

Article

Improving the Quality of Turkey Meat via Storage Temperature, Packaging Atmosphere, and Oregano (*Origanum vulgare*) Essential Oil Addition

Samir A. Mahgoub ¹, Mohamed E. Abd El-Hack ², Zohair S. Mulla ³, Waleed R. El-Ghareeb ³, Ayman E. Taha ⁴, Muath Q. Al-Ghadi ⁵, Ahmad R. Alhimaidi ⁵, Ramzi A. Amran ⁵, Bader Almutairi ⁵, Vincenzo Tufarelli ^{6,*} and Ayman A. Swelum ^{7,8,*}

- ¹ Department of Agricultural Microbiology, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt; mahgoubsamir@gmail.com
- ² Department of Poultry, Faculty of Agriculture, Zagazig University, Zagazig 44111, Egypt; dr.mohamed.e.abdalhaq@gmail.com
- ³ Department of Public Health, College of Veterinary Medicine, King Faisal University, Hofuf 31982, Saudi Arabia; zmulla@kfu.edu.sa (Z.S.M.); welsaid@kfu.edu.sa (W.R.E.-G.)
- ⁴ Department of Animal Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine, Alexandria University, Edfina 22758, Egypt; Ayman.Taha@alexu.edu.eg
- ⁵ Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia; malghadi@ksu.edu.sa (M.Q.A.-G.); ahimaidi@ksu.edu.sa (A.R.A.); ramran@ksu.edu.sa (R.A.A.); bomotairi@ksu.edu.sa (B.A.)
- ⁶ Department of DETO, Section of Veterinary Science and Animal Production, University of Bari 'Aldo Moro', Valenzano, 70010 Bari, Italy
- ⁷ Department of Animal Production, College of Food and Agriculture Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia
- ⁸ Department of Theriogenology, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt
- * Correspondence: vincenzo.tufarelli@uniba.it (V.T.); aswelum@ksu.edu.sa (A.A.S.)

Received: 24 July 2020; Accepted: 21 September 2020; Published: 9 October 2020



Abstract: The provision of plentiful good-quality food is a primary issue in the modern world. This work was planned to study the influence of packaging atmosphere and oregano (Origanum vulgare) essential oil addition [(vacuum packaging: T1 or modified atmosphere packaging or T2 ($CO_2/N_2 =$ 4:6) or T3, T2 with oregano essential oil (T2 + EO)] under various storage temperatures (0, 5, 10, and 15 °C) on the control of survival of Escherichia coli O157:H7 and associated spoilage flora in sliced smoked turkey meat. The pathogen increased by only <1.0 log colony-forming unit (CFU)/g under all packaging and temperature combinations. Moreover, T1, T2, and T3 exerted practically similar inhibitory activity against the pathogen and dominating bacteria, with a relatively low growth of E. coli O157:H7 in sliced smoked turkey during the shelf life under all storage regimes compared to the control. However, the pathogen survival was highest on the sliced smoked turkey under T1, decreasing by only 0.67, 0.74, 0.63, and 1.30 log CFU/g within 37 days if kept at 0, 5, 10, and 15 °C, respectively. Under T2 and the same condition, E. coli O157:H7 in the product declined by only 0.31, 0.50, 0.72, and 1.10 log CFU/g within 37 days of storage, respectively. In the T3 samples, the pathogen was reduced by only 0.33, 0.67, 1.72, and 3.46 log CFU/g through 37 days of storage, respectively. Under T3 were E. coli O157:H7 populations in smoked turkey eliminated (negative by enrichment) under all conditions (after 129, 95, 95, and 43 days maintained at 0, 5, 10, and 15 °C, respectively) compared with other packaging temperature combinations. Thus, T3 contributed to developing ready-to-eat smoked turkey with enhanced product quality and eliminating the pathogen.

Keywords: Escherichia coli O157:H7; smoked turkey meat; oregano; packaging



1. Introduction

Consuming ready-to-eat (RTE) meat products is related to commonly recognized risk factors for infection by foodborne microorganisms. The antimicrobial packaging (AP) is a kind of active packaging, which minimizes the microorganisms' growth in the packaged food via placing antimicrobial agents into the packaging. It is a useful tool to control the development of foodborne pathogens as well as spoilage bacteria in RTE products [1]. The application of AP is related to food safety and shelf life extension. The AP systems include materials of packaging, these systems can inhibit microorganisms, which cause foodborne illness [2–4]. The reference of legal requirements of smoked turkey ham is found on this website: https://www.ams.usda.gov/sites/default/files/media/Smoked%20Turkey% 20Ham%20March%202015.pdf. A major synergistic effect of high hydrostatic pressure, liquid smoke, and freezing to eliminate Listeria monocytogenes in smoked and raw trout reached a 5 or 2 log CFU/g reduction, respectively. Moreover, high injury levels of more than 5-log CFU/g among treatments reach up to 56% [5]. Vacuum or modified atmosphere packaging (MAP) could use to decrease food deterioration. Preventing oxidation is based on eliminating exposure of food products to oxygen by removing oxygen from the packaging headspace and/or adding antioxidants to films and coatings (i.e., oregano oil) to enhance the antioxidant properties of food surfaces. Thus, using the proper packaging materials with different environmental conditions and for different food products is important to avoid oxygen [6]. In response to the World Trade Centre tragedy in 2001, food technology experts suggested a revision of package designs and systems of packaging to enhance food security and safety. Therefore, many applications of AP will be developed on a commercial scale for the enhancement of safety and security of food products. Innovative technological developments have been made using AP by the combination among the MAP and natural antimicrobial agents. *Escherichia coli* O157:H7 (EHEC) has recognized firstly in 1982 as a human pathogen [7]. Since this time, the EHEC was and is still considered as one of the most severe pathogens [8,9]. Additionally, O157 and non-O157 Shiga toxin-producing *E. coli* strains were reported for their antimicrobial resistance [10].

