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Combined effects of thymol, carvacrol and packaging on the shelf-life of marinated chicken

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A B S T R A C T

The demand for marinated chicken worldwide, is continuously growing. To date, limited data on addition of active components of Essential Oils (EOs) to marinades for chicken preservation are available. The antimicrobial effect of carvacrol and thymol, added at 0.4 and 0.8% v/w to marinated fresh chicken, stored in air and under vacuum packaging (VP), for 21 days at 4 °C, was examined. The samples were monitored for microbiological (total viable count (TVC), lactic acid bacteria (LAB), *Brochothrix thermosphacta*, *Pseudomonas* spp., total coliforms, *Escherichia coli*, yeasts and molds) and sensory attributes (odor characteristics). Our data supports that among the tested microorganisms, *Pseudomonas* spp., LAB and *B. thermosphacta* were the most dominant microbiota in the marinated chicken samples. Additionally, the use of active EOs components, especially the higher concentration (0.8% v/w) in combination with VP, retarded the growth of spoilage microbiota and resulted in a significant reduction of about 2.9–3.1 log cfu/g and a microbiological shelf-life extension of marinated chicken by > 6 days, as judged by TVC data. Interestingly, the combination of active components of EOs at the lower concentration (0.4% v/w) and packaging (air or vacuum) resulted in a significant sensorial shelf-life extension of 15 and > 21 days, as compared to the controls' shelf-life of 9 days. The results of our study demonstrated the potential of the active components, carvacrol and thymol, as natural effective antimicrobial hurdles to control the growth of spoilage microorganisms in marinated chicken meat.

1. Introduction

It is well established that poultry meat has a short shelf-life, and this is due to microbial activity that can be manifested as visible growth, textural changes, off odors leading to significant economic losses for the poultry industry. Meat industries are looking for effective, natural preservation methods that provide meat, including fresh poultry and its products an extended shelf-life and at the same time satisfying consumers' demands for high quality, convenience and overall safety.

Over the last years, various studies have focused on use of EOs in foods in order to either extend the shelf-life or improve the food safety of these products (Martínez-Graciá et al., 2015; Sharma et al., 2017). While a number of studies have reported promising data regarding the application of EOs as natural antimicrobial agents, much less data have been reported on the use of active components of EOs (i.e. carvacrol, thymol or combinations of those) in extending the shelf-life of perishable foods (fresh poultry, fish etc.). In one study, the combined use of thymol, carvacrol, and temperature of storage on the quality of non-conventional poultry patties was evaluated (Mastromatteo et al., 2009). The meat patties mixed with the active EOs components and stored at low temperature (0–3 °C) showed a reduction of the cell load of about 1–1.5 log cfu/g.

Another possible option for the incorporation of the active components of EOs is through meat marination. Marinating is a process of incorporating or immersing meat in a liquid mixture, often called a marinade. A marinade may contain ingredients added to improve the quality, flavor, texture, and other sensory attributes, such as color or juiciness and appearance of the meat product. This includes salt, organic acids, water, seasoning, spices, sugar, binders, antimicrobial agents and aroma enhancers (Yusop et al., 2010). In general, the use of salt and acid in marinade formulation is important to achieve the mentioned quality characteristics.

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The demand for marinated chicken in Europe, is continuously growing (Yusop et al., 2012a, 2012b) and marinated meat is becoming a popular kind of ready to cook meat product (Alvarado and Sams, 2004; Magdelaine et al., 2008).

Moreover, according to Pathania et al. (2010), the growing demand for convenience products and longer shelf-life has increased interest in the use of spices, salts and organic acids in marinades, in order to increase food security. According to Björkroth (2005), marinade sauces prevent the growth of spoilage microorganisms based on a low pH, high concentration of salts (NaCl), sorbates and benzoates, and various spices. Antibacterial activity of marinades and spices depends on several factors such as pH, temperature, and duration of storage.

