ORIGINAL ARTICLES

Improving the Quality and Shelf-Life of Refrigerated Chicken Meat by Marjoram Essential Oil

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ABSTRACT

Nowadays consumers are demanding more natural foods; the present study was designed to investigate the effect of marjoram essential oil (MEO) dipping treatments (based Nano-emulsion technique) on the quality and shelf-life of raw chicken drumsticks during refrigerated storage for 12 days. Identification of chemical constituents and phenolic compounds responsible for antioxidant and antimicrobial activities were also investigated in marjoram extracts using GC/MS and HPLC analytical systems. The results indicated that significant (p<0.05) incremental pattern was observed in TVB-N, TBARS and pH values in all chicken samples during subsequent cold storage by different rates. The lowest significant (p<0.05) incremental rate was recorded in samples soaked in 0.2% stabilized MEO. The results also reveal that both dipping treatments(T1 and T2) significantly reduced the microbial load of aerobic plate counts (APC), psychrotrophic counts (PTC) and Enterobacteriaceae accounts (EBC) just after dipping and throughout the storage period in comparison with the control. However, samples dipped in MEO at 0.2% showed the lowest incremental pattern in bacterial counts at any time of the refrigerated storage. A small but statistically significant (P<0.05) reduction in the color parameters L*, a* and b* values was recorded throughout the storage, indicates increased graying over storage days particularly in control samples. MEO dipping significantly reduced color loss in chicken drumsticks. Finally, there was also a significant (p<0.05) enhancement in sensory attributes and shelf-life stability of chicken drumsticks due marjoram oil dipping, the untreated drumsticks would have a refrigerated shelf life between 6 and 7 days while after natural active dipping, the shelf life extended to 9 days in T1-drumstick samples and 12 days in T2-samples. The major identified components by GC/MS were γ-terpinene, α-terpinene and terpinen-4-ol, followed by α-terpinolene, sabine hydrate, p-cymene, α-phellandrene and limonene. While HPLC results indicated that catchin, cinnamic, gallic, caffic, chlorogenic, vanillic, ferulic, and cumaric acids were positively identified in the present study. Among the positive effects of these compounds on chicken meat characteristics are retarding lipid oxidation, color loss, off-odor formation and microbial growth occurring during refrigerated storage and may offer a promising choice in food safety and preservation.

Key words: Nano-emulsion, Lamiaceae, natural preservatives, marjoram oils, phenolic compounds, quality attributes.

Introduction

Consumption of fresh foods like seafood, meat and poultry products have increased due to the need of consumers for convenient ready-to-cook or ready-to-eat foods and the desire to lead a healthy lifestyle. The challenges of these fresh muscle foods are their limited storage life, microbiological safety and quality deteriorations (Pavelkova, 2013). Lipid oxidation is one of the main factors limiting the quality and acceptability of lipid-containing foods as it affects the sensory quality and safety (Aguirrezabal et al., 2000). Muscle foods are also susceptible to microbial contamination leading to food-borne illness and economic loss in terms of food poisoning and spoilage (Barbosa et al., 2009). Therefore, the application of agents with both antioxidant and antimicrobial activities may be useful for maintaining meat quality, extending shelf-life and preventing economic loss (Teixeira et al., 2013).

Due to consumers demand, food manufacturers aim to process additive free, natural tasting food which has a longer shelf life along with microbiological safety. So, there is a growing interest in the utilization of natural origin for preservation of foods as replacements for synthetic agents whose use is being restricted because of their potential carcinogenic and toxicity for consumers (Parke and Lewis, 1992; Holley and Patel, 2005). Much attention has been focused on the use of the extracts from herbs and spices to prolong shelf-life and improve sensory properties of perishable foods (Burt, 2004; Mohamed et al., 2011; Darwish et al., 2012). 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 (Arts and Hollman, 2005; Camo et al., 2008).

Marjoram (Origanum majorana L.), a member of the Lamiaceae family is one of the most familiar kitchen herbs, which contains up to 3% of volatile oil, other compounds like flavonoid, arbutin, tannins, caffeine acid, labiatic acid, rosmarinic acid, ursolic acid, carnosic acid, and carnosol can be found in the herb (Shan et al., 2005; Vagi et al., 2005). Marjoram essential oil is a natural product classified as generally recognized as safe (GRAS) and known to possess antimicrobial and antioxidant activities (Burt, 2004; Chan et al., 2012). In Egypt many essential oils including MEO are produced and some are imported for increasing income from foreign currency (El-Aeshmawiy et al., 2009). Marjoram “herb of happiness” is cultivated for use of its aromatic leaves and their essential oil for flavoring; industrial uses (natural components of these plants are used as food additives, herbal tea, pigments, dyes, insecticides, cosmetics, perfumes and natural preservatives); as well as medicinal and other culinary purposes (Cutler, 1995; Tapsell et al., 2006; Buchbauer, 2010).

In our previous investigations, it was found that marjoram essential oil (MEO) and their components possess relatively strong antioxidant activities which can adequately be measured by several in vitro methods, MEO also shows promising activities against many food-borne pathogens and spoilage microorganisms when tested in vitro (Badee et al., 2013). However, the concentration of EOs used in food application could vary from 2 to 100 fold of that used in vitro assays depending on the herb type and food system used, since the presence of fats, carbohydrates, proteins, salts and pH strongly influence the effectiveness of these agents (Gutierrez et al., 2009). EOs can be incorporated on to the food system by direct addition; by using active packaging; or by dipping, spraying, coating treatment of food with active solutions (Kun, 2012; Pavelkova et al., 2013).