In several countries, this serotype was recognized as a principal reason for hemorrhagic colitis, and, in severe cases, death. It was documented that these pathogens resulted in about 75,000 cases of diarrhea and many hundred annual death cases in the United States alone [11,12]. Shiga toxin-producing *E. coli* shared in about 265,000 annual foodborne cases in the United States [13]. In addition, antibacterial drug resistance among *E. coli* O157:H7 has been evaluated by Amézquita-López et al. [14] and Mora et al. [15]. People of all ages can be stricken with the diarrhea due to the apparent low infective dose of <100 cells and the severity of these illnesses [16]. Below 7 °C, the EHEC does not multiply. This is the endpoint accepted by the EU as a suitable temperature for preventing the growth of mesophilic pathogens [17]. The conditions at both wholesale and retail level can help numbers to multiply due to temperature fluctuation or temperature abuse [18]. In a previous study, it was assessed that the EHEC could survive on ready-to-cook barbecued chicken at refrigerator conditions (3 °C) and significantly increased at higher temperatures like 8 to 20 °C as reported by Shekarforoush et al. [19].

In the reduced oxygen packaging, there were extra concerns. These factors are the impact of competitive spoilage flora as well as the extended storage life on the growth and survival of EHEC [20]. Barrera et al. [21] examined the impact of various storage conditions on EHEC growth and the indigenous microflora on the meat of lambs. The authors showed that the behavior of the EHEC in air packaging demonstrated that the control of effective temperature is essential to guarantee that there is no growth of EHEC on mutton, regardless of rapid increase in the spoilage microorganisms. Regardless of the population of lactic acid bacteria (LAB) and temperatures fluctuating, the cold storage in modified atmosphere packaging (MAP) or vacuum packaging (VP) reduced the growth of EHEC compared to AP products. A sizeable number of background bacteria existing in ground meat caused an inhabitation in EHEC growth, either anaerobically or aerobically. The inhibition was clearer under the anaerobic conditions [22]. These findings suggested the main role of natural background flora in meat for inhibiting EHEC growth. Choosing the packaging system is one of the key factors to achieve ideal modifications in the atmosphere as well as avoiding the low O₂ levels or

high CO₂ levels, which can alter the anaerobic metabolism leading to a generation of an off-flavor or the hazard of the proliferation of anaerobic microorganisms. The main component of oregano essential oil (OEO) was carvacrol (65.1%), followed by p-cymene (12.0%), γ-terpinene (6.8%), and thymol (3.4%), confirming results of Silva et al. [23]. However, Souza et al. [24] investigated that the inhibitory effects of the essential oil from Origanum vulgare L. and showed that the major components of the samples were carvacrol (69.0%), thymol (14.12%), γ -terpinene (3.71%), and p-cymene (3.67%). Furthermore, Siroli et al. [25] report that the scientific literature evidences p-cymene, carvacrol, thymol, and γ -terpinene as the main components present in oregano. Carvacrol is usually reported as the major component of the oregano essential oil. It is a hydrophobic phenolic compound, with well-documented antimicrobial activity against bacteria, fungi, and yeasts [26] and antioxidant activity [27], showing a high potential to promote the extension of the shelf life and safety of food products [28]. There are many studies on the effect of essential oils and packaging in different food stuff [1,5,6]. However, there are a few studies on the survival of *E. coli* on sliced smoked turkey meat and all studies on the frequency and spread the pathogens in meat [14,15]. Therefore, the innovative character in this study is that in situ studies are still scarce and they need more studies in situ concerning the behavior of pathogen in RTE meat under different regimes and conditions. Especially, there are a modern methods and alternatives ways for preserving food stuff.

The vacuum packaging: T1, modified atmosphere packaging: T2 ($CO_2/N_2 = 4:6$), and T2 with oregano essential oil: T3 could be a viable method to control foodborne pathogens in low-salt ready-to-eat meat during maintaining, transporting, and consumption, especially at different temperatures. However, ready-to-eat meat (smoked turkey) could be contaminated during keeping at different temperatures. Use a combining T2 technology with oregano essential oil for controlling the survival of *E. coli* O157:H7 in RTE food could be a new technology to enhance the meat quality and microbiological shelf life. Therefore, the objectives were as follows: (1) to investigate the effect of T2 combined with EO to control *E. coli* O157:H7 in ready-to-eat meat; (2) to compare the influence of the packaging atmosphere T1, T2, and T2 with oregano essential oil (T3) under various storage temperatures on controlling the EHEC and spoilage flora on low-salt smoked turkey meat.

2. Materials and Methods

2.1. Procedures for Sampling

2.1.1. Preparation of Inoculum

E. coli O157: H7 NCTC 13, 125 was kept in broth at a temperature of -80 °C until its use. Before utilizing, the cultures were tested for viability by re-culturing in a fresh medium of tryptic soy broth (Difco Laboratories) at 37 °C for 24 h. Cultured cells were gathered using the centrifugation process (20,000× g for 10 min at 4 ± 0.1 °C), cleaned with Ringer's solution for three times, and re-suspended in Ringer's solution (Lab M). Cultured cell numbers are estimated by direct plating on Tryptone Bile X-Glucuronide Agar (TBX, Lab M's, HAL003) in duplicate. Further, colonies were counted 24 h post incubation at 37 °C. The last inoculation was done by serially diluting in Ringer's solution depending upon selected cell numbers and the count was between 1.0 to 1.5 × 10⁸ CFU/mL.