To date, limited data on addition of active components of EOs to marinades for meat preservation, including poultry are available (Pathania et al., 2010). In recent studies, cinnamon, oregano and thyme EOs, were applied in a marinade for meat/fish products. The combination of the marinade and EOs at low concentrations showed potential in extending the microbial shelf-life of those products by slowing the growth of spoilage microorganisms (Van Haute et al., 2016). The preservative effect of EOs may be achieved by using lower concentrations in combination with other preservation technologies such as low temperature, modified atmosphere packaging and vacuum packaging (VP) (Mastromatteo et al., 2009; Skandamis and Nychas, 2001). VP has been used to improve the shelf-life of meat and poultry due to its effect in the exclusion of air and thus retarding the growth of aerobic bacteria, the major spoilage microorganisms (Narasimha Rao and Sachindra, 2002). Thus, the goal of this study was to determine the combined effects of thymol, carvacrol and vacuum packaging on the shelf -life of fresh marinated chicken meat at refrigerated temperature (4 °C).

2. Materials and methods

2.1. Raw material, marination, preparation of marinated chicken

Skinless chicken breast fillets were purchased from a local fresh meat market in Lebanon. Fillets were received free from visible blood splash or bruising and ranged in weight from 130 to 200 g and in pH from 5.4 to 5.6. Any remaining surface fat was physically removed after visual inspection. Chicken breast fillets were cut manually into cubes (ca. 10 cm^2 area or 10 g ± 1 g), using a knife that was washed and sterilized with alcohol and flaming between samples. Before marinating, all chicken samples were kept under refrigerated conditions (4 °C). A traditional Mediterranean marinade recipe was prepared using the following ingredients: tomato paste (9.2% w/w), yogurt (4.6% v/w), vinegar (5.7%) v/v, oil (4.6% v/v), cornstarch (3.4% w/w), white pepper spices (1.9% w/w) and garlic cloves (1.15% w/w). Chicken cubes were immersed in the entire marinade (in the proportion of 100 g chicken to 40 g of marinade) and mixed together in a sterile glass tub under sterile conditions (Biosafety cabinet). The pH values of the marinade and marinated chicken were 3.5 and 4.4, respectively. The samples were covered with a lid and marinated under refrigeration for 24 h at 4 °C.

2.2. Active EOs components

Thymol (≥99%, CAS 89-83-8, Sigma Aldrich, Darmstadt, Germany) and carvacrol (≥98%, CAS 499-75-2, Sigma Aldrich, Darmstadt, Germany) were added at equal amounts (50% of each) to prepare the final mixture of thymol and carvacrol. This mixture was subsequently added to yield concentrations of 0.4% (v/w) and 0.8% (v/w) of active EOs components in the marinated chicken samples (described below). Thymol and carvacrol were added using a micro pipette and mixed with the marinade, so as to distribute the mixture of active components uniformly. Concentrations of thymol and carvacrol used in our study were established after preliminary experiments, in which determination of microbiological (TVC), as well as sensory data (results not shown) showed their antimicrobial effectiveness and that EOs components had higher sensorial acceptability at the lower concentrations used.

2.3. Packaging of samples and addition of EOs active components

Samples (25 g) were stored both in the absence or presence of thymol and carvacrol, under aerobic or vacuum packaging conditions (Vacuum machine, Henkelman, Holand), in high density polyethylene bags (Suntec, Beirut, Lebanon). Six treatments of marinated chicken were used in this study, as following: MA (marinated chicken under aerobic conditions); MV (marinated chicken under vacuum packaging conditions) both served as controls; MAE1 (with thymol and carvacrol, added at 0.4% v/w), MAE2 (with thymol and carvacrol, added at 0.8% v/w), MVE1 (with thymol and carvacrol, added at 0.4% v/w), and MVE2 (with thymol and carvacrol, added at 0.8% v/w). Both untreated (MA, MV) or treated (MAE, MVE) samples were stored under refrigerated conditions at 4 °C for a period of 21 days. Sample preparation and handling procedures were carried out following good hygienic practices in order to reduce the risk of both contamination or cross-contamination.

