Quality Enhancement and Shelf-Life Extension of Raw Beef Patties Formulated with Lactate/Thyme Essential Oil during Refrigerated Storage

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INTRODUCTION

Consumer demand for fast food has been increasing rapidly in recent years due to changes of life style. Minced beef patty is one of the most popular meat products that are under extensive consumption as fast meals in Egypt [39], however meat products are highly perishable [36]. Microbial growth, lipid oxidation and color changes are factors important for shelf-life and consequently for consumer acceptance of fresh meat [26,42]. Physicochemical and sensory qualities are also quite important in the manufacture of meat products [38]. There is at present a growing interest, both in the industry and in the scientific research, for using natural compounds in food protection instead of chemical preservatives [14,16].

Fresh meat products are commonly marketed at refrigerated temperatures (2–5°C). However, many undesirable changes of the products can occur during refrigeration due to microbial contamination leading to food-borne illness and economic loss in terms of food poisoning and meat spoilage [3,45].

Muscle foods are also susceptible to lipid oxidation that can precipitate major deterioration in many quality characteristics such as flavor, color, texture, nutritive value and safety of these products [2,40]. Thus, the application of suitable agents possessing both antioxidant and antimicrobial activities is a major concern for the meat industry for maintaining meat quality, extending shelf-life and preventing economic loss [25,36].

Minimizing product contamination and delaying or inhibiting growth of pathogenic and spoilage organisms in the product are major keys for
improving the shelf-life and increasing consumer safety. Recently, organic acids and their salts have been the preferred antimicrobial ingredients [8,34]. Commercial usage levels of lactates (2.0%) have been increasingly used in ready-to-eat (RTE) meat, poultry, and smoked seafood products to control the growth of foodborne pathogens, extend shelf-life, as well as flavor agents [23,28]. Lactates had been permitted, as a natural preservative, hence they are naturally present in meat and many other fermented foods; at a level of up to 4.8 g/100 g meat, without any health risk for consumers [54,9].

Much research indicated that addition of sodium lactate (NaL) at the level of 1.5–3.0 g/100 g of meat weight could improve flavor, color, tenderness, juiciness and cooking yields of ground meat products [17,28]. However, higher concentration levels of lactates (> 3 %) had a negative effect on sensory properties [41]. In addition, NaL has been shown to delay growth of spoilage as well as pathogens organisms and extend the shelf life of meat products [10,45]. Moreover, other authors reported that NaL has inhibitory effect on lipid oxidation and maintain the chemical quality of the product during storage [31,21]. However, there is a current demand from consumers for new methods of reducing or eliminating food-borne pathogens, possibly in combination with existing methods [23,8]. One such possibility is the use of naturally occurring antimicrobial and antioxidant compounds derived from plant sources [7]. They have been preferably employed in meat products because of their potential health benefits and safety compared with synthetic preservatives, since their use is being restricted in foods; because of their potential carcinogenic and toxicity for consumers [48,30]. Natural preservatives can protect the human body from free radicals and could retard the progress of many chronic diseases as well as lipid oxidation and microbial growth in foods due to their phenolic compounds [6,15].

Much attention has been focused on the use of the extracts from herbs and spices in the range of 0.05–0.1%, to control pathogens, prolong shelf-life and improve sensory properties of perishable foods [13,51]. Among several essential oils, thyme oil (Thymus vulgaris L.), a low cost food ingredient belongs to the Lamiace family may have the greatest potential for use in industrial applications [27,18]. Thyme essential oil (TEO) during refrigeration has proved to be an effective preservative to improve flavor and shelf-life of muscle-based foods [1,49]. TEO is well known for its high phenolic compounds content including carvacrol, thymol, p-cymene and γ-terpinene with strong antioxidant and antimicrobial activities against a wide range of spoilage and pathogenic microorganisms [46,52].

Both NaL and TEO are classified as GRAS (Generally Recognized As Safe) ingredients in Ready-To-Cook (RTC) and Ready-To-Eat (RTE) meat and poultry products [13,54]. Although, several studies have demonstrated the beneficial effects of such natural preservatives on microbiological safety, sensory properties, lipid oxidation and color preservation, there is a lack of information about their effects when applied in mixture in meat based products. Therefore, the objective of the present research was to evaluate the addition effects of sodium lactate (NaL, 2%), thyme essential oil (TEO, 0.05%), either alone or in mixture, on lipid oxidation (TBARS), total volatile basic nitrogen (TVBN), pH values, color stability (L*, a* and b* values), microbial analysis (Aerobic Plate Counts “APC”, Psychrotroph Counts “PTC”, and Enterobacteriaceae Counts “EBC”), sensorial properties (appearance, odor, texture, taste and overall acceptability) of raw ground beef patties. Shelf-life of raw ground beef patties during refrigerated storage at 4±1°C for 15 days was also investigated based on microbiological analysis and chemical indices.