2.1.2. Experimental Design

Three treatments [(vacuum packaging: T1, modified atmosphere packaging; T2 (CO₂/N₂ = 4:6); and T3, T2 with oregano essential oil (T2 + EO)] under various storage temperatures (0, 5, 10, and 15 °C) on the control of survival of *Escherichia coli* O157:H7 and associated spoilage flora in smoked turkey meat. The essential oregano oil was purchased from Sigma-Aldrich (Sigma chemical Co., St. Louis, MO, USA). The study was planned as complete randomized design having three treatments; each treatment had 40 animals allotted to four replicates each of 10 animals. Sliced smoked turkey samples were 120 samples/treatment (5 samples/replicate). A 0.1 mL of 6 log CFU/mL of *E. coli* O157:

H7 NCTC 13, 125 was used to inoculate the sliced smoked turkey products, so that the total cell counts on smoked turkey samples were ca. 5 log CFU/g. Then, T1 smoked turkey samples were placed in a plastic pouch. After that, control treatment (without inoculation) or inoculated samples with the pathogen were repackaged with T1, T2 ($CO_2/N_2 = 4:6$), or T3 ($CO_2/N_2 = 4:6$) in a mixture with oregano essential oil (OEO; 2%). The samples were divided into two groups: packs repackaged with T2 ($CO_2/N_2 = 4:6$) as a control, and packs repackaged with T3 ($CO_2/N_2 = 4:6$) with essential oil of 0.8% v/w). Each pouch contained two 20 g slices, 0.8 cm thick. The samples were treated with OEO as following: Whatman paper no. 6 was cut into 2×2 cm square pieces and 0.8 mL of essential oil was added to each piece. Excesses of essential oil were allowed to drain for 30 s from each piece, and then they were placed into the plastic pouches without touching the meat slices. Samples were placed into separate plastic pouches, complemented or not with the OEO. The packaging was performed using pouches size of 30×20 cm, with oxygen permeability of 1.7 cm³ for m⁻² 24 h⁻¹ at 23 °C and 75% relative humidity. Each pouch was emptied and washed in three changes before filling. Post samples filling, the pouches were heat-sealed twice. The whole vacuum packaging process was done using Henco Vac Machine. After repackaging, the samples were separated into four groups and stored at 0, 5, 10, or 15 °C until microbial and physicochemical analysis. On day 0, three smoked turkey pouches, after repackaging with T1, T2 ($CO_{2/}N_2 = 4:6$), or T2 with essential oil (0.8% v/w) (T3), were randomly selected for microbiological analysis and E. coli O157:H7 enumeration within five months, and pH and redox potential measurements. During storage, three pouches from each temperature (0, 5, 10, and 15 °C) were randomly selected for pH, redox potential, and microbiological analysis. At the end of experiments, each pouch was weighted for verification.

2.1.3. Microbiological Analysis

For microbiological analyses, meat samples weighing 25 g were moved and placed to sterilized stomacher bags (Sewared, London, UK); 225 mL of sterile Ringer's solution (Lab 100 Z) was added, homogenized for 1 min using a stomacher machine (Lab. Blender 400; Seward Medical, London, UK) at ambient temperature. The serial dilution volume in Ringer's solution was prepared and duplicate 1 mL or 0.1 mL samples of proper dilutions were spread on selective or non-selective media agar plates.

Determinations were performed such as the total mesophilic bacteria (TMB) on Plate Count Agar (PCA; Merck, 1.05463) incubated for 72 h at 25 °C; *E. coli* on Tryptone Bile X-Glucuronide agar (TBX, LAB) incubated for 24 h at 37 °C; and lactic acid bacteria (LAB) on de Man, Rogosa, Sharpe (MRS Biolife) overlain with the same medium (5 mL) and incubated for 72 h at 25 °C. For experimental purposes, under the detection limit of these techniques were 2 log CFU/g except for LAB for which the limit was 1 CFU/g. The bacterial populations shown are the mean of three replicates and transferred to log_{10} CFU/g.

2.1.4. E. coli O157: H7 Enrichment Technique

Samples were analyzed via the protocol described in ISO 16654. In summary, samples weighing 25 g were added to buffered peptone water (BPW; 225 mL) (Merck) and then incubated for 24 h at 37 °C. Post incubation, 0.1 mL of each BPW inoculum was transferred onto Tryptone Bile Glucuronide (TBX) agar and/or the culture was then plated on TBX agar, incubated for 4 h at 37 °C, and then directly incubated for 37 h at 44 °C.

2.1.5. Measurement of pH and Eh Values

For each sampling period, the values of pH and redox potential (Eh) of products were recorded. In brief, 25 g of the sliced turkey samples were mixed with sterile Ringer's solution (225 mL) for 1 min. Redox model 827 pH LAB (Metrohm) a pH meter (model RL150) have been used for the determination. The redox values were converted reported as redox potential (mV).

2.1.6. Carbon Dioxide and Oxygen Measurements

Carbon dioxide and oxygen values in the T2 and T3 were measured using a PBI Dansensor (Checkmate 9900 O_2/CO_2).

2.1.7. Statistical Analysis

Data from microbiological analyses were uploaded into Microsoft Excel software and converted into log CFU/g for all experiments. Analysis of variance (ANOVA) was used to estimate the significant variation (p < 0.05) in bacterial numeration and changes in pH value through the storage of smoked turkey slices at various temperature degrees. Logarithmic means were divided using SPSS 10.0 for WindowsTM via Tukey's multiple range tests.

3. Results

3.1. Effect of Packaging Atmosphere, OEO, and Storage Temperatures on Growth/Survival of E. coli O157:H7 on Ready-to-Eat Smoked Turkey

Our aim was to study the impact of vacuum packaging: T1, modified atmosphere packaging: T2 ($CO_2/N_2 = 4:6$), and T2 with essential oil: T3 on growth and survival of *E. coli* O157:H7 in RTE slices of smoked turkey stored at 0, 5, 10, and 15 °C. The sliced smoked turkey was inoculated with *E. coli* O157:H7 at ca. 4.78, 5.67, and 5.76 log CFU/g and packaged in T1, T2, and T3, respectively. At the end of the experiments, each pouch was weighted for verification that the weight of each sample had not changed from the beginning until the end of shelf life. The associated flora, physicochemical, and T2 and T3 gas composition were measured throughout the period of storage. *E. coli* O157:H7 have checked the recovery rate by replating and culturing on Tryptone Bile Glucuronide Agar (TBX).

3.2. E. coli O157:H7 in Ready-to-Eat Smoked Turkey

Investigation of control sliced smoked turkey (without artificially inoculated of the pathogen) demonstrated that there is no *E. coli* O157:H7 was found during storage for 96 days at any temperature, which showed that *E. coli* O157:H7 was not initially found in the smoked turkey utilized.