2.4. Microbiological analysis

Samples (25 g) were transferred aseptically into individual stomacher bags (Interscience, Saint Nom la Breteche, France), containing 225 mL of sterile Buffered Peptone Water (BPW) solution (0.1% w/v) and homogenized in a stomacher (Lab Blender 400, Seward Medical, London, UK) for 60 s. For each sample, appropriate serial decimal dilutions were prepared in sterile BPW solution (0.1% w/v). The amount of 0.1 mL (surface plated technique) of the serial dilutions of the chicken homogenate samples was spread on the surface of agar plates. Total Viable Count (TVC) were determined using Plate Count Agar (PCA-D, Ref 51,072, Biomerieux, Craponne, France), after incubation for 3 days at 30 °C. Yeasts and molds were enumerated on Sabouraud Chloramphenicol agar (SAB CHL 2-D, Ref 51021, Biomerieux, Craponne, France), after incubation at 25 °C for 5 days. *Pseudomonas* spp. were determined using Pseudomonas Agar Base (PCB, Oxoid, Hampshire, UK) with CFC supplement (SR0103, Oxoid, Hampshire, UK) and incubated at 25 °C for 48 h. *Brochothrix thermosphacta* were enumerated on STAA Agar Base (CM 00881, Oxoid, Hampshire, UK) with STAA Selective Supplement (SR0151E, Oxoid, Hampshire, UK), after incubation at 22 °C for 48 h. For the enumeration of *Escherichia coli* and total coliforms, the RAPID *Escherichia coli* method (BIO-RAD 3555299, California, USA) was used at 37 °C for 24 h. Finally, Lactic Acid Bacteria (LAB) were determined on MRS agar (double-layered, pour plated) after incubation of plates for 3 days at 30 °C (CM 361, Oxoid, Hampshire, UK). After incubation, plates having 25–250 colonies (cfu) were counted and multiplied by the dilution factor to determine cfu/g of marinated chicken. All plates were examined visually for typical colony types and morphological characteristics associated with each growth medium. Microbiological data was transformed into logarithms of the number of colony-forming units (cfu/g).

2.5. Sensory analysis

Sensory analysis was carried out, as described by Mastromatteo et al. (2009), by ten untrained panelists from the staff and laboratory on each sampling day. The panelists were regular consumers of the marinated products. The odor of raw, marinated chicken was tested upon bag opening and panelists were asked to score the odor of untreated and treated chicken samples using a 1–3 intensity scale with 3 corresponding to the most liked sample and 1 corresponding to the least-liked sample. A score of 2 was considered the lowest limit of acceptability. Therefore, sensory scores < 2 demonstrated an unacceptable (off odor) chicken product (Mastromatteo et al., 2009).