Materials and Methods

Materials:

Fresh deboned beef lean (from the round position) and fat (from beef trim) were purchased one day after slaughtering from local market at Giza, Egypt, and immediately transported in ice box to the laboratory, the external fat, bone and connective tissues were removed from meat samples, chilled for 3h before processing into patty on the day of purchase. Air-dried thyme leaves were purchased from Al-Dahlia Co., Egypt, and stored at 4±1 °C until use. Salt (NaCl) and potato starch were purchased from local market at Dokki, Giza. Food grade sodium lactate powder form (C,H,NaO, 98.7% purity) was purchased from Morgan for Chemicals, Cairo, Egypt. All other solvents and chemicals were of analytical grade.

Methods:

Preparation of Thyme Essential Oil (TEO):

One hundred grams of air-dried thyme leaves were hydro-distilled in a Clevenger type apparatus for 3h until no further increase in the oil was observed. After finishing the distillation process the apparatus was left to be cooled, then the essential oil was collected and dried over anhydrous sodium sulphate before held in dark sealed glass vials and stored at 4±1 °C until use.

Patties Processing and Treatments:

A base batter was prepared by using a simple traditional formulation as follows: 650 gm/kg lean beef, 200 gm/kg beef fat, 20 gm/kg (w/w) sodium chloride, 100 mL/kg (v/w) ice water, 30 gm/kg potato starch. Chilled meat and fat portions needed to formulate 10 kg patties were minced twice through a 6-mm plate (coarse), using a Moulinex meat mincer (Model 320, cod 25, France). Afterward, water, salt
and potato starch were added and the mixture was mixed by hand for 5 min, re-minced through a 3-mm holes (fine), then the homogenized meat mixture was divided into four batches (2.5 kg each), packed in polyethylene bags and tempered in a freezer for 10 min before treatments [24]. Meat mixture of each group was comminuted again through a 3-mm mincer steel plate, then treated with either 20 gm/kg NaL (Lactate) or 500 mg/kg thyme essential oil (TEO) or 20 gm/kg NaL plus 500 mg/kg TEO mixture. Control minced meat (C) did not contain NaL or TEO.

The concentrations of lactate and TEO; which are sensorially acceptable and also effective against pathogenic bacteria, were based on results from studies by Tajkarimi et al., [51] and Perumalla et al., [45] on minced beef patty. First TEO was dispersed in about 5 ml of distilled water, added to the raw minced beef and mixed thoroughly [26]; all other meat batches were formulated to contain the same amount of water, and chilled again. The mixture of each formula was shaped manually into patties using a Moulinex patties former (30 patties of approximately 80 gm weight, 9 cm in diameter and 1 cm thickness). The patties were packed separately in polyethylene bags (with two patties in each bag), then labeled and stored at ±1 °C for 15 days. Two bags of each group were withdrawn at three days intervals over storage period for analysis. The chemical and microbiological determinations were made on finely ground samples. Averages of three replicates were considered.

Analytical Methods:

Proximate Analysis:

Proximate analysis: Moisture, protein, fat and ash contents of raw beef patty samples were determined for each replicate at zero time, according to the standard analytical methods of AOAC [4]. For determination of moisture contents (gm/100 gm sample), 3 gm of minced meat samples were dried at 103±2 °C until constant weight was obtained (method 950-46). Protein content (gm protein/100 gm sample) was determined according to the Kjeldahl method of analysis using potassium sulphate and copper (II) sulphate as the catalysts and nitrogen-to-protein conversion factor of 6.25 (method 981-10). Fat (gm/100 gm sample) was determined by 6-cycle extraction with petroleum ether in a soxhlet apparatus and calculating the weight loss (960-39). Ash (gm/100 gm sample) was determined by incineration in a muffle furnace at 550±2 °C for 5 h (920-153), while the amount of total carbohydrates was calculated by differences, as follows:

\[
\text{Total Carbohydrates} = 100 \times (\text{Moisture} + \text{Protein} + \text{Intramuscular-fat} + \text{Ash})\]

Microbiological Analysis:

Aerobic Plate Counts (APC), Psychrotrophic Counts (PTC), and Enterobacteriaceae Counts (EBC) were determined following procedures recommended by APHA (2001) method after preparing serial dilution taking 10 gm sample. APC was determined by spread plating on plate count agar, employing an incubation condition at 37°C for 24-48 h. Plate count agar incubated at 7 °C for 10 days for enumeration of Psychrotrophic Counts (PTC). Enterobacteriaceae Counts (EBC) were determined by using violet red bile agar and incubation condition at 37 °C for 24-48 h. After specific incubation periods plates showing 25-250 colonies were counted. The number of colonies was multiplied by the reciprocal of the respective dilution and expressed as log10 cfu gm⁻¹.

