Food Microbiology 58 (2016) 128–134

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Effects of Citrox and chitosan on the survival of *Escherichia coli* O157:H7 and *Salmonella enterica* in vacuum-packaged turkey meat

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A R T I C L E I N F O

Article history:
Received 5 November 2015
Received in revised form
17 March 2016
Accepted 6 April 2016
Available online 28 April 2016

Keywords:
Antimicrobial
Citrox
Chitosan
Poultry safety
Zoonoses

A B S T R A C T

In this study, we examined the antimicrobial effects of citrus extract (Citrox®) and chitosan on lactic acid bacteria (LAB) and the pathogens *Escherichia coli* O157:H7 and *Salmonella enterica* on turkey meat during storage under vacuum packaging (VP) at 4 and 10 °C. We also examined the effects of Citrox and chitosan on pathogen contamination in tryptic soy broth (TSB). Chitosan alone or in combination with Citrox inhibited the growth of endogenous LAB in turkey meat, whereas citrus extract did not cause a major reduction in bacterial density. Citrus extract combined with chitosan yielded the lowest mesophilic total viable counts (TVCs), irrespective of temperature, showing major declines in all treated turkey samples from days 0–21 of storage. The shelf-lives of untreated, Citrox-treated, and chitosan and Citrox/chitosan-treated samples (as determined by TVC and sensory data) were 13, 17, and >21 days, respectively, at 4 °C for VP turkey. The addition of Citrox was more effective against *S. enterica* than *E. coli* in turkey, causing reductions of >0.5 and 2 log cfu/g at 4 and 10 °C, respectively, after 21 days of storage. Interestingly, the addition of chitosan had a significant inhibitory effect on *E. coli* at 4 °C and *S. enterica* at 10 °C as compared with the control (inoculated samples) resulting in dramatic reductions in *E. coli* (2 log) and *S. enterica* (5 log) cell counts on day 21. Of all the treatments examined, citrus extract in combination with chitosan showed an additive inhibitory effect against both pathogens, reducing *E. coli* and *S. enterica* populations, by approximately 2.7 or 4.5 and 2.2 or 5.6 log cfu/g, respectively, at 4 and 10 °C on day 21 of storage.

1. Introduction

With increases in both intensive poultry production and the global demand for poultry meat, the importance of poultry meat hygiene and safety is increasing worldwide (Sofos and Geornaras, 2010). Poultry meat is a highly perishable food commodity that provides an almost perfect medium for microbial growth, including both spoilage and pathogenic microorganisms (Vasilatos and Savvaidis, 2013).

Escherichia coli O157:H7 and *Salmonella enterica* are two major food-borne pathogens (Doyle, 1991; Doyle and Beuchat, 2007). Verocytotoxin-producing *E. coli* (VTEC), which is one of the most serious causes of human infections globally, particularly in Europe and the United States of America (USA), was found in 9.3% of cloacal swabs taken from poultry at a slaughter in Slovakia (Pilipcinec et al., 1999). *E. coli* O157:H7 is a major cause of bacterial diarrhea, and the spectrum of illnesses caused by this pathogen includes asymptomatic carriage, nonbloody diarrhea, hemorrhagic colitis, thrombotic thrombocytopenic purpura, and hemolytic uremic syndrome. In particular, children and the elderly are at a high risk of infection.

Members of the genus *Salmonella*, associated with poultry and poultry products, are the causative agents of salmonellosis (typhoid fever, enteric fever, and gastroenteritis) (Doyle, 1991). Although most outbreaks cause mild to moderate self-limited illness, serious disease resulting in death does occur in elderly and immunocompromised populations. During 1998–2008, 30% of *Salmonella*-associated foodborne outbreaks were attributed to poultry (CDC,

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http://dx.doi.org/10.1016/j.fm.2016.04.003
0740-0020/

2013).

In recent years, concerns over the safety of chemical additives have arisen, and consumers have increasingly demanded the use of natural products as alternative preservatives in foods. Accordingly, the product Citrox (14WPlus, ProGarda), manufactured recently to meet this demand, consists of ingredients that are of plant origin (citrus green extract combined with citric acid and polyphenols); these ingredients comply with the requirements of European Regulation 2092/91 and EC Directive 89/107/EEC. Citrox is effective in the presence of organic matter, breaks down biofilm, extends shelf-life, reduces pathogenic attack, can be applied directly to food as an additive, and conforms to BS EN 1276 (European Suspension Test).

