Preservative effects of rosemary extract (Rosmarinus officinalis L.) on quality and storage stability of chicken meat patties

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Abstract
The effect of different level of rosemary extract (RE) (Rosmarinus officinalis Linn.) cultivated in Jordan, and other preservative on quality and stability of ground chicken meat was investigated. Treatments, were involved 1) Control (No additive), 2) 300 ppm (RE), 3) 350 ppm RE, 4) 300 ppm L-Ascorbic acid (E-300), 5) 200 ppm Sodium nitrite (E-250), 6) 5 ppm butylatedhydroxyanisole (BHA) for breast, and 14 ppm for thigh meat were prepared. TBARS, total carbonyl, and color values, were measured and analyzed at 0, 4, and 7 day. Samples of cooked thigh meat were prepared, and sensory evaluation was reported. Cooking loss %, ultimate pH, and total aldehydes were analyzed. Both RE and E-250 were showed the highest significant effect maintaining low values of TBARS and total carbonyl at 7 day. However, no significant differences were found among all treatments measuring ultimate pH values, and their cooking loss %. The RE and E-250 also showed the highest significant effect delaying aldehydes formation, and positively affect meat sensory attributes. In conclusion, RE (350 ppm) was very effective antioxidant and comparable to the other commercial antioxidants. Thus, RE could be a good substitution to many synthetic antioxidants used in meat industry.

Keywords: lipid oxidation; protein oxidation; rosemary extract; ground chicken.

Practical Application: Improve meat storage stability and their quality characteristics using safer replacement to the synthetic antioxidant.

1 Introduction
Improving storage stability of both fresh and processed meat products consider important economically and nutritionally. However, the antioxidant capacity of fresh meat depends on the activities of endogenous reducing enzymes at early postmortem time (Ahn et al., 2009; Serpen et al., 2012; Kumar et al., 2015). In addition, this capacity will decrease when muscle cells lost their homeostasis, then free radical formation increase causing meat spoilage and deterioration. However, meat storage stability depends on many internal factors such as metal catalysts, free iron, fatty acid profile, pH, and presence of other inhibitors (Xiong, 2000; Min & Ahn, 2005). Lipid and protein oxidation considered first priority issues affecting meat quality characteristics (Ahn et al., 2009; Estévez, 2011; Lund et al., 2011). Color, flavor, odor and other meat quality characteristics are highly affected by the oxidation (Lund et al., 2011; Guyo et al., 2016). In meat industry they have been extensively used synthetic antioxidants (SA) such as butylatedhydroxyanisole (BHA), and butylatedhydroxytoluene (BHT) to prevent any oxidative changes (Monahan & Troy, 1997). However, because of the negative impression regarding the use of SA; natural antioxidants (NA) consider the suitable replacement (Velasco & Williams, 2011). Several research studies were reported possible carcinogenic and toxicological effects of the SA used in human foods (Altmann et al., 1986; Kumar et al., 2015). Currently, sodium nitrite (E-250) is the most important preservative and curing ingredients used in the meat industry (Al-Shuibi & Al-Abdullah, 2002). It is also responsible on meat color formation and their flavor after curing application (Honikel, 2008; Sindelar & Milkowski, 2011).

Recently, in curing application researchers were investigate finding a good combination with sodium nitrite or decrease their residual in finished meat products (Honikel, 2008; Oostindjier et al., 2014). Furthermore, increasing in consumer demands on the natural food products support this approach. For example, natural antioxidant from medical plants such as sage, rosemary, green tea, and oregano were tested previously (Zheng & Wang, 2001; Abdel-Hamied et al., 2009). Rosemary herbs and their extract were used as flavoring and preventing rancidity development in the meat (Yu et al., 2002; Jongberg et al., 2013). In general, there is no limitation on using the rosemary essential oil or their extract in meat products (Jordan Standards and Metrology Organization, 2016). Rosemary oleoresin, extract, and their essential oils were reported as a potential antioxidant used widely in the food industry (Yu et al., 2002; Balentine et al., 2006; Hussain et al., 2010). In addition, it had been reported that rosemary extract (RE) was effective delaying lipid oxidation in meat (Georgantelis et al., 2007; Keokamnerd et al., 2008; Kahraman et al., 2015). This high antioxidant property of RE associated with their content of phenolic diterpenes such as carnosic acid, carnosol, rosmarinquinone and rosmaridiphenol, ursolic acid, and caffeic acid (Aruoma et al., 1992; Basaga et al., 1997; Rašković et al., 2014). However, 90%...
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of the antioxidant properties of RE were reported to be from their carnosic acid and carnosol constituents (Aruoma et al., 1992; Erkan et al., 2008). The mechanism of this antioxidant activity is related to its phenolic diterpene structure which works as hydrogen donor and scavenging free radical compounds (Houlihan et al., 1984; Schwarz & Ternes, 1992; Hall et al., 1998; Richhelmer et al., 1999; Birtic et al., 2015).