Sensory Panel Evaluation:

The sensory attributes (appearance, odor, texture, taste and overall acceptability) of the freshly cooked beef patty samples (on day zero only for safety precautions) were evaluated using a 10-point numerical scale; where ten corresponded to “the highest quality”, a score less than 4 indicate that the patty is rejected [55]. The panel consisted of 10 members of the staff who were familiar with meat characteristics. Five beef patties from each formula were cooked by grilling (to simulate normal fast food restaurants), using a HG 230 Kenwood multi cooker, with turning every 3 min until cooked to an internal temperature of 72°C. Each patty was cut into six rectangular pieces from the center, labeled with 3-digit random numbers and served warm to the panelists at room temperature in random order; water was served for rinsing the mouth between samples.

Deterioration Criteria Indices:

Total volatile basic nitrogen (TVBN) and thiobarbituric acid reactive substances (TBARS) were determined according to the methods described by Pearson, [44] using micro-Kjeldahl distillation apparatus. For pH determination 10 gm of fatty samples were homogenized in 100 mL distilled water for 1 min in a warring blender, and the pH values of the slurry were measured at room temperature as described by Hayes et al. [26], using a standardized electrode (at pH 4.0 and 7.0) attached to a digital pH meter (Haana, HI9002; Germany).

Instrumental Color Evaluation:

Color profile was determined after allowing patties surfaces to bloom for 30 min., using a Hunter Lab Scan XE Colorimeter (Hunter Laboratory Inc. Restonova). Three readings per sample were taken and the mean values of lightness (L*), redness (a*), and yellowness (b*) were calculated.

Statistical Analysis:

Results were expressed as means and standard deviation (M±SD) from triplicate determinations.
Analysis of variance (ANOVA) was performed to compare the effect of the treatments. Significant differences were defined as P<0.05, according to PC-STAT, 1985.

Results and Discussions

Proximate Analysis:

Beef patty is a very popular food commodity around the world. Mean values for proximate composition of raw different formulas of beef patties are presented in Table 1. The targeted value of ca. 20% for fat content was achieved. Elevated moisture content and an increase in ash content were observed in all treated samples as compared to control samples. However, formula ingredients, fat level as well as method of processing affect to a great extent the proximate composition and quality attributes of formulated patties [33]. Results indicate that the addition of sodium lactate (NaL), thyme essential oil (TEO) or both to raw fresh patties samples had a slight effect (P<0.05) on their proximate composition, the values for moisture, protein, fat ash and carbohydrate contents in treated patties indicating that all treatments were similar in composition except for used NaL and/or TEO. Similar trend of changes in proximate composition was achieved in beef patty samples by other authors [35,39].

Sensory Evaluation of Cooked Beef Patties:

One of the essential properties of a food ingredient is not to cause any undesired sensorial changes in a food product. In this study, the sensorial criteria (appearance, odor, texture, taste, and overall-acceptability) of the freshly cooked beef patty samples were evaluated and presented in Table 2. The addition of NaL or TEO did not cause any undesired changes in the sensory characteristics of the product but a partial improvement in appearance and odor scores were achieved in the treated patty samples.

Results in Table 2 indicate that, all tested patty samples were acceptable as evidenced by the higher overall acceptability scores. Texture and taste were unaffected (P > 0.05) by addition of sodium lactate, the results for odor and taste may be related to the effect of thyme essential oil more than sodium lactate. Panelists judged lactate samples better in appearance with no differences found overall in taste and texture (Table 2). Our results confirmed the findings reported by other authors [11,34] who reported that the addition of NaL enhanced the typical meat flavor and exhibited no negative effect on sensory characteristics, while minimized the flavor deterioration during storage.

Microbiological Analysis:

1- Aerobic plate counts (APC):

Microbiological evaluation, together with chemical indices, has been used extensively to assess the quality and shelf-life of meat products [34]. Aerobic plate counts “APC” of minced beef patty under investigation were evaluated and the counts (as log10 CFU/gm) were presented in Fig. 1. The shelf-life of raw meat is usually limited by microbial spoilage. Depending on hygiene and preservation

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**Table 1:** Proximate chemical composition (%) of beef patties formulated with NaL and TEO or both to control patties samples (on fresh weight basis).

