few studies were concerned with chamomile green parts (leaves). The present study was performed to investigate the antioxidant and antibacterial effect of chamomile leaf extract on the preservation of chicken breast meat stored under refrigerated conditions.

MATERIALS AND METHODS

Preparation of extract

Chamomile plants were collected; the green parts (leaves) were separated from the flowers and washed to remove any adherent dirt. The plant leaves were then oven dried until constant weight to remove moisture. The dried leaves were crushed and then soaked (overnight) in 80% ethanol. The resulting solution was collected after filtration and remaining solvent was evaporated. The chamomile leave extract (CLE) was obtained and kept in a freezer until further use.

Determination of the antioxidant properties of the extract

The total antioxidant capacity of CLE was determined by the “phosphomolybdenum” method (Prieto et al., 1999). The results were calculated from a standard curve using Ascorbic acid as a reference antioxidant material and results were expressed as mg Ascorbic acid equivalent/g of extract (mg AAE/g).

The total phenols content of CLE was determined using the Folin-Ciocalteau method (Turkmen et al., 2006). A calibration curve of Gallic acid was prepared and the results, determined from regression equation of the calibration curve were expressed as mg Gallic acid equivalents per gm of the extract (mgGAE/g).

The total flavonoids content of the extract was determined by the aluminium chloride test (Mohdaly et al., 2010) using Quercetin as standard and the results were calculated as mg Quercetin equivalent/g of extract (mgQE/g).

Determination of the antibacterial activity of the extract

The antibacterial effect of CLE was examined using the Kirby–Bauer disc diffusion method as described by El-Chaghaby et al. (2014). In this procedure, bacterial suspensions are spread onto in Petri plates. Then a known concentration of CLE is put on a sterile paper disc and placed on the agar surface. The plates are finally incubated for 18 h at 37°C and the antibacterial activity was determined by measuring the diameter (mm) of the clear zone of inhibition of growth around each disc.

Preparation of chicken breast meat

Raw chicken breast meat samples were purchased from a local supermarket in Egypt and were transferred to the laboratory within 1 h of production. Chicken breast meat was cut into portions of 5 g each. The samples were divided into five groups and with three replicates per group. The concentrations of CLE were prepared by dissolving the appropriate amount of extracts in a very small amount of 100% ethanol; the resulting solutions were mixed with vegetable oil. The ethanol added was removed using a rotary evaporator before adding the stock solution to chicken breast meat samples.

The samples were subjected to the following treatments:

Group 1: negative control without any preservative.
Group 2: positive control treated with BHT (100 ppm).
Group 3: treated with CLE (100 ppm).
Group 4: treated with CLE (200 ppm).
Group 5: treated with CLE (400 ppm).

After treatment, samples were left for 1 min to drip and were then separately packaged in sterile bags and stored at 4°C. Samples were analyzed on storage day 0, 7 and 15.

Determination of the antioxidant capacity of chicken meat

The antioxidant capacity of chicken breast meat samples was determined according to the procedures described by Jang et al. (Jang et al., 2008) with some modifications.

DPPH scavenging activity

According to Jang et al. (2008), 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity was
estimated by spectrophotometric method. The absorbance of the samples was measured at 517 nm using UV/Vis spectrophotometer. The percentage of DPPH radical scavenging was calculated as: [1- (absorbance value of sample/absorbance value of control)] x100. The control sample contained 1 ml of distilled water and 1 ml of methanolic DPPH solution (0.2 mM).

Thiobarbituric Acid-Reactive Substance (TBARS)

The lipid oxidation analysis was determined as milligrams of malonaldehyde per kilogram of meat using the spectrophotometric method of TBARS determination (Tres et al., 2013).

Determination of total bacterial count in chicken meat

The total bacterial count of chicken breast meat treated with CLE was determined in comparison with control groups. The total bacterial count was determined by the conventional diluting pouring plate technique and enumerating microbes in samples (Abdalla et al., 2007). The plates were incubated for 48 h at 30°C, the colonies were then counted and the average of two replicates from the same dilution was calculated directly by colony forming unit (log_{10}CFU/g).

Statistical analysis

All measurements were done in triplicate and the mean values were presented. The data were subjected to statistical analysis using the CoStat software to determine the significance at level (P<0.05). The differences were classified by Duncan multiple comparison test.