The initial number of *E. coli* O157:H7 on artificially inoculated smoked turkey samples were ca. 5 log CFU/g. This count was decreased significantly (p < 0.05) by one log CFU/g under T1, T2, and T3 after (51, 129, and 43 days and 10, 6, and 5 days) of storage at 0 and 15 °C, respectively. Regardless of the rapid proliferation of the background microflora on the smoked turkey, the pathogen increased by only <1 log CFU/g under all packaging and temperature combinations (Figures 1–3). Moreover, T1, T2, and T3 exerted practically similar inhibitory activity against the pathogen and dominating bacteria, with a relatively low growth of *E. coli* O157:H7 in smoked turkey products during the shelf life under all storage regimes (Figures 1–3). However, the pathogen survival was highest on the sliced smoked turkey under T1, decreasing by only 0.60, 0.77, 0.86, and 0.84 log CFU/g within 44, 59, 51, and 27 days at 0, 5, 10, and 15 °C, respectively (Figure 1). Under T2 and the same storage temperatures, E. coli O157:H7 in the product declined by only 0.50, 0.76, 0.77, and 0.97 log CFU/g within 95, 95, 59, and 17 days of storage, respectively (Figure 2). In the T3 samples, the pathogen was reduced by only 0.87, 0.82, 0.57, and 0.91 log CFU/g through 72, 43, 17, and 14 days of storage, respectively (Figure 3). Only under T3 were E. coli O157:H7 populations in smoked turkey eliminated (negative by enrichment) under all conditions (after 129, 95, 95, and 43 days at 0, 5, 10, and 15 °C, respectively) compared with other packaging temperature combinations; however, T2 did eliminate the pathogen at 15 °C after 95 days of storage.

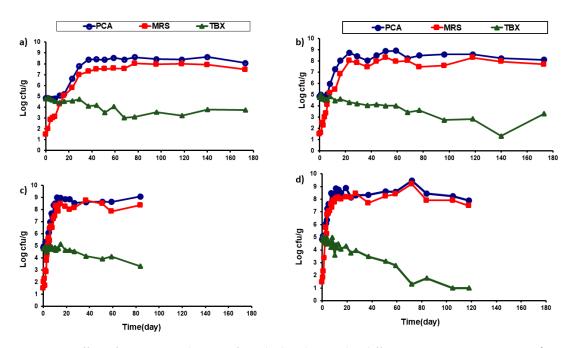


Figure 1. Effect of vacuum packaging of smoked turkey under different storage temperatures 0 °C (**a**), 5 °C (**b**), 10 °C (**c**), and 15 °C (**d**) on the growth of *E. coli* (TBX), total mesophilic bacteria (PCA), and lactic acid bacteria (MRS).

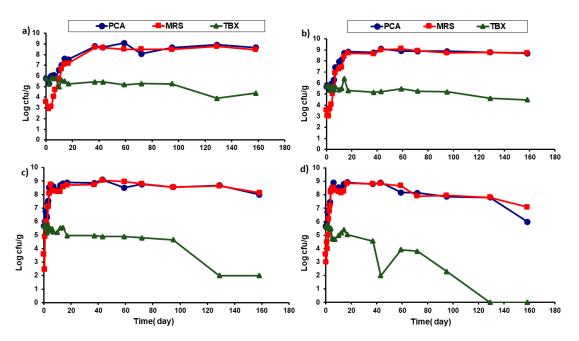


Figure 2. Effect of modified atmospheric packaging (CO₂/N₂ = 4:6) of smoked turkey under different storage temperature 0 °C (**a**), 5 °C (**b**), 10 °C (**c**), and 15 °C (**d**) on the growth of *E. coli* (TBX), total mesophilic bacteria (PCA), and lactic acid bacteria (MRS).

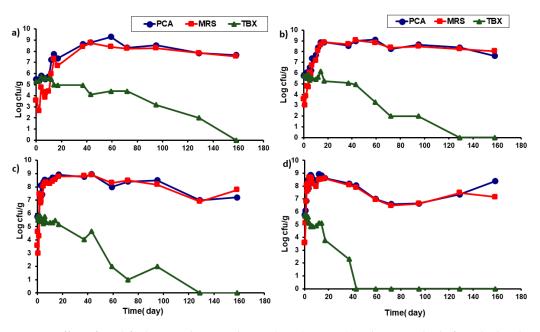


Figure 3. Effect of modified atmospheric packaging(CO₂/N₂ = 4:6) with essential oil of smoked turkey under different storage temperature 0 °C (**a**), 5 °C (**b**), 10 °C (**c**), and 15 °C (**d**) on the growth of *E. coli* (TBX), total mesophilic bacteria (PCA), and lactic acid bacteria (MRS).

3.3. The Development of Endogenous Flora in Packaging Smoked Turkey During Storage at Different Temperature Regimes

Changes in TMB and LAB counts in the control (without inoculation of the pathogen) and treated samples (inoculated with E. coli O157:H7) VP were recorded during storage regimes. Our results indicated that the TMB and LAB count increased in T1 smoked turkey with the length of storage, regardless of the storage temperature (Figure 1). In the control experiment, the initial TMB levels were 2 log CFU/g. The TMB was elevated to more than 7 log CFU/g in the meat at 0, 5, 10, and 15 °C at the days 29, 23, 8, and 5, respectively. The LAB increased steadily from 1.3 log CFU/g to the spoilage limit (>7 log CFU/g) at 51, 23, 11, and 7 days under 0, 5, 10, and 15 °C, respectively, in all samples. However, the samples treated with E. coli O157:H7, the level of TMB in the smoked samples was similar to the levels of the pathogen populations of ca. 5 log CFU/g. The TMB in smoked samples has reached the spoilage limit (>7 log CFU/g) after at 0, 5, 10, and 15 °C at 23, 8, 6, and 4 days, respectively. While the LAB in smoked turkey samples reached the spoilage limit after 29, 16, 8, and 5 days of storage under similar conditions (Figure 1). The impacts of T2 temperature combinations on the growth of TMB and LAB populations in the smoked turkey stored at 0, 5, 10, and 15°C was similar to those under T2 with essential oil. At 0 °C, TMB (5.77 log CFU/g) was identical to the levels of E. coli O157:H7 (5.67 log CFU/g) under all the storage regimes (Figure 2). The TMB and LAB counts in smoked turkey reached higher than 7 log CFU/g after 12 days of storage at 0 °C, six days of storage at 5 °C, two days of storage at 10 °C, and after only one day of storage at 15 °C. Our results indicated that the combination of essential oil with T2 did not delay the growth of LAB compared to T2 alone under the same temperature (Figure 3).