This study is the first evaluating Jordanian RE effectiveness on storage stability of ground chicken meat. However, different composition of this Jordanian cultivated oil of Rosmarinus officinalis L. (Lamiaceae) by these phenolic compounds may give unique antioxidant activity. The essential oil and RE composition of these phenolic compounds (mono or di-terpenes) has been reported in a variable % which associated with several other factors such as genetic, storage condition, extraction method (Mena et al., 2016). In addition, it was reported in several study that the level of using RE or their essential oil in the food need more investigation (Kahraman et al., 2015). The previous suggested concentration of using RE is ranging between 200-1000 mg/kg in different food and meat products (Stoick et al., 1991; Shahidi et al., 1992; Sebranek et al., 2005; Georganetelis et al., 2007). In addition, ground chicken meat of both raw and cooked is more susceptible for oxidation. So economically, it is important to increase meat storage stability where the consumer found these products more acceptable. The objectives of current study were 1) to investigate the effect of two different level of Rosmarinus officinalis L. extract on storage stability and quality characteristics of ground chicken meat during storage time and 2) to compare the antioxidant effect of Rosmarinus officinalis L. with the most popular antioxidant used in the meat industry.

2 Materials and methods

2.1 Meat patties preparation

All broilers (140/6-wk-old) were fed a corn-soybean meal diet and, slaughtered at Mu'tah University (Agriculture Collage / Department of Animal Production-farm facilities). All chicken were checked and veterinary being qualified for health and welfare. The chicken carcases were immersed in ice water for 1 h and drained in a cold room, and then deboned muscles (breast and thigh) were vacuum packaged in oxygen impermeable bags, and stored at -18 °C.

After thawing, the meats were double grounded a through a 8-mm and a 3-mm plates (Moulinex, Type DKA1, France) before use. Six different treatments, including 1) Control (No additive), 2) 300 ppm Rosemary extract (RE), 3) 350 ppm RE, 4) 300 ppm L-Ascorbic acid (E-300), 5) 200 ppm Sodium nitrite (E-250), 6) 5ppm Butylatedhydroxyanisole (BHA) for both breast, and 14ppm thigh meat were prepared. Rosemary pure extract was obtained from a certified company in Jordan (Green Fields Factory for oils, Amman, Jordan) using the most efficient purification, extraction, and steam distillation methods. The HPLC analysis of the RE was measured by the method of Okamura et al. (1994) at the Royal Scientific Society, Jordan, Amman (RSS) research institution, and RE was containing 26 ± 3% as the average of phenolic diterpenes (carnosol (4%), carnosic acid (6%), rosmanol (8%), and rosmarinic acid (8%)). L-ascorbic acid (Fisher Scientific, fair Lawn, N.J., USA), and sodium nitrite (Gainland Chemical company-GCC, factory road; UK) powder were dissolved in de-ionized distilled water (DDW) first, then oil emulsion (water in oil) using mineral oil were prepared to make their aqueous solution. BHA and RE were dissolved 100% ethanol, and then mixed with mineral oil to make their stock solutions. The ethanol added was removed using a rotary evaporator (Heidolph, Model Laborato 4001-efficient) at (70 °C, 175 mbar vacuum pressure). Each additive was added to the ground, and then mixed for 3 min in a bowl mixer (Model KM-331; Kenwood Limited, New Lane, Havant, PO9 2NH, UK). Similar amounts of mineral oil and water (oil emulsion) were added for all treatments to provide the same conditions. The prepared meat samples (50 g each) were individually packaged in oxygen-permeable bags (polyethylene, Size: 11 × 25 cm, Future for Plastic Industry, Al-Mountaz bags, Co. L.T.D, Amman, Jordan), stored at 4 °C cooler for up to 7 days, and analyzed for TBARS and total carbonyl, and color at 0, 4, and 7 days. In addition, separate samples of raw meat from each treatment were used to measure their ultimate pH. In cooked meat study, the raw meat samples were packaged in oxygen impermeable vacuum bags (Ehsan & Tahssin Baalbaki Co, Bayader Wadi Al-Seer, Amman, Jordan), and the meats were cooked in-bag in a 90 °C water bath (Memmert, WNB 14; GMBH + Co. KH, D-91107 Schwabach, Germany) until the internal temperature of the meat reached to 75 °C. After cooling, the cooked meat samples (50 g) was transferred to a new oxygen-permeable bag (polyethylene, Size: 11 × 25 cm, Future for Plastic Industry, Al-Mountaz bags, Co. L.T.D, Amman, Jordan), and stored at 4 °C for up to 7 days, and analyzed for TBARS and Total carbonyl at 0, 4, and 7 days of storage. Same preparation method was done for all cooking loss and sensory analysis treatments samples. However, the ground chicken (raw thigh) meat patties stored at 4 °C up to 4 days before cooking and for each evaluation session.