RESULTS AND DISCUSSION

Antioxidant properties of chamomile leave extract

Free radicals are major cause of a variety of pathological symptoms. While, antioxidants fight against free radicals by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms (Saeed et al., 2012). In the present work, the antioxidant properties of chamomile leave extract were evaluated through determining its total antioxidant capacity, total phenolic content and total flavonoids. The results of total antioxidant capacity (TAC), total phenols content (TPC) and total flavonoids (TF) of chamomile leave extract are shown in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>TAC (mgAAE/g)</th>
<th>TPC (mgGAE/g)</th>
<th>TF (mgQE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>202±5.09</td>
<td>43.46±1.14</td>
<td>25.37±1.09</td>
</tr>
<tr>
<td>CLE</td>
<td></td>
<td></td>
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</tbody>
</table>

Data are mean of three replicates ± standard deviation.

The total antioxidant capacity of CLE was evaluated using the phosphomolybdenum method based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm. The total antioxidant capacity of CLE was found to be 202 mg AAE/g. The antioxidant capacity of chamomille is related with several groups of active components that it contains mainly the phenolic compounds, primarily the flavonoids apigenin, quercetin, patuletin, luteolin and their glucosides (Sharafzadeh and Alizadeh, 2011; Zeković et al., 2014). The total phenolic content of CLE was found to be 43.46 mg GAE/g. This high phenolic content of the extract indicates its high antioxidant potential because the phenolic compounds can react with active oxygen radicals and there is high correlation between antioxidant activity and phenolic content (Aliyu et al., 2013). Also, phenolic compounds play an important role in stabilizing lipid peroxidation (Subhadradevi et al., 2010). This study shows that chamomile leave extract contains large quantity of total flavonoids (25.37 mgQE/g). The total flavonoids are major contributors to the antioxidant activity neutralizing the free radicals (Subhadradevi et al., 2010). Flavonoids have several biological properties; they are known for their anti-inflammatory, antioxidant, antihepatotoxic and antiviral activities together with their vasculo-protector and spasmolitic effects (Farideh et al., 2010).

It is generally agreed that free radicals cause auto-oxidation of unsaturated lipids in food (Jayaprakasha et al., 2003). From the antioxidant properties results obtained, CLE can be studied as natural preservative for food systems. In the present work, the extract was applied to chicken breast meat at three levels to study its effect on the antioxidant properties of chicken meat during storage.

Antibacterial activity of CLE

The antibacterial activity of CLE was tested against three Gram positive (Staphylococcus aureus, Streptococcus mutans and Bacillus subtilis) and three Gram negative bacteria (Escherichia coli, Neisseria gonorrhoeae and Pseudomonas aeruginosa). The results of antibacterial activity of CLE as measured by paper disk method are presented in Table 2. The results revealed that CLE exhibits antibacterial effect against both Gram negative and Gram positive bacteria.
Table 2. Antibacterial activity of CLE.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Gram reaction</th>
<th>Inhibition zone diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>G-</td>
<td>13</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoeae</em></td>
<td>G-</td>
<td>13</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>G-</td>
<td>12</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>G+</td>
<td>12</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>G+</td>
<td>11</td>
</tr>
<tr>
<td><em>Streptococcus mutans</em></td>
<td>G+</td>
<td>11</td>
</tr>
</tbody>
</table>

Figure 1. Effect of storage time on DPPH scavenging activity of chicken breast meat [C (-): negative control, C (+): positive control, T1 (100ppm CLE), T2 (200ppm CLE), T3 (400ppm CLE)].

Due to this inhibitory effect of CLE against these food-borne and food-spoilage bacteria, CLE can be used as natural food preservative to reduce these food poisoning bacteria and control their contaminations in foods (Mith et al., 2014).

**DPPH scavenging activity of chicken breast meat**

The stable free radical DPPH has been used in order to evaluate the antioxidative effect of CLE on chicken breast meat. The effect of storage period on the DPPH scavenging activity of chicken breast meat of all groups is depicted in Figure 1. The results in Figure 1 indicated that increasing the storage period resulted in a significant ($P<0.05$) decrease in the DPPH scavenging activity of chicken breast meat in all groups. Similar results were also reported by Fasseas et al. (2008).

The results of free radical-scavenging effect of chicken breast meat supplemented with CLE are given in Table 3. The results showed that in the first day of storage there were no significant differences ($P>0.05$) between the DPPH scavenging activities of breast meat of T1, T2, T3 and the positive control treated with BHT.
Table 3. DPPH scavenging activity of chicken breast meat at different storage time.

<table>
<thead>
<tr>
<th>Time</th>
<th>Negative control without preservative (100 ppm BHT)</th>
<th>Positive control (100 ppm CLE)</th>
<th>T1 (200 ppm CLE)</th>
<th>T2 (400 ppm CLE)</th>
<th>T3 (400 ppm CLE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>73.759&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.137&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.539&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.205&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.376&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Day 7</td>
<td>51.256&lt;sup&gt;c&lt;/sup&gt;</td>
<td>66.804&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.759&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61.220&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65.061&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Day 15</td>
<td>24.752&lt;sup&gt;c&lt;/sup&gt;</td>
<td>43.132&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.925&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.513&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.612&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

a, b, c: difference in significance within same raw.