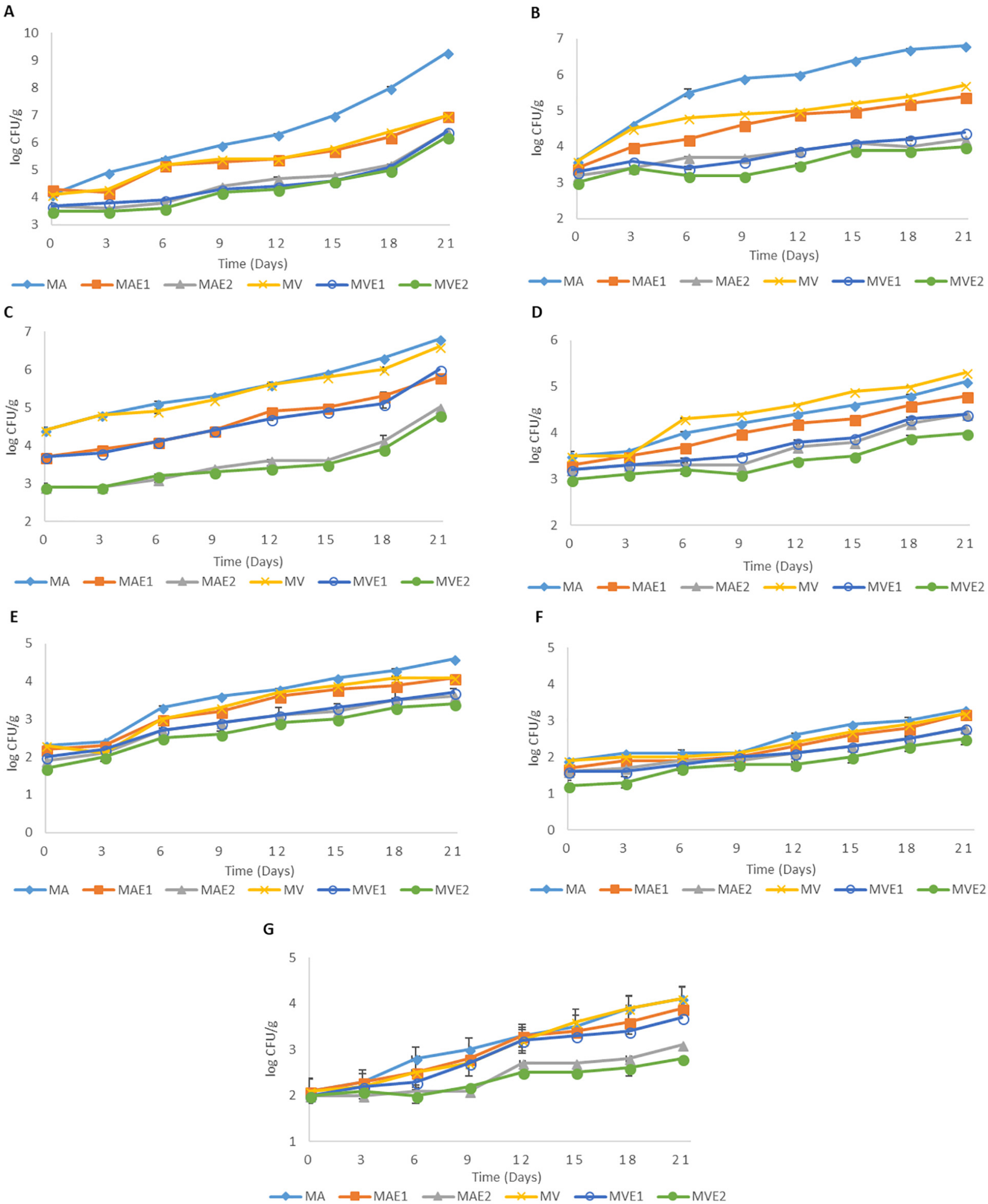

Fig. 1. Changes in (A) Total Viable Count, (B) *Pseudomonas* spp., (C) *Brochothrix thermosphacta*, (D) lactic acid bacteria, (E) total coliforms, (F) *Escherichia coli* and (G) yeasts and molds of marinated chicken during storage at 4 °C for 21 days. Treatments: marinated chicken stored in air (MA, -◆-); in air with active EOs added at 0.4% v/w (MAE1, -■-), in air with active EOs added at 0.8% v/w (MAE2, -▲-); under vacuum (MV, -X-); under vacuum with active EOs added at 0.4% v/w (MVE1, -○-), under vacuum with active EOs added at 0.8% v/w (MVE2, -●-). Data shown represent the mean of three samples taken from two replicate experiments (n = 3 × 2 = 6). Error bars show the standard deviation (SD).

2.6. Statistical analysis

The effects of active EOs concentration and packaging on the microbial populations, as well as, on sensory (odor) scores were analyzed using Statgrahics® plus version 5.1 (Statistical Graphics Corp., Rockville, MD, USA). For each of the different treatments, experiments were replicated twice (n = 2) and tests were run in triplicate for each replicate. Results were reported as mean values ± standard deviation (S.D.). Experimental data were subjected to one-way analysis of variance (ANOVA) to compare means. Pairwise comparisons were made with Fisher's least significant difference (LSD) test, to determine significant differences between means. Significance was defined at $P < 0.05$.