2.2 Thiobarbituric acid-reactive substances (TBARS) measurement

Lipid oxidation in the meat was determined using a TBARS method (Ahn et al., 1998) with minor modification. The amounts of TBARS were expressed as mg of malondialdehyde (MDA) per kg of meat.

2.3 Protein oxidation (Total carbonyl)

Protein oxidation was determined by the general method of total carbonyl value proposed by Lund et al. (2008) with minor modifications. The carbonyl content was calculated as nmol/mg protein using absorption coefficient of 22,000/M/cm as described by Levine et al. (1994).

2.4 Color measurement

The color was measured using a Konica Minolta Color Meter (CR-400, Konica Minolta, Osaka, Japan). The colorimeter was calibrated using an illuminant source C on a standard white ceramic plate. The color were expressed as CIE L* (lightness), a* (redness), and b* (yellowness) values (American Meat Science Association, 2012). The obvious defects areas were
2.7 Cooking loss %

Chicken thigh meat samples (3g) were weighed and packaged in oxygen impermeable vacuum bags. The meat was cooked at constant temperature using preheated water bath (Memmert UN 42, Germany). The internal temperature of 20 °C, then water blotted or purged until the meat samples were cooled in water bath using cold water to the water loss expected (Murphy & Marks, 2000). After cooking, the cooked meat samples (10 g each) were evaluated by the panelists for the room temperature at 25 °C for all treatment separated days to decrease any variability.

2.8 Total aldehydes

The identification of each peak was achieved by Wiley Library. The total area of each peak was integrated by GC-MS. The area of each peak was reported to be an indicator of volatiles.

3.1 Lipid oxidation

Meat scientists are focusing on lipid oxidation and their free radicals due to their major effect on meat quality and their freshness (Ladikos & Lougovois, 1990; Ahn et al., 2009). Recently, improvement in antioxidant capacity of the meat by incorporating suitable and safer antioxidant getting more attention. Rosemary extract was extensively studied, and reported as a strong antioxidant decreasing the TBARS values in various meats (Zubillaga et al., 1992; Aruoma et al., 1992; Decker et al., 2010). On addition RE extract incorporates the suitable and safer antioxidant getting more attention. Rosemary extract was extensively studied, and reported as a strong antioxidant decreasing the TBARS values in various meats (Zubillaga et al., 1992; Aruoma et al., 1992; Decker et al., 2010).

2.9 Statistical analysis

Data were analyzed using the procedures of generalized linear model (Proc. GLM, SAS program, version 9.3, SAS Institute, Inc., Cary, NC, USA). The significance was defined at P < 0.05 and Tukey’s Multiple Range test or Tukey’s Multiple Range test were used to determine the significant differences between the mean values.
reported the level 200-1000 mg/kg of RE in different food and meat products (Stoick et al., 1991; Shahidi et al., 1992; Estévez et al., 2005; Kahraman et al., 2015). The effective level of RE to prevent meat deterioration depends on many internal and external factors which may change the previous recommended levels. There were no significant differences (P > 0.05) between all treatments additives using raw meat samples at day 4 of storage time. This may be due to the low malonaldehyde formation in raw meat compared to the cooked ground chicken meat (Al-Hijazeen et al., 2016a, b). In addition, E-250 showed the highest antioxidant effect at 7 day of storage time. Furthermore, L-ascorbic acid (E-300) showed the lowest effect in delaying lipid oxidation. Generally, RE (350 ppm) was equally effective like BHA, and comparable to E-250 maintaining low TBARS values of cooked and raw meat samples.