<table>
<thead>
<tr>
<th>Patty Treatment</th>
<th>Moisture ±SD</th>
<th>Protein ±SD</th>
<th>Fat ±SD</th>
<th>Ash ±SD</th>
<th>Carb. ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>58.36 ± 0.82</td>
<td>16.78 ± 0.64</td>
<td>20.40 ± 0.47</td>
<td>1.12 ± 0.11</td>
<td>3.54 ± 0.19</td>
</tr>
<tr>
<td>Lactate (2% NaL)</td>
<td>58.78 ± 1.14</td>
<td>16.75 ± 0.58</td>
<td>20.16 ± 0.62</td>
<td>1.23 ± 0.13</td>
<td>3.10 ± 0.32</td>
</tr>
<tr>
<td>Thyme Oil (0.05 % TEO)</td>
<td>58.54 ± 1.27</td>
<td>16.69 ± 0.14</td>
<td>20.30 ± 0.15</td>
<td>1.34 ± 0.24</td>
<td>3.79 ± 0.26</td>
</tr>
<tr>
<td>Lactate/TEO mixture</td>
<td>58.83 ± 1.09</td>
<td>16.56 ± 0.82</td>
<td>19.98 ± 0.78</td>
<td>1.46 ± 0.60</td>
<td>3.17 ± 0.14</td>
</tr>
</tbody>
</table>

Mean ±SD= All values determination & standard deviation (SD) are mean of triplicate determinations.

**Table 2:** Sensory scores of freshly cooked control and treated beef patty samples (at zero time).

<table>
<thead>
<tr>
<th>Patty Treatment</th>
<th>Appearance</th>
<th>Odor</th>
<th>Texture</th>
<th>Taste</th>
<th>Overall-acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>8.60±0.10</td>
<td>8.36±0.11</td>
<td>8.27±0.16</td>
<td>8.74±0.13</td>
<td>8.49±0.11</td>
</tr>
<tr>
<td>Lactate (2% NaL)</td>
<td>9.00±0.13</td>
<td>8.45±0.08</td>
<td>8.30±0.10</td>
<td>8.78±0.17</td>
<td>8.63±0.14</td>
</tr>
<tr>
<td>Thyme Oil (0.05% TEO)</td>
<td>8.82±0.11</td>
<td>9.14±0.14</td>
<td>8.32±0.11</td>
<td>8.92±0.11</td>
<td>8.80±0.18</td>
</tr>
<tr>
<td>Lactate/TEO mixture</td>
<td>9.14±0.12</td>
<td>9.25±0.17</td>
<td>8.35±0.14</td>
<td>8.98±0.15</td>
<td>8.93±0.10</td>
</tr>
</tbody>
</table>

All values reflect the mean and standard deviation, (n=10).

Mean values in the same column bearing the same superscript do not differ significantly (P<0.05).
Based on PTC number (Fig. 1), refrigerated ground meat produce significant reduction in growth of APC in contrast to control, lactate, thyme essential oil, and lactate/thyme mixture patty samples were 8, 12, 11, and 15 days; respectively. The present results regarding PTC confirmed the findings obtained by other authors [21,8] in their frameworks on meat patty treated with natural essential oils or sodium lactate and subjected to cold storage. However, the present study also indicates that, at any given time of refrigerated storage, NaL alone was slightly more effective against microbial growth than TEO alone.

Results of Fig. 1 also reveal that at day 15 of storage, samples containing mixture of NaL with TEO had a lower APC (6.78 log10 CFU/gm) than the maximal recommended limit, while control samples exhibited a higher count of 9.64 log10 CFU/gm, indicating that such mixture is more effective than lactate or TEO alone. This result might have been due to the synergistic effect of the two adverse factors. This finding is consistent with El-Dessouky et al., [21] and Barros et al., [8], who reported similar effect of Sodium lactates in combination with natural essential oils on APC of ground beef patty during refrigerated storage.

On the other hand, addition of TEO alone (500 mg/kg) had significant reduction (P>0.05) on APC as compared to control samples, inclusion of thyme essential oil extended the shelf-life to about 11 days under the same condition (Fig. 1). These findings are in accordance with that of Angis and Oguzhan, [3]; Roby et al., [46] and Teixeira et al., [52], who claimed that TEO is well known for its high phenolic compounds content including carvacrol, thymol, p-cymene and γ-terpinene with strong antimicrobial activities against a wide range of spoilage and pathogenic microorganisms. However, the present study also indicates that, at any given time of refrigerated storage, NaL alone was slightly more effective against microbial growth than TEO alone.