There is budding interest within the food and beverage industries for the utilization of Nano-emulsions for delivering the bioactive compounds such as antimicrobials, antioxidants, colors and flavors agents to the food (Ghosh et al., 2013). Nano-emulsion is a heterogeneous system of oil-in-water and stabilized by emulsifier through ultrasound applications. It is thermodynamically stable, optically clear and transparent. Ultrasound applications to formulate Nano-emulsion do not cause alteration in the temperature, structure, physical or chemical properties of materials (Thakur et al., 2012).

Keeping in view the common use and healing potential of marjoram, the present study was carried out to investigate the influence of dipping chicken drumsticks for 10 min in zero, 0.1 or 0.2% stabilized MEO solutions (based Nano-emulsion technique) on the sensorial properties, microbial status, deterioration criteria indices, color profile and shelf-life stability of raw chicken drumsticks during refrigerated storage at 3-5°C for 12 days. Identification and quantification of the chemical constituents and phenolic compounds responsible for antioxidant and antimicrobial activities were also investigated in marjoram extracts using GC/MS and HPLC analytical systems.

Materials and Methods

Chicken Source:

Nine kg. chicken drumsticks (average 80g per drumstick) from fresh slaughtered broilers were purchased from a commercial source at Giza, transported to the laboratory on ice, washed under cold running tap water, then divided into three equal groups, and immediately cooled on ice before soaking in cold stabilized MEO solutions (based Nano-emulsion technique).

Preparation of Marjoram Essential Oil (MEO):

Air-dried marjoram leaves were purchased from Al-Dahlia Company, Nasr City, Egypt. Steam distillation using the Clevenger system over a period of 3h was used to obtain the essential oil, which was dried over anhydrous sodium sulphate before held in dark sealed glass vials and stored at 3-5°C until use.

Preparation of Stabilized MEO Solutions:

Oil-in-water Nano-emulsion was formulated using marjoram essential oil, glycerol and distilled water. The concentration of MEO was based on results from studies of El-Desouky et al., (2006) and Busatta et al., (2008) in meat products. For food applications glycerol was chosen owing to its classification as GRAS, as well as being an odorless, colorless, and high purity and emulsifying properties (EFSA, 2010). Emulsion was prepared, according to the method of Ghosh et al., (2013), through Ultrasonic Cleaner Apparatus (Model UD150SH3.8LQ, USA), using a 150 kHz for 30 min at room temperature.
Treatments of Chicken Samples:

Dipping treatment of chicken parts with active solutions is currently applied prior to packaging as a valid option (Pavelková et al., 2013). In similar way, one group was immersed in cold 0.1% stabilized* MEO solution for 10 min at 3-5°C with moderate agitation, using the drumsticks/solution ratio of 1:2 (w/v), drained well for 3 min, then packed with two chicken drumsticks in each polyethylene bag (T1). The second group was soaked in cold 0.2% stabilized* MEO solution for 10 min, drained, and packed as previous group (T2). The third group was dipped in cold distilled water solution for 10 min., to compensate for possible physical removal of bacteria and for moisture uptake, then drained and packed as the previous samples (control). Each treatment samples were chilled stored at 3-5°C. Two bags of each group were withdrawn for analysis at three days intervals over storage period of 12 days.

Preparation of Marjoram Methanolic Extract (MME):

Marjoram methanolic extract were prepared according to the method of W’glarz et al. (2006) as follow: one gram of dry marjoram powder was mixed with methanol (20 mL MeOH, HPLC grade), the suspension was left at room temperature for 1 hour, centrifuged at 10000 rpm for 10 min and the supernatant was filtered through Whatman No.4. The residue was dissolved in 10 ml of MeOH, centrifuged and filtered, then 1-3 ml from the filtrate was collected in a vial for injection use into HPLC analytical system.

GC-MS Analysis of marjoram essential oil (MEO):

Marjoram essential oil constituents were analyzed in a gas-chromatograph interfaced with a mass selective detector—GC/MS, Model (Varian 240-MS), using a capillary column VF-5, MS (30m; 0.25 mm, ID; 0.25 μm film thickness) and a flow rate of helium as the carrier gas at 1ml/min. 1μL of MEO was direct injection. The injector and detector temperatures were 250°C. The identification of marjoram essential oil compounds was accomplished by comparing their retention times with those of authentic standards, and by comparison of their mass spectra with those from the Wiley library. Compositions were then expressed as percentages of normalized peak areas.

HPLC Analysis of Marjoram Methanolic Extract (MME):

Previous prepared MME (25 µL) was injected into HPLC Hewllet Packared (Agilent Technologies, USA) equipped with an auto-sampling injector, solvent degasser, quaternary HP 1100 pump and a diode array detector. Chromatographic separation was acquired at 280 nm. On ZORBAX Eclipse XDB C18 column (15 cm x 4.6 mm I.D., 5 μm, USA). Operative conditions were: mobile phase (A, methanol; B, 2% acetic acid); flow rate, 1mL min; and chromatogram elution program, A/B (%): 0 min 5/95; 10 min 25/75; 20 min 50/50; 30 min 100/0; 40 min 5/95. Peaks identification and quantification (as peak area %) were confirmed by comparison of retention time and spectral data with adequate parameters of standards.

Analytical Methods:

Proximate analysis:

Moisture, protein, intramuscular-fat and ash contents were determined at zero time for raw and grilled chicken drumstick samples using standard analytical methods (AOAC, 1995), while the amount of total carbohydrates was calculated by differences.