3.4. pH and Redox Values in Smoked Turkey

The initial pH of smoked turkey was 6.32, 6.07–6.09, and 6.16–6.20 for the samples packaged with T1, T2, and T3, respectively. A slight decrease in the pH values was observed in smoked turkey samples in all cases. At the end of experiments, a high drop in the pH levels had occurred under all storage temperature and packaging systems (Figures 4–6). On day 0, the redox potential of smoked turkey was 267.11, 249.11–255.11, and 252.11–254.11 for the samples packaged with T1, T2, and T3, respectively. A slight reduction in the redox values was noted in all smoked turkey meat samples

through the shelf life. By the end of storage, redox levels had significantly decreased at all storage temperatures and packaging conditions.

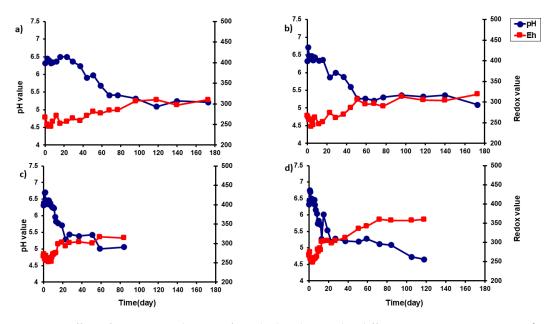


Figure 4. Effect of vacuum packaging of smoked turkey under different storage temperature 0 °C (**a**), 5 °C (**b**), 10 °C (**c**), and 15 °C (**d**) on pH value and redox value (Eh).

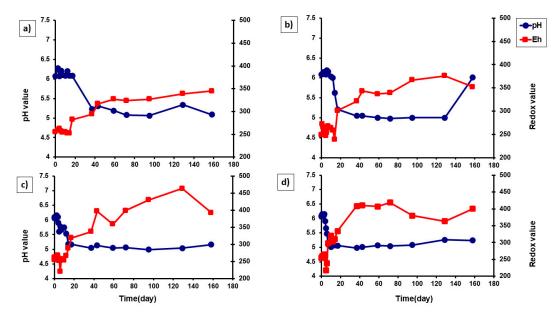


Figure 5. Effect of modified atmospheric packaging (CO₂/N₂ = 4:6) of smoked turkey under different storage temperature 0 °C (**a**), 5 °C (**b**), 10 °C (**c**), and 15 °C (**d**) on pH value and redox value (Eh).

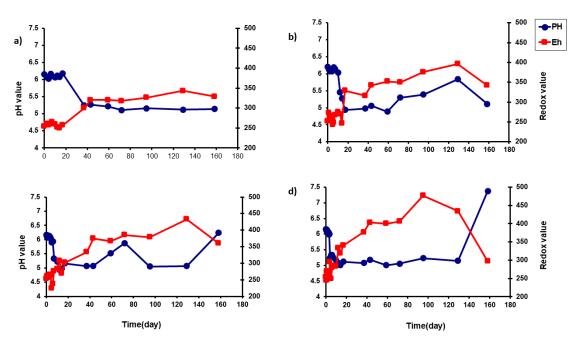


Figure 6. Effect of modified atmospheric packaging (CO₂/N₂ = 4:6) with essential oil of smoked turkey under different storage temperature 0 °C (**a**), 5 °C (**b**), 10 °C (**c**), and 15 °C (**d**) on pH value and redox value (Eh).

3.5. MAP Gases Alterations in MAP and MAPEO Smoked Turkey at Different Storage Systems

The level of gases in the T2 and T3 throughout the storage period of the samples was monitored and these showed only small changes from the beginning until the end of the product shelf life in both O_2 and CO_2 .

4. Discussion

In the current study, the direct influence of oregano oil was not determined against E. coli O157:H7 in vitro, but the impact of this oil has been examined against this pathogen in situ (i.e., sliced smoked meat turkey) indirectly via oregano oil vapor compared to the control without oil. Each treated sample and the control were placed into a separate plastic pouch then emptied and washed in three changes before filling and heat-sealing twice. Cattelan et al. [29] showed that many natural compounds have biological in vitro properties evidenced, but in situ studies are still scarce. At various storage regimes, the T3 has seemed to be efficiently preventing growth and led to the death of E. coli O157:H7. Beuchat et al. [30] concluded that low temperatures stimulated the preventive activity of plant extracts. However, the antimicrobial ability of natural extracts downregulated when such extracts are applied to food systems [31]. According to Solomakos et al. [32], the treatment of beef meat with essential oil at 0.6% had preventive activity against *E. coli* O157:H7 at 10 °C, but not at 4 °C. The cause of this behavior has not yet been adequately elucidated. Our results indicated that the inhibition of the pathogen took place at all the storage temperatures under T3 from the beginning up to the end of storage when compared to T1 and T2. E. coli O157:H7 was shown to survive in ready-to-cook chicken [19], minced or chopped bison meat [33], and ground beef [20] at refrigeration condition (3 °C) and multiplied at ambient temperature (20 °C) and unsuitable refrigeration condition (8 °C). The treated smoked meat samples with T1, T2, or T3 combined with different storage temperatures (0, 5, 10, or 15 °C) led to injured E. coli O157:H7. Therefore, the pathogenic bacteria may not count at some intervals, which led to fluctuating in *E coli*. Thus, the treatment used in this study resulted in injuring the bacteria, and maybe the conventional method is not sensitive enough to determine the damaged cells. Therefore, the special recovery steps are needed when analyzing smoked meat samples, which may contain the bacterium. In addition, using amplified by PCR with selective media may be considered as