Storage time had a significant effect on APCs of raw beef patties in aerobic storage (Fig. 1). However, by the day 9 of storage, APC of control (7.38) exceeded the maximal recommended limit of 7 log10 CFU/gm for APC in raw meat [29], indicating a shelf life of about 8 days. While NaL-treatment significantly delayed the microbial growth and extended the shelf life of the product up to 12 days at which the APC was 6.84 versus 8.73 log10 CFU/gm, and signs of spoilage started to appear for control patties. Addition of NaL has been reported to produce significant reduction in growth of APC in refrigerated ground meat [10,28].

### 2- Psychrotrophic Counts (PTC):

The psychrotroph counts (PTC) in minced beef patty were slightly lower than the APC, this was true for all of the four groups analyzed (Fig.2). During chilling storage a similar trend of PTC was found as APC. The development rate of PTC was progressively increased more than that of APC under the same refrigerated conditions, ascribed to optimum temperature condition in chilling storage. In this concern, it is worth mentioning that, according to microbiological standard for this type of raw meat product in Egypt, the psychrotrophic counts (PTC) should not be more than 10³ cfu/gm meat [20,18]. Based on PTC number (Fig. 2) the shelf-life of refrigerated (at 3-5°C) control, lactate, thyme essential oil, and lactate/thyme mixture patty samples were 8, 12, 11, and 15 days; respectively. The present results regarding PTC confirmed the findings obtained by other authors [21,8] in their frameworks on meat patty treated with natural essential oils or sodium lactate and subjected to cold storage.

### 3- Enterobacteriaceae Counts (EBC):

Enterobacteriaceae (Fig.3), considered as a hygiene indicator [7]. The growth of Enterobacteriaceae was slower than that of APC, or PTC. The initial EBC increased from 2.17 log10 CFU/gm in control samples at day 0 to a higher
Fig. 3

count of 4.28 log10 CFU/gm by day 15 of storage, while it reached significant (P<0.05) lower counts of 3.08 or 3.37 log10 CFU/gm in ground beef treated with either NaL or MEO; respectively when compared with control (Fig. 3). This indicated that addition of NaL was more effective (P<0.05) against Enterobacteriaceae than MEO. However, a mixture of NaL and MEO restricted the growth of the Enterobacteriaceae to a lower level of 2.96 log10 CFU/gm, and appeared to be the most effective (P<0.05) among the other treatments against the growth of Enterobacteriaceae. These results are in accordance with those of Sallam and Samejima, [47], and El-Desouky et al. [21], who reported inhibitory effects of NaL against Enterobacteriaceae counts in minced meat patties during refrigerated storage, and also with Angis and Oguzhan [3], who reported that thyme essential oil had a strong effect in the reduction of Enterobacteriaceae counts during refrigerated storage.

Fig 3 also reveal that the mixture effect of sodium lactate with thyme essential oil were efficient in the reduction of the microbial contamination and extend the shelf life of minced beef patties during refrigerated storage. These findings are also on line with other authors [8,52]. The most significant benefit of applying sodium lactate and/or thyme essential oil to minced meat products was to inhibit the growth of Enterobacteriaceae bacteria, which is a very desirable quality since this group contains a large number of pathogens.

Quality Indices Alterations:

pH Changes:
Meat pH is considered as one of the most important technological properties as it alters pigment and lipid stability [22]. Changes in pH values in ground beef during cold storage at 4±1°C for 15 days are illustrated in Fig. 4, from which it is clear that the initial pH value of control patty (5.90) was significantly (P<0.05) higher than those of samples treated with NaL (5.78), TEO (5.84) or mixture of NaL with TEO (5.73). These results confirmed the findings of El-Desouky et al. [21]; Bingol and Bostan [10] in their frameworks in beef patties treated with sodium lactate and/or thyme essential oil.

Fig. 4 further shows that during refrigerated storage pH values of raw patty samples tended to decrease up to 6 days, and then gradually increased till the end of chilling period. The decline in pH values could be due to the formation of carbonic and lactic acids [32], while the increase in pH values thereafter could possibly due to decomposition of amino and nitrogenous compounds caused primarily by microbial activity, leading to the increase in alkaline groups and ammonia in patty samples [44,22].

Display of data demonstrated in Fig. 4 it is obvious that at any given time of refrigerated storage control samples exhibit significantly higher (P<0.05) pH values than treated samples; conversely lactate/thyme in mixture treatment had the lowest pH increment during the course of refrigerated storage. Similar trends of pH changes have been observed [22,49]. However, the lower pH values of treated patty samples reflect antimicrobial properties of NaL or TEO [28,3]. Fig. 4 also indicates that, over the storage time, addition of NaL or TEO maintained the ground beef at almost constant pH (5.73–5.96), while the pH of control samples significantly increased (5.90-6.40). NaL, or thyme essential oil has been shown to stabilize pH during storage of meat products; the researchers concluded that these findings were probably due to the buffering capacity of NaL as well as to antimicrobial activity of TEO [7,52].