Microbiological Status:

Aerobic plate count (APC), psychrotrophic count (PTC), and Enterobacteriaceae count (EBC) were determined following procedures recommended by APHA (2001) after preparing serial dilution taking 10g sample. APC was determined by spread plating on plate count agar, employing an incubation condition at 37°C for 24-48h. 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 24-48h. After specific incubation periods plates showing 25-250 colonies were counted. The number of colonies were multiplied by the reciprocal of the respective dilution and expressed as log10 cfu g⁻¹.
Sensory panel evaluation:

Samples of freshly cooked chicken drumsticks were organoleptic evaluated by aid of 10 semi-trained panelists from the staff members. Only edible chicken drumsticks from control and marjoram treated groups (T1 & T2) were grilled (to simulate a normal fast food restaurants), using a HG 230 Kenwood multi cooker with turning every 3 min until cooked to an internal temperature of 80°C. Chicken samples were labeled with 3-digit random numbers and served warm to panelists in individual booths. Panellists were instructed to cleanse their palates with water between samples. They were asked to score appearance, odor, texture, taste and overall acceptability according to Watts et al., (1989), as follows: very good (8-9), good (6-7), fair (4-5), poor (2-3) and very poor (0-1).

Deterioration criteria indices:

Total volatile basic nitrogen (TVBN), and thiobarbituric acid reactive substances (TBARS) were determined according to Pearson, (1991). For pH determination 10g of drumstick meat samples was homogenized in 100 mL distilled water for 1 min in a blender, and the pH was measured using a digital pH meter (Haana, HI9002, Germany).

Instrumental color evaluation:

Color profile was determined after allowing the muscle surface to bloom for 30 min., using a Hunter Lab Scan XE Colorimeter (Hunter Laboratory Inc. Restonva). 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 Discussion

GC/MS Studies:

The chromatogram obtained by GC/MS (Fig. 1) shows that there were 15 identified components from marjoram essential oil; a chemical profile very similar to that found by Darwish et al., (2012). The essential oil assayed here possesses γ-terpinene, α-terpinene and terpinen-4-ol as its major compounds, followed by α-terpinolene, sabinene hydrate, p-cymene, α-phellandrene and limonene. Fig. 1 also reveals the presence of myrcene, α-thujene, α-terpineol, and β-caryophyllene in appreciable amounts, while α-pinene, methyl benzene, and trans-cocimene were traces (less than 1%), these identified compounds accounted for 96.54% of the total oil. GC/MS results indicate that Egyptian marjoram oil belonged to terpinen-4-ol/sabinene hydrate chemotype. However, the content of active substances in herb essential oils can vary among others due to plant species variability, its growth phase, the country of origin, part of the plant, season of harvesting, and also the extraction method (Bakkali et al., 2008).

Marjoram has a high antioxidant capacity due to its high phenolic contents (Shan et al. 2005; Badee et al., 2013). HPLC was used to identify and quantified the phenolic compounds that were present in the studied marjoram methanolic extract. Fig.2 indicates that catchin, cinnamic, gallic, caffic, chlorogenic, vanillic, ferulic, and cumaric acids were positively identified in the present study. The HPLC results obtained also reveal that the dominant phenolic compound was cumaric acid, while the peak produced for caffic acid was very low suggesting that it is found in very small quantities. Such results are in agreement with those reported by Petr et al (2008) and Hafez, (2012) who reported that HPLC analysis of marjoram alcoholic extract showed the presence of: salicylic, caffeine, vanillic, cumarin, feurilic, p-cumaric, chlorogenic, ellagic, pyrogallol and catechol in varying quantities.

It can be deduced from the results illustrated in Figs.1&2 that the presence of high phenolic compounds including flavonoids, phenolic acids and volatile compounds might play a major role in their antioxidant and antimicrobial effects (Jun et al., 2001; Burt, 2004; Shan et al., 2005; Schmidt et al., 2008). These results confirmed the findings obtained by other researchers (Vagiet al., 2005; Busattaet al., 2008; Barbosa et al., 2009; Chan et al., 2012; Teixeira et al., 2013) who stated that marjoram essential oil is a natural product classified as generally recognized as safe (GRAS) and known to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant activities.
Fig. 1: Chemical composition of hydro-distilled marjoram essential oil obtained by GC/MS:

Retention time, component identifications and their relative proportions (Area %) of MEO as follows:
5.16—$\alpha$-pinene (0.74%); 5.31—$\alpha$-thujene (2.41%); 11.28—$\alpha$-phellandrene (5.43%); 11.51—Myrcene (2.91%); 12.13—$\alpha$-terpinene (19.71%); 12.93—Limonene (5.40%); 13.29—SabineneHydrate (6.56%); 15.08—$\gamma$-terpinene (23.20%); 15.35—Trans-Ocimene (0.72%); 15.82—P-Cymene (5.81%); 16.34—$\alpha$-terpinolene (6.76%); 20.92—Methylbenzene (0.73%); 24.96—$\beta$-Caryophyllene (1.58%); 25.12—Terpinen-4-ol (12.64%); 27.09—$\alpha$-Terpineol (1.94%).

HPLC Analysis:

Fig. 2 Chemical components of marjoram methanolic extract as detected by HPLC:

Retention time, component identifications and their relative proportions (Area %) of marjoram methanolic extract as follows:
4.401—Catchin (15.08%), 10.729—Gallic (6.81%), 12.661—Caffic (2.18%), 13.652—Chlorogenic (8.72%), 14.757—Vanillic (13.24%), 18.470—Ferulic (7.43%), 19.700—Cumaric (18.36%) and 25.010—Cinnamic (17.11%) were positively identified in the present study by HPLC analytical system.