3. Results and discussion

3.1. Effect of active EOs and packaging on microbial changes in marinated chicken

TVC values for all the six different chicken meat treatments are given in Fig. 1A. The initial value of TVC (day 0) for the fresh marinated chicken meat was 4.1 log cfu/g, indicative of good quality chicken meat (Dawson et al., 1995). TVC reached a value of 7.0, considered as the upper microbiological limit for good quality fresh chicken meat on day 15 for MA. This limit was reached on day 21 for MV and MAE1 with a log reduction of 2.3, as compared to the control MA. Interestingly, the TVC limit value was not reached for the treated MAE2, MVE1 and MVE2 chicken samples during the entire period of storage. In addition, those three treatments resulted in significant ($P < 0.05$) reductions of 2.9–3.1 log cfu/g in the TVC (compared to the control MA), by the final day of storage (Fig. 1A).

The active components of the EOs tested at 0.4% and 0.8% (v/w) had an antimicrobial effect on the inhibition of TVC in fresh marinated chicken meat, especially with the highest concentration of active EO and when combined with VP. In other studies, the phenols thymol and carvacrol showed a high antimicrobial effectiveness and potential to be used as natural antimicrobials for the preservation of food (Aligiannis et al., 2001; Burt, 2004; Holley and Patel, 2005). In addition, the antimicrobial effect of oregano and thyme EOs, can be attributed to thymol and carvacrol, the major compounds found in these EOs (Burt, 2004). However, the synergy among EO components could contribute to the antimicrobial efficiency of an EO (Burt, 2004; Lambert et al., 2001; Periago et al., 2004).

Our results (TVC data) showed that significant extension of the microbiological shelf-life of marinated chicken by > 6 days can be achieved for the treatments MAE2, MVE1 and MVE2, as compared to the control's shelf-life (15 days). In other studies, the combination of thymol and carvacrol acted synergistically to give TVC log reductions of 1.0–1.5 in poultry patties stored at 0–3 °C (Mastromatteo et al., 2009). Recently it was reported that cassia EO resulted in the lowest microbial (TVC) count in vacuum packaged fresh chicken sausages (Sharma et al., 2017). Cassia EO is obtained from *Cinnamomum cassia*, and its active components such as cinnamaldehyde, are known to inhibit the development of spoilage and pathogenic organisms and this may be due to the presence at high percentage of phenolic compounds (Sharma et al., 2017). In another study, Van Haute et al. (2016) reported that a marinade (pH 4.5) composed of 10% NaCl, 2% lactic acid and low concentrations of cinnamon, oregano and thyme EOs, enabled the shelf-life extension of the meat/fish products. These authors did not however explain why the marinade itself (without the addition of the EOs) was not successful in inhibiting microbial growth in the meat/fish products.

As previously noted for TVC, MVE2, MAE2 and MVE1 were the most effective treatments against *Pseudomonas* spp., a strictly aerobic bacterial group involved in the spoilage of fresh meat, including chicken stored in air and under refrigeration (Fig. 1B). Significant reductions ($P < 0.05$) of 2.4–2.8 log cfu/g were noted among the above treatments as compared to the aerobically packaged, marinated chicken samples on day 21 (Fig. 1B). VP reduced the pseudomonads count due to the decrease in the amount of oxygen available. Moreover, essential oil addition was more effective than VP alone and the combination of factors exerted a synergetic effect on the reduction of pseudomonads counts. In other studies, the combination of thymol and carvacrol reduced the population of *Pseudomonas* spp. in poultry patties packaged in air and MAP at 0–3 °C (Mastromatteo et al., 2009). Recently, cassia oil incorporated into fresh chicken sausages delayed the growth of psychrophilic bacteria in the product throughout the storage period (Sharma et al., 2017).