### 3.2 Protein oxidation

The DNPH (2,4-Dinitrophenylhydrazine) or total carbonyl method showed no significant differences (P > 0.05) among all treatments at day 0 of storage time. All treatments additives were showed significant effect (p < 0.05) on total carbonyl values at day 4 using cooked meat samples compared to the control. Similar with the TBARS results, both E-250 and RE (350 ppm) were showed the highest effect maintaining low total carbonyl values during storage of cooked meat (Table 2). On the other hand, there were no significant differences (P > 0.05) between all treatment additives at day 4 of storage time using raw meat samples. This was in agreement with Xiao et al. (2011) who also reported low total carbonyl value (0.46 to 0.81 nmol/mg protein) using raw ground chicken meat. The total carbonyl values were reported in the range of 2-5 nmol/mg protein using various cooked meat products (Requena et al., 2003; Sun et al., 2010; Estévez, 2011; Al-Hijazeen et al., 2016a, b). However, E-250 showed the lowest total carbonyl values at day 7 compared to the other treatments using the raw meat samples. Both RE and E-250 showed the highest effect decreasing total carbonyl formation during storage time of the raw meat samples. RE was reported to have protection ability against formation of aldehydes and protein carbonyls (Estévez et al., 2005; Jongberg et al., 2013).

### 3.3 Color measurement

In this study there were no significant differences (P > 0.05) among all treatments when measuring a*, b*, and L* values (thigh meat) at day 0 of storage time (Table 3). So, any significant differences during storage time could happen due to the treatment effect. Rosemary extract (RE) and E-250, BHA treatments additives were showed higher significant (P < 0.05) values of L* values at day 7 compared to the control. The decrease in L* values using chicken meat after day 5 was reported in previous research studies (Chouliara et al., 2007; Al-Hijazeen et al., 2016a, b).

### Table 1. *TBARS values of cooked ground chicken meat at different storage time at 4 °C.

<table>
<thead>
<tr>
<th>Time</th>
<th>Control without</th>
<th>300 PPM RE</th>
<th>350 PPM RE</th>
<th>300 PPM E-300</th>
<th>200 PPM E-250</th>
<th>14 PPM BHA</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooked</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>0.94**</td>
<td>0.96**</td>
<td>0.94**</td>
<td>0.95**</td>
<td>0.95**</td>
<td>0.98**</td>
<td>0.071</td>
</tr>
<tr>
<td>Day 4</td>
<td>3.38**</td>
<td>1.96**</td>
<td>1.35**</td>
<td>1.84**</td>
<td>1.34**</td>
<td>1.62**</td>
<td>0.109</td>
</tr>
<tr>
<td>Day 7</td>
<td>6.26**</td>
<td>2.94**</td>
<td>2.23**</td>
<td>2.98**</td>
<td>2.21**</td>
<td>2.62**</td>
<td>0.091</td>
</tr>
<tr>
<td>SEM</td>
<td>0.09</td>
<td>0.127</td>
<td>0.075</td>
<td>0.108</td>
<td>0.064</td>
<td>0.073</td>
<td></td>
</tr>
</tbody>
</table>

| Raw  |                 |            |            |              |             |           |     |
| Day 0 | 0.198**         | 0.177**    | 0.192**    | 0.183**      | 0.187**     | 0.203**   | 0.019 |
| Day 4 | 1.24**          | 0.297**    | 0.246**    | 0.318**      | 0.215**     | 0.268**   | 0.032 |
| Day 7 | 2.15**          | 0.87**     | 0.658**    | 1.00**       | 0.641**     | 0.81**    | 0.048 |
| SEM  | 0.047           | 0.054      | 0.015      | 0.062        | 0.03        | 0.021     |     |

** Value with different letters within a row are significantly different (P < 0.05); *** Value with different letters within a column are significantly different (P < 0.05), n= 4; *TBARS value in mg malonaldehyde/kg meat.

### Table 2. Effect of adding different level of rosemary extract on protein oxidation in cooked ground chicken thigh meat.

<table>
<thead>
<tr>
<th>Time</th>
<th>Control without</th>
<th>300 PPM RE</th>
<th>350 PPM RE</th>
<th>300 PPM E-300</th>
<th>200 PPM E-250</th>
<th>14 PPM BHA</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooked</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>1.17**</td>
<td>1.23**</td>
<td>1.15**</td>
<td>1.19**</td>
<td>1.23**</td>
<td>1.21**</td>
<td>0.069</td>
</tr>
<tr>
<td>Day 4</td>
<td>3.36**</td>
<td>1.80**</td>
<td>1.60**</td>
<td>1.74**</td>
<td>1.37**</td>
<td>2.30**</td>
<td>0.056</td>
</tr>
<tr>
<td>Day 7</td>
<td>4.36**</td>
<td>2.26**</td>
<td>1.92**</td>
<td>2.31**</td>
<td>1.82**</td>
<td>2.59**</td>
<td>0.091</td>
</tr>
<tr>
<td>SEM</td>
<td>0.078</td>
<td>0.042</td>
<td>0.074</td>
<td>0.107</td>
<td>0.053</td>
<td>0.071</td>
<td></td>
</tr>
</tbody>
</table>

| Raw  |                 |            |            |              |             |           |     |
| Day 0 | 0.635**         | 0.638**    | 0.623**    | 0.638**      | 0.630**     | 0.643**   | 0.07 |
| Day 4 | 1.19**          | 0.85**     | 0.76**     | 0.91**       | 0.76**      | 0.95**    | 0.078 |
| Day 7 | 2.06**          | 1.18**     | 0.96**     | 1.14**       | 0.94**      | 1.25**    | 0.06 |
| SEM  | 0.064           | 0.071      | 0.089      | 0.064        | 0.067       | 0.058     |     |