Proximate Composition:

Poultry meat is a very popular food commodity around the world. Mean values for proximate composition of raw and grilled chicken drumstick are presented in Table1. For raw chicken parts the moisture, protein, intramuscular fat, and ash contents were 72.46, 21.48, 4.80, and 1.16 % (on fresh weight basis). However, soaking chicken drumsticks in active solutions (MEO) had no effect on their proximate composition (results not shown in a tabular form). Similar results were achieved in chicken thigh by Keokamnerd et al., (2008). On the other hand, the corresponding proximate composition in grilled drumsticks were 67.30, 25.16, 5.54 and 1.35 %; respectively. This indicate that, grilling of chicken parts caused reduction in their moisture content leading to apparent increase in protein, Int. fat and ash contents (on fresh weight basis). The present results regarding proximate composition of grilled chicken meat also confirmed the findings obtained by Latif (2010).
Table 1: Proximate Chemical Composition of Fresh Raw and Grilled Chicken Drumsticks.

<table>
<thead>
<tr>
<th>Chemical Constituents</th>
<th>Raw Chicken Drumstick</th>
<th>Grilled Chicken Drumstick</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wt. Weight</td>
<td>Dry Weight</td>
</tr>
<tr>
<td>Moisture %</td>
<td>72.46±0.78</td>
<td>--</td>
</tr>
<tr>
<td>Protein %</td>
<td>21.48±0.47</td>
<td>78.00</td>
</tr>
<tr>
<td>Int. Fat %</td>
<td>4.80±0.19</td>
<td>17.83</td>
</tr>
<tr>
<td>Ash %</td>
<td>1.16±0.11</td>
<td>4.21</td>
</tr>
<tr>
<td>Carbohydrates %</td>
<td>0.10±0.08</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.D. from triplicate determinations.

Microbiological Status:

Aerobic Plate Count (APC):

Amongst the studied microbial categories, the population counts in control as well as in treated chicken parts followed the order: APC > PTC > EBC throughout the storage period. Results depicted in Table 2 reveal that the initial value of APC (day 0) for the fresh chicken meat was 4.2 log CFU/g, indicative of good quality chicken meat (Chouliara et al., 2007). It is evident that dipping chicken parts in MEO solutions (T1 and T2) resulted in Significant (p<0.05) reductions of APC by 0.9 and 1.02 logs, respectively, at zero time of cold storage. The Enterobacteriaceae were the most affected organisms, where MEO-dipping reduced their initial counts in chicken meat by 1.12 and 1.24 log CFU/g, respectively. On the contrary, psychrotrophs were the least affected bacteria as the T1- and T2-dipping achieved mean log reductions of 0.62 and 0.75 log CFU/g in their initial counts, respectively, which indicate that MEO caused sudden lethal effect for microorganisms.

The increase in storage time produced significant proliferations in APC of chicken drumsticks. From the results in Table 2 it could be observed that control sample had always higher microbial counts when compared with marjoram treated samples. On the sixth day of storage, APC of control samples reached a log mean count of 6.87, which is closed to the maximal recommended limit (7 log CFU/g) set by ICMSF (1986) for APC in processed chickens. On the ninth day of storage T1- and T2-dipped samples demonstrated mean APC of 6.75 and 6.40, respectively vs. 8.28 for control, while signs of spoilage started to appear as a slight foul smell for control chicken. By the end of storage (day 12), T1- and T2-dipped samples exhibited log mean APC of 7.88 and 6.04, respectively vs. 9.48 in control, indicating a shelf life between 6-7 days for the control samples, 9 days for T1 and 12 days for T2 treated samples.

Table 2: Microbiological Analysis of Chicken Drumsticks during Cold Storage (count as log CFU/g).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bacterial Categories</th>
<th>Z-Day</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>APC</td>
<td>4.20±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.60±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.87±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.28±0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.48±0.13&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td>3.30±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.42±0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.69±0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.75±0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.88±0.09&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td>3.18±0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.20±0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.30±0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.40±0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.04±0.11&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>PTC</td>
<td>3.62±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.80±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.74±0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.58±0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.46±0.17&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td>3.00±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.95±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.62±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.35±0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.18±0.14&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td>2.87±0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.48±0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.06±0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.80±0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.52±0.11&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>EBC</td>
<td>2.35±0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.67±0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.93±0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.27±0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.53±0.10&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td>1.23±0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.51±0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.76±0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.10±0.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.44±0.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td>1.11±0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.34±0.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.58±0.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.82±0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.15±0.15&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Where: APC (aerobic plate count), PTC (psychrotrophic count), EBC (Enterobacteriaceae count). Control (chicken samples soaked in distilled water), T1 (chicken samples soaked in 0.1% MEO solution), T2 (chicken samples soaked in 0.2% MEO solution). 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.