clearly after 60 days of storage at 0, 5, and 15 °C under T1 while this fluctuation can be noticed after 20 and 40 days of storage under modified atmosphere packaging without oil and with essential oil at 10 and 15 °C and 5 and 10 °C, respectively. Therefore, it can be stated that the fluctuations in *E coli* did not appear in the case of packaging with a modified atmosphere with essential oil at 15 °C. These results indicated that the treatment with T3 could eliminate the bacteria from meat samples. However, there are reports that the survival and growth of pathogenic bacteria, including *E. coli* O157:H7, in other types of food products depends on various factors, such as temperature and background flora [22], pH, and the type of essential oil used [34,35]. The antifungal and antimicrobial efficacy of essential oils is available for *E. coli* O157:H7 in the literature [36]. Addition of active compounds to film formulations is a fundamental way to improve mechanical and chemical resistance of films to oxygen. Thus, using the proper packaging materials with different environmental conditions and for different food products is important to avoid oxygen [6].

At the end of storage, the pH values in the smoked turkey were recorded at lower levels compared with the initial level and the pathogen was not detected by enrichment. The low pH level at the end of storage is apparently quick due to anaerobic and microaerophilic microbial cross-contamination due to lactic acid formation as a consequence of carbohydrate breakdown by microbial metabolism [7,8,37,38]. This finding agrees with findings reported by Conner and Beuchat [37], which showed that essential oil with decreased pH, is more efficiently prevented the microbial growth. Solomakos et al. [32] and Shekarforoush et al. [19] found that essential oils have an antibacterial efficiency against spoilage and pathogenic bacteria [38–47]. It could be concluded that the effect of modified atmosphere packaging with essential oil and different temperature combinations even more effectively controlled the pathogen growth in the smoked turkey. These results highlight the importance of effective temperature control and additional essential oils in preventing pathogen growth on RTE smoked turkey during slicing, transport, and home use.

The control of *E. coli* O157:H7 growth in RTE smoked turkey would be under active modified atmosphere packaging incorporated with oregano essential oil under the different temperatures of storage. *E. coli* O157:H7 up-regulated no significance by <1 log CFU/g under all packaging and temperature combinations compared to the control. Moreover, T1, T2, and T3 have extended the shelf-life of RTE meat. However, the pathogen survival was highest on the RTE smoked turkey. However, only under T3, the *E. coli* O157:H7 strain in smoked turkey was not detected (negative by enrichment) under all conditions compared with other packaging temperature combinations. The result indicating that the gas exchange mechanical barriers were effective during the study (data are not shown).

Author Contributions: S.A.M. conceived and designed the experiments; S.A.M. and M.E.A.E.-H. performed the experiments; M.E.A.E.-H. analyzed the data; A.A.S., Z.S.M., W.R.E.-G., and A.E.T. contributed to analysis tools; M.E.A.E.-H., M.Q.A.-G., A.R.A., R.A.A., B.A., V.T., and A.E.T. wrote the paper. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Deputyship for Research & Innovation, "Ministry of Education" in Saudi Arabia through the project number IFKSU-RG1442-002.

Acknowledgments: The authors extend their appreciation to the Deputyship for Research & Innovation, "Ministry of Education" in Saudi Arabia for funding this research work through the project number IFKSU-RG1442-002. The Authors fully acknowledge George John-Nychas and the research team of the Lab of Microbiology and Biotechnology of Foods, Department of Food Science and Human Nutrition, Era Odos 75, Athens 11855, Greece, for their support to Samir Mahgoub. Authors extend thanks to their respected institutes and universities.

Conflicts of Interest: The authors have no competing interests to declare.

References

 Appendini, P.; Hotchkiss, J.H. Review of antimicrobial food packaging. *Innov. Food Sci. Emerg. Technol.* 2002, 3, 113–126. [CrossRef]