** Value with different letters within a row are significantly different (P < 0.05); *** Value with different letters within a column are significantly different (P < 0.05), n= 4.
Several research studies have reported that RE have a potential antioxidant activity which help to maintain meat color stability and especially a* values (Estévez et al., 2005; Rohlik & Pipek, 2012; Kumar et al., 2015). However, the effect of E-300 was very low and not significant (P > 0.05) at day 7 of storage time. Higher discoloration in the redness (a* values) of control sample appeared after day 4 of storage time. Increasing of the total myoglobin oxidized to metmyoglobin (discoloration) was causing this loss.

This was in agreement with Keokamnerd et al. (2008) who study the effect of adding commercial rosemary oleoresin on ground chicken thigh meat quality. All treatments additives were showed significant (P < 0.05) effect maintaining higher a* values compared to the control treatment. However RE showed the highest color stability maintaining a* values compared to the other treatments at day 7. Changing in b* values during storage time were not constant, and no significant differences (P > 0.05) were appeared between all treatments additives.

### 3.4 Sensory evaluation

All treatment additives were showed significant differences (P < 0.05) compared to the control using cooked meat color attribute. The panelist were more likely prefer cooked meat color of E-250, and this due to their curing effect on pink color formation. Furthermore, both RE treatments levels showed highest significant values of spice odor, but with no ability to distinguish between both levels by the panelists (Table 4). The control samples were showed the highest significant scores of oxidation odor attribute compared to the other treatments. This effect due to the antioxidant treatments additives decreasing off-odor and specific volatiles that cause rancidity development.

These volatile such as aldehydes, hydrocarbons, sulfuric compounds usually increase by storage time due to lipid peroxidation and their secondary compounds (Ahn et al., 2009). However, E-250 was showed the highest overall acceptability scores compared to the other treatments. Overall, RE of both levels had comparable effect on panelist evaluation with E-250. Finally, E-300 and BHA samples treatment was less acceptable by panelist compared to the other treatments additives. This effect of RE on the sensory attributes of different meat preparation had been reported in various studies (Thongtan et al., 2005; Estévez et al., 2005; Feng et al., 2016). In addition, RE prevents the alteration in protein functionality caused by oxidation which may affect meat color and their texture characteristics (Estévez et al., 2005).

### 3.5 Ultimate pH, cooking loss %, and total aldehydes

There were no significant differences (P > 0.05) of the ultimate pH values (raw meat samples) among all treatments before storage time at day 0 (Table 5).

So, all treatments samples were having the same pH value, which enhance the univariate analysis between treatments. In addition no significant differences (P > 0.05) were appeared...
when measuring cooking loss % among all treatments. This may be due to the low effect of free radical on protein net charge (amino acids charges) and their functionality. Finally the control sample of cooked thigh meat showed the highest significant (P < 0.05) values of total aldehydes formation at day 7 of storage time. However, both RE and E-250 were showed the highest antioxidant effect maintaining low values of aldehydes formation. The results in total aldehyde had similar trend to the TBARS, and total carbonyl at the end of storage time for both RE and E-250. The RE (350 ppm) antioxidant effect delaying aldehydes formation such as hexanal is due to their composition of phenolic compounds. However, hexanal was reported the major aldehyde correlated with meat deterioration and TBARS values in chicken meat (Du et al., 2003).

4 Conclusions

Rosemary extract (350 ppm), and E-250 treatments were showed the highest significant (P < 0.05) effect maintaining low TBARS and total carbonyl values during storage time. L-Ascorbic acid and BHA also showed significant effect compared to the control treatment at day 7, but lower than E-250 or RE treatments. In term of meat storage stability, both RE and E-250 showed better sensory evaluation scores compared to the other treatments. Lower total aldehydes values were found when using RE (350 ppm) and E-250 compared to the other treatments. Overall, RE (350 ppm) could be an excellent natural antioxidant substitution or partial replacement to the SA used currently in meat preservation.

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References


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