Psychrotrophic Count (PTC):

The psychrotrophic counts in broiler chicken carcasses were slightly lower than the APC. This was true for all of the three groups analyzed. As the storage time increased, the PTC steadily increased in the control group from a mean count of 3.62 log CFU/g at the beginning of the storage (day 0) to higher counts of 5.74 log CFU/g on the sixth day of storage (Table 2). On the other hand, both T1 and T2-dipped samples exhibited significantly (p<0.05) lower PTC in comparison with the control throughout the storage time. By the end of the storage time (day 12), the mean PTC in T1-treated samples was 1.28 logs lower than that of the control (6.18 vs. 7.46), while the mean PTC in T2-treated samples was 1.94 logs lower than that of the control (5.52 vs. 7.46). In this concern, it is worth mentioning that psychrotrophic counts (PTC) should not be more than 10<sup>c</sup> CFU/g meat (Darwish et al., 2012; Kamel, 2013).
Enterobacteriaceae Count (EBC):

Finally, Enterobacteriaceae (Table 2), considered as a hygiene indicator (Barbosa et al., 2009), the initial counts were 2.35 log cfu/g, indicative of good quality chicken meat. The EBC of control chicken samples increased to a high count of 2.93 on the sixth day of storage (Table 2). Chicken samples dipped in 0.1 or 0.2% MEO solutions, however, exhibited significantly lower counts (p<0.05) in comparison with control throughout the storage time and they remained below the count of 2.5 log CFU/g even on the end of storage period (day 12). Similarly, Chouliara et al., (2007) reported that marjoram oil had a strong effect in the reduction of Enterobacteriaceae counts of chicken meat during refrigerated storage. The most significant benefit of applying essential oils to stored chicken products was to inhibit the growth of Enterobacteriaceae bacteria (by 1.09–1.38 log cycle), which is a very desirable quality since this group contains a large number of pathogens.

Our results confirmed the findings of Kim and Marshall, (1999), who reported that raw control chicken legs stored in refrigerated conditions has a shelf-life less than 8 days. However, the present study indicated that both T1 and T2 are efficient in the reduction of the microbial contamination and extend the shelf life of chicken drumsticks during refrigerated storage. These findings are also on line with (Chouliara et al., 2007; Busatta et al., 2008; Barbosa et al., 2009; Mohamed et al., 2011; Teixeira et al., 2013), who found that among several essential oils marjoram oil (Origanum majorana L.) have the greatest potential antimicrobial effect against foodborne pathogens and spoilage microorganisms on their frame works on meat products. Generally, most of the antimicrobial activity of MEO is found in the oxygenated terpenoids and some terpenic hydrocarbons, alcohols, ketones, and p-cymene (Fig. 1; Badeet al., 2013), due to their hydrophobic characteristics effects on the cellular membrane structure and permeability, causing loss of cellular constituents, and cell death (Burt, 2004; Koroch et al., 2007).

Sensory Evaluation:

Taste, odor and appearance of a product can be the criteria for rejection of any kind of food if they differ significantly from what is expected by the consumers (Chouliara et al., 2007). As in all foods, the organoleptic tests are generally the final guide of the quality from the consumer’s point of view. Thus, it is beneficial to make a comparison between sensory evaluation for untreated and treated chicken drumsticks with the essential oil. To study the acceptability of untreated and treated chicken samples, appearance, odor, taste, texture and overall acceptability were organolitically evaluated; the obtained data are tabulated in Table 3.

Table 3: Sensory scores* of Freshly Cooked Refrigerated Chicken Drumsticks.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sensory Properties</th>
<th>Z/Day</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Appearance</td>
<td>8.4±0.27†</td>
<td>7.1±0.29&quot;</td>
<td>5.7±0.43'</td>
<td>Rejected</td>
<td>Rejected</td>
</tr>
<tr>
<td>T1</td>
<td>Appearance</td>
<td>8.4±0.43&quot;</td>
<td>7.6±0.58&quot;</td>
<td>6.9±0.58&quot;</td>
<td>6.0±0.47&quot;</td>
<td>Rejected</td>
</tr>
<tr>
<td>T2</td>
<td>Appearance</td>
<td>8.6±0.35&quot;</td>
<td>8.0±0.34&quot;</td>
<td>7.3±0.37&quot;</td>
<td>6.5±0.31&quot;</td>
<td>5.9±0.53</td>
</tr>
<tr>
<td>Control</td>
<td>Odor</td>
<td>7.2±0.16</td>
<td>6.4±0.58</td>
<td>5.3±0.22</td>
<td>Rejected</td>
<td>Rejected</td>
</tr>
<tr>
<td>T1</td>
<td>Odor</td>
<td>8.5±0.25&quot;</td>
<td>7.8±0.29&quot;</td>
<td>6.5±0.14&quot;</td>
<td>5.8±0.18&quot;</td>
<td>Rejected</td>
</tr>
<tr>
<td>MT2</td>
<td>Odor</td>
<td>8.7±0.48&quot;</td>
<td>8.0±0.47&quot;</td>
<td>7.1±0.51&quot;</td>
<td>6.3±0.47&quot;</td>
<td>5.6±0.34</td>
</tr>
<tr>
<td>Control</td>
<td>Taste</td>
<td>8.1±0.35&quot;</td>
<td>7.0±0.15&quot;</td>
<td>5.8±0.34</td>
<td>Rejected</td>
<td>Rejected</td>
</tr>
<tr>
<td>T1</td>
<td>Taste</td>
<td>8.8±0.68&quot;</td>
<td>8.1±0.30&quot;</td>
<td>7.0±0.16&quot;</td>
<td>6.2±0.58&quot;</td>
<td>Rejected</td>
</tr>
<tr>
<td>T2</td>
<td>Taste</td>
<td>9.0±0.31&quot;</td>
<td>8.4±0.26&quot;</td>
<td>7.6±0.25&quot;</td>
<td>6.7±0.37&quot;</td>
<td>5.0±0.37</td>
</tr>
<tr>
<td>Control</td>
<td>Texture</td>
<td>8.5±0.47&quot;</td>
<td>7.4±0.29&quot;</td>
<td>6.2±0.09&quot;</td>
<td>Rejected</td>
<td>Rejected</td>
</tr>
<tr>
<td>T1</td>
<td>Texture</td>
<td>8.5±0.58&quot;</td>
<td>7.7±0.44&quot;</td>
<td>6.8±0.30</td>
<td>5.9±0.33&quot;</td>
<td>Rejected</td>
</tr>
<tr>
<td>T2</td>
<td>Texture</td>
<td>8.7±0.35&quot;</td>
<td>8.1±0.51&quot;</td>
<td>7.2±0.64&quot;</td>
<td>6.4±0.29&quot;</td>
<td>5.7±0.47</td>
</tr>
<tr>
<td>Control</td>
<td>Overall Acceptability</td>
<td>8.1±0.27&quot;</td>
<td>7.0±0.19&quot;</td>
<td>5.8±0.25</td>
<td>Rejected</td>
<td>Rejected</td>
</tr>
<tr>
<td>T1</td>
<td>Overall Acceptability</td>
<td>8.6±0.54&quot;</td>
<td>7.8±0.25&quot;</td>
<td>6.8±0.34&quot;</td>
<td>6.0±0.14&quot;</td>
<td>Rejected</td>
</tr>
<tr>
<td>T2</td>
<td>Overall Acceptability</td>
<td>8.8±0.36&quot;</td>
<td>8.1±0.12&quot;</td>
<td>7.3±0.14&quot;</td>
<td>6.5±0.33&quot;</td>
<td>5.6±0.33</td>
</tr>
</tbody>
</table>