Total Volatile Basic Nitrogen (TVBN) Changes:
TVBN is considered the most commonly used biochemical methods for assessing meat spoilage [44]. TVBN changes of beef patty samples are illustrated in Fig. 5, at the beginning of storage (day zero) control, lactate, thyme essential oil and lactate/thyme combination patty samples exhibit 10.84, 10.28, 10.45 and 10.16 mg N/100g flesh; respectively. These values are indicative of good
quality raw material used in this assay and they are similar to those found by other authors [37,21].

Fig. 5 also reveals that, TVBN of all patty samples gradually increased with different rates depending on the nature of the treatments and time of storage. By the end of storage (15 days), TVBN of the above mentioned patty samples reached figures of 27.20, 21.84, 22.42 and 18.56 mg N/100 gm flesh; respectively. However, the increase in TVBN values is related to the activity of spoilage bacteria and endogenous enzymes [44,22]. On the other hand, the lower TVBN for treated samples may be due to the effectiveness of the essential oil or sodium lactate on microorganisms [28,3]. The present results confirmed the findings of Choi and Chin [17] and Baross et al. [8], on their frameworks on other meat products treated with essential oil and/or lactate.

Regarding TVBN values as a spoilage index for meat products, a level above 20 mg N/100 gm flesh is usually regarded spoiled minced meat [20]. Accordingly, control beef batty samples still acceptable with regard to TVBN index for eight days in comparison to 11 days for thyme essential oil-treated patties, and 12 days for lactate-treated ones, whereas lactate/thyme mixture exceeded the shelf-life to 15 days under the same conditions. These results confirmed the findings of El-Desouky et al. [21], who reported that treatment of beef patties by lactate and/or thyme essential oil were very effective in inhibiting microbial growth, reduced chemical and enzymatic deterioration reactions and prolonged the shelf-life.

Lipid Oxidation:

TBARS value is used as an index of lipid oxidation in meat products during storage [44]. Data presented in Table 3 showed the changes that took place in TBARS values of raw ground beef patties during refrigerated storage for 15 days. Storage time had a significant effect on TBARS values, which tended to increase with storage. Results indicate that TBARS values increased over time in all raw (uncooked) patties, with the control (C) patties oxidizing most rapidly and to the greatest extent as compared to treated samples, control raw patties exhibit significantly (P<0.05) higher TBARS values at any given time of chilling study as compared to lactate (NaL) or thyme essential oil (TEO) containing patties. Conversely, lactate/thyme mixture-treated samples had the greatest oxidative stability as shown by the consistently significantly (P<0.05) lower TBARS values initially and during the refrigerated study. Table 3 further shows that thyme essential oil was more effective (P<0.05) in retarding lipid oxidation than sodium lactate. The present results (Table 3) confirmed the findings obtained by other researchers [22,3], who reported that sodium lactate or thyme essential oil has a significant (P<0.05) effect in retarding lipid oxidation and maintaining the chemical quality of the product during storage.

**Table 3**: TBARS values changes of beef patty samples during refrigerated storage at 4±1 °C for 15 days.

<table>
<thead>
<tr>
<th>Patty Treatment</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>0.45 ± 0.10*</td>
<td>0.56 ± 0.12*</td>
<td>0.73 ± 0.12*</td>
<td>0.92 ± 0.10*</td>
<td>1.12 ± 0.11*</td>
<td>1.42 ± 0.14*</td>
</tr>
<tr>
<td>Lactate (2% NaL)</td>
<td>0.41 ± 0.14*</td>
<td>0.48 ± 0.10*</td>
<td>0.61 ± 0.11*</td>
<td>0.74 ± 0.12*</td>
<td>0.89 ± 0.10*</td>
<td>1.02 ± 0.12*</td>
</tr>
<tr>
<td>Thyme Oil (0.05%)</td>
<td>0.38 ± 0.11*</td>
<td>0.42 ± 0.13*</td>
<td>0.53 ± 0.14*</td>
<td>0.66 ± 0.11*</td>
<td>0.78 ± 0.08*</td>
<td>0.94 ± 0.10*</td>
</tr>
<tr>
<td>Lactate/TEO mixture</td>
<td>0.34 ± 0.12*</td>
<td>0.39 ± 0.11*</td>
<td>0.48 ± 0.10*</td>
<td>0.59 ± 0.13*</td>
<td>0.71 ± 0.12*</td>
<td>0.85 ± 0.11*</td>
</tr>
</tbody>
</table>

Mean ±SD=All values determination ± standard deviation (SD) mean of triplicate determinations. Mean values in the same column bearing the same superscript do not differ significantly (P>0.05).