It is well documented that when fresh meat or meat products are stored under vacuum/modified atmospheres or aerobic (to a lesser extent) packaging conditions, they may encourage the shifting of natural microbiota to mainly Gram positive species, such as facultative anaerobic species, e.g. *Brochothrix thermosphacta* and LAB. Our data supported this hypothesis and these species were found in marinated untreated (MA, MV) and treated (MAE1, MAE2, MVE1 and MVE2) chicken samples, stored in air or under vacuum (Fig. 1C and D). *B. thermosphacta* reached relatively significant high counts (6.6–6.8 logs) in the untreated marinated chicken (MV and MA) on final day, and our results were in agreement with those of Hernández-Macedo et al. (2011) that stated that *B. thermosphacta* is an important species playing a key role in the spoilage of vacuum packaged refrigerated meat. MAE2 and MVE2 treatments retarded the growth of *B. thermosphacta* in marinated chicken resulting in significantly lower ($P < 0.05$) counts compared to the untreated samples, with differences of 1.8–2.0 logs recorded on final day 21 (Fig. 1C). MAE1 and MVE1 showed lower log difference of 0.8–1.0 on the same day of storage. VP exerted no additional effect in retarding the growth of *B. thermosphacta* counts. LAB, facultative anaerobes and naturally present in the yogurt added to the marinade, constituted a substantial part of the natural microbiota of marinated chicken, both in the absence or presence of active EOs, and in aerobic and vacuum packaging. The highest final counts of 5.1–5.3 log cfu/g were reached in the untreated MV and MA and the lowest ones ($P < 0.05$) of 4 logs were in the MVE2 chicken samples (Fig. 1D). Lower LAB populations ($P < 0.05$) of 4.4 log cfu/g were also recorded in MVE1 and MAE2. In similar studies, chicken treated with thyme essential oil showed a remarkable inhibition of LAB growth (Fratianni et al., 2010). Zaika et al. (1983) demonstrated, as well, a reduction of 4 log cfu/g in LAB populations in pure culture, after adding oregano oil at a concentration of 4 g/L. Therefore, the microbial population reduction of *Brochothrix thermosphacta* and LAB can be related to the addition and concentration of the active EOs. The mode of action of carvacrol and thymol was described in several studies. Thymol and carvacrol have similar structures with the hydroxyl group at a different location on the phenolic ring. They interact with the lipid bilayer of cytoplasmic membranes, due to their hydrophobic nature, and can thus cause the leakage of cellular components (Martínez-Graciá et al., 2015; Mastromatteo et al., 2009).

It was found that the control marinated chicken products (MA, MV) and MAE1 had significantly higher total coliforms (4.1–4.6 log cfu/g) and *Escherichia coli* (3.2–3.3 log cfu/g) counts than the other treated samples on final storage day 21 (Fig. 1E and F). MVE2, MVE1 and MAE2 resulted in lower ($P < 0.05$) counts of 3.4–3.7 and 2.5–2.8 for total coliforms and *E. coli*, respectively. This may be due to the action of vacuum packaging and/or the active EOs present in the marinated chicken samples. Sharma et al. (2017) did not observe any coliforms up to day 15 in control (fresh chicken sausages) samples and up to day 30 of storage in holy basil oil, thyme oil and clove oil products. Coliform count was not at all observed in cassia oil incorporated fresh chicken sausages throughout the storage period. In another study, raw chicken meat microbiota was dominated by Enterobacteriaceae and spices extracts were used for shelf-life extension (Radha krishnan et al., 2014). Chicken samples treated with a combination of spice extracts showed lower counts ($P < 0.05$) when compared with the samples treated with individual extracts. In general, the antimicrobial activity of spice extracts is not fully understood, however, cell membrane disruption by phenolics or metal chelation by flavonoids could lead to the inhibition of the growth of microorganisms (Cowan, 1999). Finally, increasing amounts of thymol and carvacrol added to poultry patties stored in air and under MAP conditions decreased the final cell load of Enterobacteriaceae in these products (Mastromatteo et al., 2009).