All values reflect the mean and standard deviation. (n=10). Control (chicken samples soaked in distilled water), T1 (chicken samples soaked in 0.1% MEO solution), T2 (chicken samples soaked in 0.2% MEO solution). *Any two means at the same row or at the same column have the same letter are not significantly different at P<0.05.

As shown in Table 3 ANOVA indicated that there are high significant differences (P<0.05) between different MEO concentrations in their sensory scores. Table 3 reveals that at the begging of storage (zero time) there were high scores, which matches the initial microbial load, pH, TVBN, and TBARS values. It is evident that odor and taste of grilled treated chicken parts were improved as shown by its higher scores comparing to control. Moreover, the samples which dipped in higher MEO (T2) had the highest scores in all sensory attribute as compared with T1 and control samples.

From the same given results in Table 3 it is apparent that throughout the storage period there were decreases and significant changes (p<0.05) in all sensorial criteria (appearance, odor, taste, texture, and overall acceptability), the lowest significant (p<0.05) negative changes was recorded in samples soaked in 0.2% MEO. The sensory results indicate that these natural functional ingredients can be incorporated into chicken products.
without having a detrimental effect on product quality producing healthy chicken products. This is due to the action of marjoram oil in retarding lipid oxidation, color loss, off-odor formation and microbial growth occurring during refrigerated storage (Burt, 2004; Vagi et al., 2005; Busatta et al., 2008).

Our results (Table 3) are in agreement with other researchers (El-Desouky et al., 2006; Chouliara et al., 2007; Mohamed et al., 2011; Darwish et al., 2012; Pavelková et al., 2013), who stated that incorporating of lower concentrations of essential oils in meat products in combination with refrigeration also had positive effects on their sensory traits as well as improving the quality, safety and shelf-life stability. Generally, Sensory data were in a very good agreement with microbiological and quality indices data (Tables 2&4).

Quality indices Alterations:

pH Changes:

The mean pH values of chicken drumsticks are given in Table 4. Inclusion of MEO (T1&T2) in chicken drumsticks had almost slight effect on their initial pH values. Table 4 also reveals that with the progression of cold storage pH values gradually increased for all investigated treatments with different rates depending on the initial treatments. Control samples showed the highest (P<0.05) incremental rate compared to other treatments (T1 & T2). Changing in pH values may be due to the activation effect of microbial load which may cause protein hydrolysis and/or decomposition of nitrogenous compounds with the appearance of alkaline groups and ammonia formation (Pearson, 1991; Chouliara et al., 2007).

Similar trends of pH changes have been observed in meat, poultry and fish products treated with natural essential oils (El-Desouky et al., 2006; Keoknamerd et al., 2008; Shalaby et al., 2013). However, the lower pH values of treated chicken samples reflect antimicrobial properties of marjoram oil, which reduces the accumulation of basic substances (Burt, 2004; Busatta et al., 2008; Barbosa et al., 2009; Mohamed et al., 2011; Darwish et al., 2012; Teixeira et al., 2013). Display of data demonstrated in Table 4 it is obvious that control samples were of good and acceptable quality with regard to pH up to six days in comparison to 9 days for T1-treated samples, whereas T2 treatment exceeded the critical pH value (>7.0) after 12 days of cold storage.

Total Volatile Basic Nitrogen (TVBN) Changes:

TVBN is considered the most commonly used biochemical method for assessing meat spoilage (Pearson, 1991). The mean values of TVBN for raw chicken drumsticks are given in Table 4, from which it is clear that the initial value for control samples was 11.2 mgN/100g flesh, indicative of good quality chicken meat (Chouliara et al., 2007; Keoknamerd et al., 2008). While as the period of cold storage increased the TVBN of all samples progressively increased with different rates depending on the nature of treatments. However, control samples showed the highest (P<0.05) incremental rate compared to other treatments and had TVBN value of 18.4 mgN/100g at 6 days. However, the TVBN values above 20 mg N/100g raw samples indicate spoilage of chicken products as recommended by Egyptian Standards (ES, 2910 - 2005). Long shelf life storage (9 days) was found for samples treated with 0.1% marjoram oil, which had 19.3 mgN/100g flesh. Moreover, as the concentration of MEO increased in the dipping solutions (0.2%) the accumulation of basic volatile nitrogen in the T2-treated drumsticks decreased, reaching value of 19.4 mgN/100g flesh in the twelfth day (Table 4).