**Thiobarbituric acid reactive substances (TBARS, as mg malonaldehyde ”MDA”/kg meat):**

It is clear from the results in Table 3 that incorporation of thyme essential oil in raw patties, alone or in mixture with 2% NaL had significantly...
(P<0.05) lower and acceptable TBARS values (less than 0.9 mg MDA kg⁻¹ meat) as required by the Egyptian Standards [20] for meat products compared to control (C) patties. Moreover, sodium lactate alone or thyme essential oil only containing patties still showed good quality with regard to TBARS values even after 12 days of refrigerated storage versus to only eight days for control samples; whereas NaL/TEO mixture exceeded the shelf-life to 15 days under the same conditions. These results are in agreement with the findings achieved by Darwish et al., [18]; and Shalaby et al., [49], who reported that TEO incorporation retarded lipid oxidation in muscle based products. However, the accumulation of malonaldehyde (MDA) in patty samples during refrigerated storage could be due to hydrolytic and oxidative processes in the lipid fraction, as well as to the increase in “free” iron ions of meat products [12]. Lactate potentially retarded the oxidation in patty samples through the chelation of pro-oxidant metal ions [50]. On the other hand, the high efficiencies found in thyme essential oil were closely related to the high content of phenolic compounds, confirming the key role of phenolic compounds as scavengers of free radicals and as primary, chain breaking antioxidants [46,52].

**Color Profile Changes:**

The main factor determining consumer acceptance in the selection of meat purchased is meat color [19], consequently desirable color must be maintained throughout the storage of meat products. Color values of all beef patty treatments at selected sampling days are given in Table 4. During refrigerated storage the L* values which refers to the lightness decreased gradually up to day 15 of storage, indicative of the fact that the color of the product became more dull. For the samples containing thyme essential oil (TEO), either alone or in mixture, the reverse trend was recorded resulting in higher L* value toward the end of storage. In addition, samples treated with lactate alone exhibited moderate increase in their L*, a* and b* values as compared to control samples; which confirming the findings obtained by other researchers [19,34].

As shown in Table 4, Redness was increased (P<0.05) after addition of TEO, thyme oil containing patties showed significantly (P< 0.05) higher a* values compared to lactate or untreated control samples throughout the storage. The best synergistic effect was again obtained from the combination of TEO with lactate, which exhibits the lowest deterioration effect on Hunter a* values. Thyme essential oil might work as reducing agent [46,52], which could reduce metmyoglobin formation to some degrees, consequently resulted in more stability of a* values [3]. Regarding Hunter b* values of beef patties treated with TEO were initially higher (P< 0.05) than lactate or control samples, and remained higher at the end of the storage period indicating increased yellowness of TEO-treated patties, which could be attributed to the natural yellowish color of TEO positively affecting the patty color. Similar observations have been also made by Georgantelis et al., [24]. In contrast, control samples showed, however, a poorer color stability giving initial lower L*, a* and b* values.