In our study, yeasts and molds had the lowest contribution to the microbiota of the marinated chicken meat. They reached the highest population in the marinated products stored in air and under VP. Even a small percentage of oxygen under VP may account for yeasts presence and this may be the reason for their presence in the marinated product stored under VP. It is noteworthy that the treatments with the highest concentrations of active EOs (MVE2, MAE2), were the most effective in reducing significantly ($P < 0.05$) yeasts and molds numbers in the marinated chicken samples (Fig. 1G). Conner and Beuchat (1984) have reported the strong inhibitory action of oregano and thyme oils on growth of yeasts. Recently, Sharma et al. (2017) reported that yeast and mold count was below the limit of detection in vacuum packaged fresh chicken sausages treated with cassia EO, throughout the storage period.

Our data supports that among the tested microorganisms, *Pseudomonas* spp., LAB and *B. thermosphacta* were the most dominant microbiota in the marinated chicken samples. Additionally, the use of active EOs (carvacrol, thymol) especially the higher concentration (0.8% v/w) in combination with VP resulted in a microbiological shelf-life extension, as judged by TVC data and retarded the growth of spoilage microbiota in the marinated chicken.

Finally, it must be stated that the present results obtained on the effects of active EOs against the microbial parameters analyzed, cannot convey information on the mechanism of action of the compounds used. As reported in previous studies, phenolic compounds from different plant sources have been highly correlated with antimicrobial activity (Shan et al., 2007). The possible modes of action of the phenolic compounds may include degradation of the cell wall, disruption of the cytoplasmic membrane, loss of cell integrity, change in fatty acid and phospholipid constituents, alteration of the synthesis of DNA and RNA and destruction of protein translocation (Shan et al., 2007).

3.2. Effect of active EOs and packaging on sensory changes in marinated chicken

In this study, we assessed the sensorial quality of raw marinated chicken, by examining the odor, a commonly checked attribute by the consumers. Therefore, the odor of the uncooked marinated chicken product was evaluated by untrained panelists, to simulate the consumers' practices when buying the product from the retail shops or handling it at home.

The results of the sensory evaluation (odor attribute) of the (uncooked) marinated chicken samples untreated (MA, MV) and treated (MAE1, MAE2, MVE1 and MVE2) stored at 4° C are shown in Fig. 2. The sensory scores for both untreated (MA, MV) and treated (MAE1, MAE2, MVE2) chicken samples showed a similar pattern of decreasing ($P < 0.05$) acceptability with storage time, except for the treated (MVE1) samples that did not show significant differences ($P > 0.05$) after day 3 of storage (Fig. 2). Based on odor scores, and as judged by the panelists, untreated (MA, MV) chicken samples were acceptable during the first 9 days of storage and were rejected after that day, dropping below the sensorial limit value of 2 (Fig. 2). The treated chicken samples, MAE2 and MVE2, reached the acceptable limit value on day 2. Significantly lower ($P < 0.05$) scores of the treated marinated chicken with the higher active EOs concentration, might be due to the intense aroma imparted by carvacrol or thymol to the product. Interestingly, MAE1 chicken samples were acceptable based on odor scores until day 15, whereas, MVE1 samples received the highest sensory scores of all treatments, remaining acceptable even after day 21 of storage (final). Based on the odor score data, a shelf-life of 2; 9; 15 and > 21 days for MAE2, MVE2; MA, MV; MAE1 and MVE1 marinated chicken, respectively, was obtained. These results demonstrated that the best sustainability was found for the groups of marinated chicken samples treated with the lowest concentration of active EOs (MAE1, MVE1) either stored in air or under vacuum. Both carvacrol and thymol active EOs added to the marinated chicken samples produced higher scores than other treatments on days 15 (MAE1) and 21 (MVE1) of storage period. Therefore, the use of the lower active EOs concentration and their combination, together with low temperature storage, either under aerobic or vacuum packaging conditions resulted in a shelf-life extension of 6 and > 12 days for the marinated chicken samples under MAE1 and MVE1 treatments, respectively.

The types and quantities of EOs used for food preservation are limited by the sensory acceptability of the final product.