The present results regarding TVBN are in good agreement with the findings achieved by other authors (El-Desouky et al., 2006; Chouliara et al., 2007; Keoknamerd et al., 2008) on their framework on meat products treated with essential oils. However, the increase in TVBN values reflects the decomposition of chicken protein by microbial activities and autolytic enzymes found naturally in meat tissues (Pearson, 1991), whereas the low levels detected in treated chicken parts could be due to the high antimicrobial efficiency of marjoram oil (Chouliara et al., 2007; Busatta et al., 2008; Barbosa et al., 2009; Mohamed et al., 2011; Teixeira et al., 2013).

Table 4: Deterioration Criteria Indices of Raw Chicken Drumstick during Cold Storage.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Quality Indices</th>
<th>Z-Day</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>pH Value</td>
<td>6.10 ± 0.10</td>
<td>6.38 ± 0.13</td>
<td>6.79a ± 0.12</td>
<td>7.18a ± 0.13</td>
<td>7.56a ± 0.17</td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td>5.96 ± 0.08</td>
<td>6.23 ± 0.10</td>
<td>6.52a ± 0.11</td>
<td>6.85a ± 0.14</td>
<td>7.18a ± 0.12</td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td>5.93 ± 0.12</td>
<td>6.14 ± 0.14</td>
<td>6.34a ± 0.15</td>
<td>6.52a ± 0.10</td>
<td>6.74a ± 0.11</td>
</tr>
<tr>
<td>Control</td>
<td>TVBN (mgN/100g)</td>
<td>11.2a ± 0.31</td>
<td>14.7a ± 0.40</td>
<td>18.4a ± 0.16</td>
<td>23.0a ± 0.14</td>
<td>26.1a ± 0.15</td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td>10.6a ± 0.19</td>
<td>12.8a ± 0.37</td>
<td>15.9a ± 0.21</td>
<td>19.3a ± 0.36</td>
<td>22.7a ± 0.26</td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td>10.5a ± 0.16</td>
<td>11.9a ± 0.26</td>
<td>13.6a ± 0.35</td>
<td>17.2a ± 0.19</td>
<td>19.4a ± 0.40</td>
</tr>
<tr>
<td>Control</td>
<td>TBARS (mgMD/kg)</td>
<td>0.42 ± 0.11</td>
<td>0.61 ± 0.10</td>
<td>0.87 ± 0.13</td>
<td>1.14 ± 0.14</td>
<td>1.42 ± 0.11</td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td>0.30 ± 0.12b</td>
<td>0.45 ± 0.08b</td>
<td>0.58a ± 0.11b</td>
<td>0.74a ± 0.12b</td>
<td>0.93b ± 0.06b</td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td>0.28 ± 0.14b</td>
<td>0.35 ± 0.12b</td>
<td>0.46a ± 0.16b</td>
<td>0.60 ± 0.10a</td>
<td>0.82a ± 0.13b</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.D. from triplicate determinations. Control (chicken samples soaked in distilled water), T1 (chicken samples soaked in 0.1% MEO solution), T2 (chicken samples soaked in 0.2% MEO solution). *Any two means at the same row or at the same column have the same letter are not significantly different at P < 0.05. Total volatile basic nitrogen (TVBN, as mgN/100g flesh), thiobarbituric acid reactive substances (TBARS, as mg malonaldehyde “MD”/kg).
Lipid Oxidation:

The effect of marjoram essential oil dipping on the lipid oxidation of raw chicken drumsticks during refrigerated period was depicted in Table 4. In spite of that chicken meat have less fat compared to other meat animals it have relatively high polyunsaturated fatty acids which may play an important role in increasing the rate of lipid oxidation (Melton, 1983). During cold storage there was an incremental pattern in TBARS values in different samples which indicated oxidation in chicken drumsticks, with the control parts oxidizing most rapidly and to the greatest extent as compared to marjoram oil-treated samples. It is clear from the same results that control samples exhibited the highest (P<0.05) TBARS values initially and at any given time of refrigerated storage. Conversely, T2-treated samples had the greatest oxidative stability as shown by the consistently lower TBARS values.

Data demonstrated in Table 4 also reveals that, on the sixth day of storage the control samples had TBARS value of 0.87 mg malonaldehyde/kg, which is closed to the maximal (0.9 mg MD/kg) recommended limit by Egyptian Standards for chicken products (ES, 2005). The samples treated with marjoram oil (0.1%) still acceptable until 9 days as indicated by TBARS values. Whereas, samples treated with high concentration of marjoram oil (0.2%) still showed good quality even after 12 days of refrigerated storage. These results are in agreement with the findings achieved by other researchers (El-Desouky et al., 2006; Mansour et al., 2006; Mohamed et al., 2011; Kamel, 2013) who reported that marjoram addition retarded lipid oxidation in meat products, and it is advised that marjoram essential oil could be used as an antioxidant additive in industrial processing of food.