- 2. Han, J.H. Antimicrobial food packaging. Food Technol. 2000, 54, 56–65.
- 3. Han, J.H. Antimicrobial packaging materials and films. In *Novel Food Packaging Techniques;* Ahvenainen, R., Ed.; Woodhead Publishing Ltd: Cambridge, UK, 2003; pp. 50–70.
- 4. Han, J.H. Design of antimicrobial packaging systems. Int. Rev. Food Sci. Technol. 2003, 11, 106–109.
- Ekonomou, S.I.; Bulut, S.; Karatzas, K.A.G.; Boziaris, I.S. Inactivation of *Listeria monocytogenes* in raw and hot smoked trout fillets by high hydrostatic pressure processing combined with liquid smoke and freezing. *Innov. Food Sci. Emerg. Technol.* 2020, 64, 102427. [CrossRef]
- 6. Sahraee, S.; Regenstein, J.M.; Kafil, H.S. Protection of foods against oxidative deterioration using edible films and coatings: A review. *Food Biosci.* **2019**, *32*, 100451. [CrossRef]
- Wells, J.B.G.; Davis, B.R.; Wachsmuth, I.K.; Riley, L.W.; Remis, R.S.; Sokolow, R.; Morris, G.K. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. *J. Clin. Microbiol.* 1983, 18, 512–520. [CrossRef]
- 8. Meng, J.; Zhao, S.; Doyle, M.P. Virulence genes of Shiga toxin-producing *Escherichia coli* isolated from food, animals, and humans. *Int. J. Food Microbiol.* **1998**, 45, 229–235. [CrossRef]
- 9. Blanco, M.; Blanco, J.E.; Mora, A.; Rey, J.; Alonso, J.M.; Hermoso, M.; Hermoso, J.; Alonso, M.P.; Dahbi, G.; González, E.A.; et al. Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing *Escherichia coli* isolates from healthy sheep in Spain. *J. Clin. Microbiol.* **2003**, *41*, 1351–1365. [CrossRef]
- Mukherjee, S.; Mosci, R.E.; Anderson, C.M.; Snyder, B.A.; Collins, J.; Rudrik, J.T.; Manning, S.D. Antimicrobial drug–resistant shiga toxin–producing *Escherichia coli* Infections, MI, USA. *Emerg. Infect. Dis.* 2017, 23, 1609–1611. [CrossRef]
- 11. Mead, P.S.; Slutsker, L.; Dietz, V.; McCaig, L.F.; Bresee, J.S.; Shapiro, C.; Griffin, P.M.; Tauxe, R.V. Food-related illness and death in the United States. *Emerg. Infect. Dis.* **1999**, *5*, 607–625. [CrossRef]
- 12. Tarr, P.I. *Escherichia coli* 0157:H7: Clinical, diagnostic and epidemiological aspects of human infection. *Clin. Infect. Dis.* **1995**, 20, 1–10. [CrossRef]
- 13. Scallan, E.; Hoekstra, R.M.; Angulo, F.J.; Tauxe, R.V.; Widdowson, M.A.; Roy, S.L. Foodborne illness acquired in the United States—major pathogens. *Emerg. Infect. Dis.* **2011**, *17*, 7–15. [CrossRef]
- Amézquita-López, B.A.; Quiñones, B.; Soto-Beltrán, M.; Lee, B.G.; Yambao, J.C.; Lugo-Melchor, O.Y. Antimicrobial resistance profiles of Shiga toxin-producing *Escherichia coli* O157 and non-O157 recovered from domestic farm animals in rural communities in northwestern Mexico. *Antimicrob. Resist. Infect. Control* 2016, 5, 1–6. [CrossRef] [PubMed]
- 15. Mora, A.; Blanco, J.E.; Blanco, M.; Alonso, M.P.; Dhabi, G.; Echeita, A. Antimicrobial resistance of Shiga toxin (verotoxin)–producing *Escherichia coli* O157:H7 and non-O157 strains isolated from humans, cattle, sheep and food in Spain. *Res. Microbiol.* **2005**, *156*, 793–806. [CrossRef] [PubMed]
- Strachan, N.J.C.; Doyle, M.P.; Kasuga, F.; Rotariu, O.; Ogden, I.D. Dose response modelling of *Escherichia* coli O157 incorporating data from foodborne and environmental outbreaks. *Int. J. Food Microbiol.* 2005, 103, 35–47. [CrossRef] [PubMed]
- Anonymous. Introduction, background and conclusions: Background and objectives. In Proceedings of the International Workshop on Heat Resistance of Pathogenic Organisms, International Dairy Federation, Brussels, Belgium, 2004; p. 14.
- 18. ICMSF. International Commission on Microbiological Specifications for Foods Microorganisms in foods 7: Microbiologic Testing Food Safety Management; Kluwer Academic/Plenum Publishers: New York, NY, USA, 2008.
- 19. Shekarforoush, S.S.; Nazer, A.H.K.; Firouzi, R.; Rostami, M. Effects of storage temperatures and essential oils of oregano and nutmeg on the growth and survival of *Escherichia coli* O157:H7 in barbecued chicken used in Iran. *Food Control* **2007**, *18*, 1428–1433. [CrossRef]
- 20. Tamplin, M.L. Growth of *Escherichia coli* O157:H7 in raw ground beef stored at 10 °C and the influence of competitive bacteria flora, strain variation, and fat level. *J. Food Prot.* **2008**, *65*, 1535–1540. [CrossRef] [PubMed]
- Barrera, O.; Rodríguez-Calleja, J.M.; Santos, J.A.; Otero, A.; García-López, M.L. Effect of different storage conditions on *E. coli* O157:H7 and the indigenous bacterial microflora on lamb meat. *Int. J. Food Microbiol.* 2007, *115*, 244–251. [CrossRef] [PubMed]
- 22. Vold, V.; Holck, A.; Wasteson, Y.; Nissen, H. High levels of background flora inhibits growth of *Escherichia coli* O157:H7 in ground beef. *Int. J. Food Microbiol.* **2000**, *56*, 219–225. [CrossRef]