In this study, the combination of active EOs added at the lower concentration (0.4%) to marinated chicken resulted in a product with a well-perceived odor. Similarly, in a related study, poultry patties with added active EOs (thymol, carvacrol) had a distinctive and pleasant flavor with respect to the control samples (Mastromatteo et al., 2009), whereas higher sensory scores in vacuum packaged fresh chicken sausages treated with EOs (cassia oil, holy basil) were obtained (Sharma et al., 2017). High concentrations of EOs added to foods, including marinated chicken, irrespective of packaging, could lead to a non-desirable food or chicken product, suggesting that when EOs are applied in food formulations, their sensorial impact should be carefully considered in terms of the amount of the EO that can be applied. In other studies, the use of *Thyme vulgaris* EO on meat, was acceptable concerning odor and taste attribute, added in the range of 0.2–0.6% v/w (Giatrakou et al., 2010; Solomakos et al., 2008) but unacceptable at 0.9% v/w on minced beef (Solomakos et al., 2008). The current challenge with the use of EOs is to reduce their amount without compromising their antimicrobial effectiveness in food formulations. One possible strategy would be to use the active components of an EO rather than the EO itself. Thus, the application of carvacrol or thymol instead of oregano or thyme EO, is worth investigating.

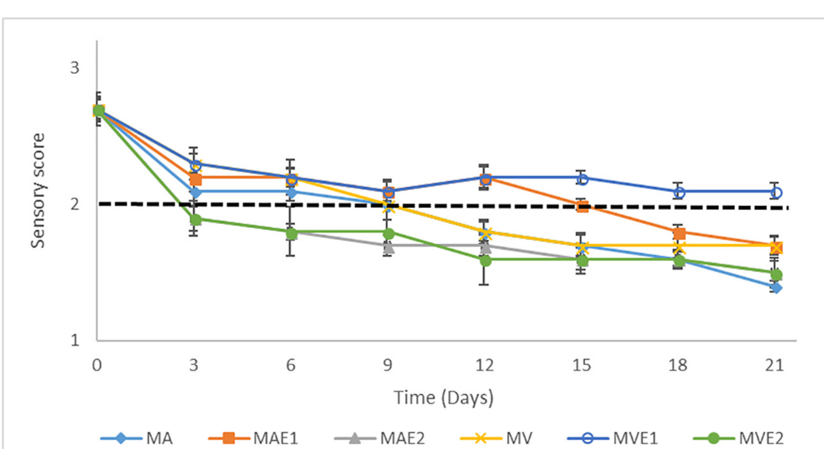

Fig. 2. Changes in sensory (odor) in uncooked marinated chicken stored in air (MA, -◆-); in air with active EOs added at 0.4% v/w (MAE1, -■-), in air with active EOs added at 0.8% v/w (MAE2, -▲-); under vacuum (MV, -X-); under vacuum with active EOs added at 0.4% v/w (MVE1, -○-), under vacuum with active EOs added at 0.8% v/w (MVE2, -●-) at 4 °C for 21 days. Line (—) represents the acceptable limit. Error bars show the standard deviation (SD).

4. Conclusions

This work permitted to study the effects of thymol, carvacrol and vacuum packaging on the shelf -life of fresh marinated chicken meat at refrigerated temperature (4 °C). The results showed that active compounds (carvacrol and thymol) of EOs combined with either air or vacuum packaging could be used to monitor the growth of the natural microbiota of the marinated chicken for 21 days. Active components of the oregano and thyme EOs, added to marinated chicken, proved effective antimicrobial hurdles over the microbiota of the poultry product. However, more studies are needed to support our findings and evaluate the effectiveness of the used treatments on different meat matrices. This can additionally include using different concentrations and combinations of active EOs and packaging conditions (such as modified atmosphere packaging and active packaging technology). Further studies should also be oriented on testing specific pathogens affecting the safety of chicken meat. On the industrial level, different options are possible and will be decided taking into consideration the desired shelf-life extension, the cost of each treatment, the handling issues, consumers' preferences, sensory aspects, and other similar practical issues.

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