The accumulation of malonaldehyde in chicken samples during refrigerated storage could be due to the hydrolytic and oxidative processes in the lipid fraction (Brake and Fennema, 1999), as well as to the increase in free iron ions during refrigerated storage of meat (Kanner et al., 1991). On the other hand, the high efficiencies found in marjoram essential oil were closely related to the high content of phenolic compounds including flavonoids, phenolic acids (Fig. 1) and volatile compounds (Fig. 2), confirming the key role of phenolic compounds as scavengers of free radicals, as chelating metal ions (such as Fe⁺), and as primary chain breaking antioxidants (Shan et al., 2005; Vagiet et al., 2005; Schmidt et al., 2008). A purified component isolated from marjoram methanol extract, T3b, is found to be a better super oxide anion radical scavenger than BHT BHA, α tocopherol, ascorbic acid and a variety of polyphenolic flavonoids (Jun et al., 2001).

Color Profile Changes:

The main factor determining consumer acceptance in the selection of poultry meat purchased is meat color (Keokammer et al., 2008). Consequently, desirable color must be maintained throughout the storage of chicken products. Color values of all chicken meat treatments at selected sampling days are given in Table 5. During refrigerated storage the L* values which refers to the lightness, decreased gradually up to day 12 of storage, indicative of the fact that the color of the product became more dull. For the samples containing marjoram oil (T1) the reverse trend was recorded resulting in higher L* value toward the end of storage. In addition, samples with increasing level of MEO exhibited more increase in their L* values.

As shown in Table 5, MEO dipping treatments showed significantly higher a* values compared to untreated samples throughout storage (P<0.05). In addition as the concentration of MEO increased the Hunter a* values also increased. Marjoram oil might work as reducing agent (Badie et al., 2013), which could reduce metmyoglobin formation to some degree, which confirms the findings of TBARS (Table4), consequently, resulted in more stability of a* values (Jun et al., 2001). Regarding Hunter b* values of chicken drumsticks treated with 0.1–0.2% were initially lower (P< 0.05) than control and remained low at the end of the storage period indicating reduced yellowness of marjoram-treated drumsticks (Table 5). In contrast, control samples showed, however, a poorer color stability giving initial lower a* values and higher L* and b* values.

Table 5: Color profile (L*, a* and b* Values) of Chicken Drumsticks during Cold Storage.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Color Profile</th>
<th>Z-Day</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>L Value</td>
<td>51.32±0.46</td>
<td>51.78±0.26</td>
<td>47.16±0.35</td>
<td>45.63±0.25</td>
<td>44.52±0.56</td>
</tr>
<tr>
<td></td>
<td>a Value</td>
<td>6.73±0.69</td>
<td>6.15±0.16</td>
<td>5.36±0.30</td>
<td>4.51±0.68</td>
<td>4.02±0.19</td>
</tr>
<tr>
<td>T1</td>
<td>a Value</td>
<td>7.62±0.74</td>
<td>7.24±0.10</td>
<td>6.78±0.11</td>
<td>6.12±0.33</td>
<td>5.74±0.16</td>
</tr>
<tr>
<td>T2</td>
<td>a Value</td>
<td>8.15±0.35</td>
<td>7.81±0.27</td>
<td>7.44±0.26</td>
<td>7.00±0.50</td>
<td>6.80±0.31</td>
</tr>
<tr>
<td>Control</td>
<td>b Value</td>
<td>10.85±0.33</td>
<td>9.64±0.31</td>
<td>8.76±0.36</td>
<td>7.72±0.44</td>
<td>7.08±0.23</td>
</tr>
<tr>
<td>T1</td>
<td>b Value</td>
<td>10.12±0.27</td>
<td>9.27±0.15</td>
<td>8.35±0.11</td>
<td>7.24±0.30</td>
<td>6.67±0.35</td>
</tr>
<tr>
<td>T2</td>
<td>b Value</td>
<td>9.73±0.15</td>
<td>9.05±0.12</td>
<td>8.13±0.10</td>
<td>6.85±0.11</td>
<td>6.18±0.12</td>
</tr>
</tbody>
</table>

*Color parameters lightness(L*), redness (a*) and yellowness (b*) Values are given as mean ± S.D. from triplicate determinations. Control (chicken samples soaked in distilled water), T1 (chicken samples soaked in 0.1% MEO solution), T2 (chicken samples soaked in 0.2% MEO solution). *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 5 also indicated that, over storage time, a* values of all chicken samples decreased, indicating that samples were becoming less red or brown due to metmyoglobin formation. Lightness (L*) values steadily decreased as expected to day 12. The yellowness (b*) values followed similar trends decreasing to day 12. The control sample had a decremental trend higher than treated sample. 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 slope 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 were observed by Chouliara et al., (2007) and Keokamnerd et al., (2008) for chicken samples treated with natural EOs.

Conclusion:

Color changes, lipid oxidation and microbial contamination are serious concern for meat producers and consumers. The application of marjoram essential oil dipping treatments showed potential in enhancing the color, lipid stability and microbial status of raw chicken drumsticks during refrigerated storage. The most significant benefit of applying essential oils to stored chicken products was to minimize undesirable sensory changes and the growth of Enterobacteriaceae bacteria (by 1.09-1.38 log cycle), which is a very desirable quality since this group contains a large number of pathogens. Sensory acceptability was extended by 2-3 and 5-6 days in T1 and T2-treated drumsticks; respectively as compared to control samples. The results of this study support many recommendations for using natural herbs in preserving poultry meat. Due to concerns regarding the safety and toxicity of synthetic antioxidants, marjoram essential oil may prove useful as safe, natural, functional ingredients to the meat industry.

References