- Silva, J.P.L.; Duarte-Almeida, J.M.; Perez, D.V.; Franco, B.D.G.M. Óleo essencial de orégano: Interferência da composição química na atividade frente a *Salmonella enteritidis*. *Cienc. Tecnol. Aliment.* 2010, 30, 136–141. [CrossRef]
- 24. Souza, G.T.; De Carvalho, R.J.; De Sousa, J.P.; Tavares, J.F.; Schaffner, D.; De Souza, E.L.; Magnani, M. Effects of the essential oil from *Origanum vulgare* L. on survival of pathogenic bacteria and starter lactic acid bacteria in semihard cheese broth and slurry. *J. Food Prot.* **2016**, *79*, 246–252. [CrossRef] [PubMed]
- 25. Siroli, L.; Patrignani, F.; Serrazanetti, D.I.; Tappi, S.; Rocculi, P.; Gardini, F.; Lanciotti, F. Natural antimicrobials to prolong the shelf-life of minimally processed lamb's lettuce. *Postharvest Biol. Technol.* **2015**, *103*, 35–44. [CrossRef]
- 26. Guarda, A.; Rubilar, J.F.; Miltz, J.; Galotto, M.J. The antimicrobial activity of microencapsulated thymol and carvacrol. *Int. J. Food Microbiol.* **2011**, *146*, 144–150. [CrossRef] [PubMed]
- 27. Baser, K.H.C. Biological and pharmaceutical activities of carvacrol and carvacrol bearing essential oil. *Curr. Pharm. Des.* **2008**, *14*, 3106–3120. [CrossRef]
- 28. Rubilar, J.F.; Cruz, R.M.S.; Silva, H.D.; Vicente, A.A.; Khmelinskii, I.; Vieira, M.C. Physico-mechanical properties of chitosan films with carvacrol and grape seed extract. *J. Food Eng.* **2013**, *115*, 466–474. [CrossRef]
- 29. Cattelan, M.G.; Nishiyama, Y.P.O.; Gonçalves, T.M.V.; Coelho, A.R. Combined effects of oregano essential oil and salt on the growth of *Escherichia coli* in salad dressing. *Food Microbiol.* **2018**, *73*, 305–310. [CrossRef]
- 30. Beuchat, R.E.; Ryu, J.H. Produce handling and processing practices. *Emerg. Infect. Dis.* **1997**, *3*, 459–465. [CrossRef]
- 31. Pandit, V.A.; Shelef, L.A. Sensitivity of Listeria monocytogenes to rosemary (*Rosmarinus officinalis* L.). *Food Microbiol.* **1994**, *11*, 57–63. [CrossRef]
- 32. Solomakos, N.; Govaris, A.; Koidis, P.; Botsoglou, N. The antimicrobial effect of thyme essential oil, nisin and their combination against *Escherichia coli* O157:H7 in minced beef during refrigerated storage. *Meat Sci.* 2008. [CrossRef]
- 33. Li, Q.; Logue, C.M. The growth and survival of *Escherichia coli* O157:H7 on minced bison and pieces of bison meat stored at 5 and 10 °C. *Food Microbiol.* **2005**, *22*, 415–421. [CrossRef]
- Erickson, J.P.; Stamer, J.W.; Hayes, M.; McKenna, D.N.; Van Alstin, L.A. An assessment of *Escherichia coli* O157:H7 contamination risks in commercial mayonnaise from pasteurized eggs and environmental sources, and behavior in low-pH dressings. *J. Food Prot.* 1995, *58*, 1054–1064. [CrossRef] [PubMed]
- 35. Juven, B.J.; Kanner, J.; Schved, F.; Weisslowicz, H. Factors that interact with antibacterial action of thyme essential oil and its active constituents. *J. Appl. Bacteriol.* **1994**, *76*, 626–631. [CrossRef] [PubMed]
- 36. Burt, S.A.; Reinders, R.D. Antimicrobial activity selected plant essential oils against *Escherichia coli* O157:H7. *Lett. Appl. Microbiol.* **2003**, *36*, 162–167. [CrossRef] [PubMed]
- Conner, D.E.; Beuchat, L.R. Effects of essential oils from plants on growth of food spoilage yeasts. *J. Food Sci.* 1984, 49, 429–434. [CrossRef]
- 38. Martin-Sanchez, A.M.; Chaves-Lopez, C.; Sendra, E.; Sayas, E.; Fenandez-Lopez, J.; Perez-Alvarez, J.A. Lipolysis, proteolysis and sensory characteristics of a Spanish fermented dry-cured meat product (salchichon) with oregano essential oil used as surface mold inhibitor. *Meat Sci.* **2011**, *89*, 35–44. [CrossRef]
- 39. Champagne, C.P. Inhibition of psychrotrophic bacteria in raw milk by immobilized lactic acid bacteria. *Biotech. Lett.* **1990**, *12*, 771–776. [CrossRef]
- 40. Abd El-Hack, M.E.; Alagawany, M.; Abdel-Moneim, A.-M.E.; Mohammed, N.G.; Khafaga, A.F.; Bin-Jumah, M.; Othman, S.L.; Allam, A.A.; Elnesr, S.S. Cinnamon (*Cinnamonum zeylanicum*) oil as a potential alternative to antibiotics in poultry. *Antibiotics* **2020**, *9*, 210. [CrossRef]
- 41. Mahgoub, S.A.M.; Abd El-Hack, M.E.; Saadeldin, I.M.; Hussein, M.A.; Swelum, A.A.; Alagawany, M. Impact of *Rosmarinus officinalis* cold-pressed oil on health, growth performance, intestinal bacterial populations, and immunocompetence of Japanese quail. *Poult. Sci.* **2019**, *89*, 2139–2149. [CrossRef]
- 42. Kishawy, A.T.Y.; Amer, S.A.; Abd El-Hack, M.E.; Saadeldin, I.M.; Swelum, A.A. The impact of dietary linseed oil and pomegranate peel extract on broiler growth, carcass traits, serum lipid profile, and meat fatty acid, phenol, and flavonoid contents. *Asian-Australas. J. Anim. Sci.* **2019**, *32*, 1161–1171. [CrossRef]
- 43. Hussein, M.M.A.; Abd El-Hack, M.E.; Mahgoub, S.A.; Saadeldin, I.M.; Swelum, A.A. Effects of clove (*Syzygium aromaticum*) oil on quail growth, carcass traits, blood components, meat quality and intestinal microbiota. *Poult. Sci.* **2019**, *98*, 319–329. [CrossRef]

- 44. Abd El-Hack, M.E.; Mahgoub, S.A.; Hussein, M.M.A.; Saadeldin I, M. Improving growth performance and health status of meat-type quail by supplementing the diet with black cumin cold-pressed oil as a natural alternative for antibiotics. *Environ. Sci. Pollut. Res.* **2018**, *25*, 1157–1167. [CrossRef] [PubMed]
- 45. Abd El-Hack, M.E.; Alagawany, M.; Ragab Farag, M.; Tiwari, R.; Karthik, K.; Dhama, K.; Adel, M. Beneficial impacts of thymol essential oil on health and production of animals, fish and poultry: A review. *J. Essent. Oil Res.* **2016**, *28*, 365–382. [CrossRef]
- 46. Alagawany, M.; Abd El-Hack, M.E.; Farag, M.R.; Shaheen, H.M.; Abdel-Latif, M.A.; Noreldin, A.E.; Patra, A.K. The usefulness of oregano and its derivatives in poultry nutrition. *World's Poult. Sci. J.* **2018**, *74*, 463–474. [CrossRef]
- 47. Alagawany, M.; Abd El-Hack, M.E.; Farag, M.R.; Shaheen, H.M.; Abdel-Latif, M.A.; Noreldin, A.E.; Khafaga, A.F. The applications of *Origanum vulgare* and its derivatives in human, ruminant and fish nutrition–a review. *Ann. Anim. Sci.* **2020**, *20*, 389–407. [CrossRef]



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).