**Table 4: Color profile (L*, a* and b*Values) changes of beef patties during Cold Storage at 4±1°C for 15 days.**

<table>
<thead>
<tr>
<th>Beef Patty Treatment</th>
<th>Color Profile</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>L*</td>
<td>44.20 ± 0.09d</td>
<td>43.86 ± 0.17d</td>
<td>43.12 ± 0.10d</td>
<td>42.27 ± 0.14d</td>
<td>41.56 ± 0.13d</td>
<td>40.28 ± 0.15d</td>
</tr>
<tr>
<td>NaL</td>
<td></td>
<td>45.61 ± 0.13c</td>
<td>44.92 ± 0.11c</td>
<td>44.37 ± 0.10c</td>
<td>43.54 ± 0.15c</td>
<td>43.16 ± 0.12c</td>
<td>42.27 ± 0.11c</td>
</tr>
<tr>
<td>TEO</td>
<td></td>
<td>46.35 ± 0.11b</td>
<td>46.02 ± 0.13b</td>
<td>45.60 ± 0.12b</td>
<td>45.15 ± 0.11b</td>
<td>44.52 ± 0.09b</td>
<td>43.40 ± 0.12b</td>
</tr>
<tr>
<td>NaL/TEO</td>
<td></td>
<td>46.78 ± 0.16a</td>
<td>46.36 ± 0.11b</td>
<td>46.00 ± 0.15a</td>
<td>45.40 ± 0.10a</td>
<td>45.18 ± 0.11a</td>
<td>44.08 ± 0.10a</td>
</tr>
<tr>
<td>Control</td>
<td>a*</td>
<td>11.62 ± 0.10d</td>
<td>11.18 ± 0.10d</td>
<td>10.74 ± 0.11d</td>
<td>10.18 ± 0.13d</td>
<td>9.56 ± 0.17d</td>
<td>8.74 ± 0.13d</td>
</tr>
<tr>
<td>NaL</td>
<td></td>
<td>11.84 ± 0.13b</td>
<td>11.42 ± 0.12c</td>
<td>11.10 ± 0.10b</td>
<td>10.73 ± 0.14b</td>
<td>10.22 ± 0.12b</td>
<td>9.28 ± 0.11b</td>
</tr>
<tr>
<td>TEO</td>
<td></td>
<td>12.57 ± 0.13b</td>
<td>12.15 ± 0.14b</td>
<td>11.62 ± 0.08b</td>
<td>11.25 ± 0.13b</td>
<td>10.78 ± 0.14b</td>
<td>10.13 ± 0.08b</td>
</tr>
<tr>
<td>NaL/TEO</td>
<td></td>
<td>12.78 ± 0.17a</td>
<td>12.52 ± 0.16a</td>
<td>12.06 ± 0.13a</td>
<td>11.68 ± 0.14a</td>
<td>11.23 ± 0.11a</td>
<td>10.91 ± 0.12a</td>
</tr>
<tr>
<td>Control</td>
<td>b*</td>
<td>15.85 ± 0.12d</td>
<td>15.17 ± 0.10d</td>
<td>14.73 ± 0.14d</td>
<td>13.90 ± 0.12d</td>
<td>13.15 ± 0.10d</td>
<td>12.49 ± 0.13d</td>
</tr>
<tr>
<td>NaL</td>
<td></td>
<td>16.18 ± 0.10c</td>
<td>15.66 ± 0.13c</td>
<td>15.18 ± 0.11c</td>
<td>14.76 ± 0.15c</td>
<td>14.25 ± 0.12c</td>
<td>13.35 ± 0.17c</td>
</tr>
<tr>
<td>TEO</td>
<td></td>
<td>17.23 ± 0.10b</td>
<td>16.75 ± 0.09b</td>
<td>16.23 ± 0.16b</td>
<td>15.60 ± 0.14b</td>
<td>15.14 ± 0.11b</td>
<td>14.28 ± 0.12b</td>
</tr>
<tr>
<td>NaL/TEO</td>
<td></td>
<td>18.12 ± 0.12a</td>
<td>17.68 ± 0.14a</td>
<td>17.25 ± 0.10a</td>
<td>16.82 ± 0.11a</td>
<td>16.15 ± 0.13a</td>
<td>15.00 ± 0.11a</td>
</tr>
</tbody>
</table>

Color parameters lightness (L*), redness (a*) and yellowness (b*) Values are given as mean ± S.D. from triplicate determinations.

*Any two means at the same row or at the same column have the same letter are not significantly different at P < 0.05.

The results in Table 4 also indicated that, over storage time a* values of all patty samples decreased, indicating that samples were becoming less red or brown due to metmyoglobin formation. Lightness (L*) values steadily decreased as expected until day 15. The yellowness (b*) values followed similar trend decreasing to day 15. ANOVA revealed significant (P<0.05) differences between control and treated samples for all color parameters during storage. This indicates increased graying over storage days particularly in control samples. Microbial spoilage, the decomposition of pigments as a result
of bacterial action and a scape of some pigments (as water soluble protein) were most likely responsible for the overall graying effects seen over storage time. Similar trends of color changes in minced meat containing lactate or essential oil compared to controls were observed by other authors [24,26].

Conclusion:
Color changes, lipid oxidation and microbial contamination are serious concern for meat producers and consumers. The exogenous application of sodium lactate (NaL) or thyme essential oil (TEO) showed potential in enhancing the color, lipid stability, sensory qualities and microbial status, while maintain the chemical indices, and extend the shelf life of minced beef patties during refrigerated storage at 4±1°C. However, the best shelf-life, antioxidative, antimicrobial and sensorial effects were obtained by the mixture of NaL with TEO. Such mixture could have for commercial use to improve preservation of beef patties during refrigerated storage. Due to concerns regarding the safety and toxicity of synthetic preservatives, NaL/TEO mixture may prove useful as safe, natural, functional ingredients to the meat industry.

References
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43. PC-STAT, 1985. Version IA copyright, University of Georgia.