Table 4. TBARS values of chicken breast meat at different storage time.

<table>
<thead>
<tr>
<th>Time</th>
<th>Negative control without preservative</th>
<th>Positive control (100 ppm BHT)</th>
<th>T1 (100 ppm CLE)</th>
<th>T2 (200 ppm CLE)</th>
<th>T3 (400 ppm CLE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>0.728</td>
<td>0.730</td>
<td>0.729</td>
<td>0.727</td>
<td>0.732</td>
</tr>
<tr>
<td>Day 7</td>
<td>1.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.769&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.798&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Day 15</td>
<td>1.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.83&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.951&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

a, b, c: difference in significance within same raw.

Table 5. Total bacterial count of chicken meat (log<sub>10</sub>CFU/g).

<table>
<thead>
<tr>
<th>Time</th>
<th>Negative control without preservative</th>
<th>Positive control (100 ppm BHT)</th>
<th>T1 (100 ppm CLE)</th>
<th>T2 (200 ppm CLE)</th>
<th>T3 (400 ppm CLE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>4.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.59&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>4.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.06&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.74&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Day 7</td>
<td>4.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.30&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.33&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Day 15</td>
<td>4.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.57&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.57&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

a, b, c: difference in significance within same raw.

While, the negative control group had the lowest significant (P<0.05) scavenging activity. After seven and fifteen days of storage, the DPPH scavenging activities of the negative control group was significantly (P<0.05) lower than all other groups. Meanwhile, there were no significant (P>0.05) differences between the positive control group and group T3. Also, there were no significant differences between the scavenging activity of T1 and T2, while both groups recorded significantly lower activities as compared to positive control and T3. The order of DPPH scavenging activity of breast meat during 7<sup>th</sup> and 15<sup>th</sup> day of storage was: Negative control < T1, T2 < positive control, T3.

**TBARS values of chicken meat**

Lipid oxidation is a critical parameter affecting the quality of chicken meat. Lipid oxidation process of chicken meat causes the development of potentially toxic substances and changes the nutritional value of meat, making it unsuitable for consumption (Ramziya, 2008). The thiobarbituric acid reactive substances (TBARS) method has been widely used to estimate the degree of lipid oxidation in meat products (Radha krishnan et al., 2015).

Table 4 shows the TBARS values of chicken breast meat belonging to the different experiment groups. At day 1 of the experiment, there were no significant differences in the TBARS values of all groups. From Table 4, it can be seen that, the TBARS values of the positive control and CLE treated groups were significantly (P<0.05) lower than the negative control group during the 7 and 15<sup>th</sup> day of storage. At the 7<sup>th</sup> day of storage there was no significant difference (P>0.05) in the TBARS value of the group treated with 400 ppm CLE (T3) and the positive control treated with BHT(C+) indicating that both treatments have similar effect against lipid oxidation. After 15 days of storage there were significant differences in TBARS values of all experimental groups in the following order: negative control>T1>T2>T3>positive control.

**Bacterial analysis of chicken breast meat (TBC)**

Table 5 shows the total bacterial count of chicken breast meat non-treated and treated with BHT and CLE. The negative control group recorded the highest significant (P<0.05) TBC as compared to all other groups. At the beginning of storage (Day 1), the total bacterial count of the positive control group was significantly lower than other groups followed by the group T3. While groups T1 and T2 had both significantly higher TBC values compared to T3. During later storage periods (days 7 and 15) the bacterial load of the group T3 was similar to that of the positive control group with no significant difference (P>0.05) between
their TBC values. This indicates the bactericidal effect of CLE at a supplementation level of 400ppm against food spoilage bacteria. These results are in agreement with the results reported by (Singh et al., 2014), who studied the effect of different natural preservatives on chicken storage quality. The economic advantages of using plant extracts as preservative include their low cost of production as they are usually prepared from a waste material. It is also worthy to note that the application of plant extract as preservative has successfully maintained chicken meat quality for fifteen days under refrigerated conditions, thus reducing the costs of freezing or other preservation techniques.

Conclusion

Chamomile leave extract has been proven to possess antioxidant and antibacterial activities that allow it to be used in food preservation systems. The use of chamomile leave extract as a natural preservative for chicken breast meat showed promising results. The applied natural preservative was effective for retarding the oxidative damage as well as delaying the bacterial spoilage of chicken breast meat. Chamomile leaves extract as a natural preservative with both antioxidant and antimicrobial activities is thus a good candidate to preserve chicken meat quality and preventing economic loss.

REFERENCES