Alternatively, chitosan and its derivatives, which are biodegradable, biocompatible, nontoxic, and bactericidal, represent promising agents for maintaining food freshness and safety. The antimicrobial activity of chitosan against both gram-positive and gram-negative bacteria is well established (Kong et al., 2010). In a recent study, the combined use of chitosan (dipping) and oregano essential oil (EO) yielded a shelf-life of 14 days for fresh chicken breast meat, stored at 4 °C, with the meat maintaining acceptable sensory characteristics (Petrou et al., 2012). Consistent with this, many studies have examined the effects of spices, EOs, natural antimicrobials, and biopreservative agents to enhance the safety of foods (Burt, 2004). Citrus Eos (CEOs) are the most widely used EOs in the world, extracted from the peels of citrus fruit. Flavonoids from citrus fruits have been shown to have strong biological activity, including antimicrobial, antioxidant, anticancer, and antiviral activities, in several studies (Burt, 2004). However, to the best of our knowledge, limited data on the survival of pathogens in fresh turkey fillets have been published, and no studies have been conducted on the use of Citrox, chitosan, and combined Citrox/chitosan on the ability to control *E. coli* O157:H7 and *S. enterica* in turkey fillets. In a recent study, carvacrol (0.25% v/v) was shown to be effective in reducing *Salmonella* and *Campylobacter jejuni* on turkey breast cutlets, by approximately 1.0 log cfu/g, when stored under modified atmosphere packaging (MAP; 95% CO_2 and 5% O_2) (Nair et al., 2015).

Therefore, the objectives of this study were to evaluate the effects of Citrox, chitosan, and combined Citrox/chitosan on the survival and growth of *E. coli* and *S. enterica* spp. in fresh turkey fillets stored under vacuum packaging (VP) at 4 and 10 °C and to determine the shelf-life of turkey meat (uninoculated) in the absence or presence of the aforementioned antimicrobials and storage temperatures.

2. Material and methods

2.1. Preparation of samples

Fresh turkey breast samples (skinless and boneless fillets, approximately 100 g or 16 cm × 8 cm each; Nitsiakos, S.A., Ioannina, Greece) were provided by a local poultry processing company within 1 h of slaughter in insulated polystyrene boxes on ice flakes. Turkey samples were subsequently kept under refrigeration in a cooling incubator (2 °C) before inoculation of the bacterial strains and the addition of the antimicrobials (see Section 2.4).

2.2. Bacterial strains and culture conditions

Two strains of *E. coli* serotype O157:H7 (NCTC 13127, isolated from human diarrhea stool; ATCC 43888, courtesy of Professor Hilde Nissen, Matforsk, Norway; both Vero cytoxin negative) and two strains of *S. enterica* (*S. enterica* serovar Typhimurium DT104, a human isolate kindly provided by Professor M. Zwietering; and *S. enterica* serovar Montevideo BAA710, purchased from American Type Culture Collection [ATCC, Wesel, Germany]) were used in each cocktail. All strains were available as frozen (−30 °C) cultures in Trypticase Soy Broth (LAB M, Lancashire, UK) with 0.6% yeast extract (TSBYE) plus 20% glycerol (Merk, Darmstadt, Germany) and were activated by transferring 1 mL of stock culture in 9 mL of TSBYE followed by incubation at 37 °C for 24 h. Working cultures were kept on slants of TSBYE at 4 °C and transferred monthly. Strains were subcultured twice in TSBYE (37 °C, 24 h) before use in the experiments.

2.3. Citrox and chitosan formulations

The formulation of Citrox was ProGarda 14WPlus, kindly supplied by Polypan Group (Athens, Greece). Citrox was added to the turkey meat at a final concentration of 2 mL/kg. Chitosan of low molecular weight (MW; 340) in powder form from crab shells was purchased from Aldrich Company (Athens, Greece). The moisture content was less than 10%, and chitosan had a deacetylation degree of 75–85%. A stock solution of chitosan was prepared by dissolving 2.0 g in 100 mL of 1% (w/v) glacial acetic acid and stirred overnight at room temperature (final chitosan concentration = 2.0% w/v).

2.4. Turkey fillet inoculation, packaging, and treatment

Samples of turkey meat fillets (approximately 100 g) were transferred aseptically into an open packaging pouch, and 1 mL cocktailed cultures (inoculum concentration, approximately 10^6 log cfu/mL), prepared with the above-mentioned method, was added onto the turkey samples, yielding a concentration of approximately 10^4 cfu/g. Citrox (2 mL/kg) and chitosan (2% w/v) were then inoculated as antimicrobial additives to the turkey samples (artificially contaminated with *E. coli* O157:H7 and *S. enterica*) prior to VP (see below). Samples of turkey without pathogen inoculation but with the above-mentioned antimicrobials were also prepared.

All samples were subsequently subjected to VP into sterile low-density polyethylene/polyamide/low-density polyethylene pouches (VER PACK, Thessaloniki, Greece) using a using a MINIPACK-TORRE model MV31 vacuum sealer (MINIPACK-TORRE, SpA, Dalmine, Italy) and finally stored in cooled incubators at 4 and 10 °C for up to 21 days. Two experiments were conducted: Experiment A, in the absence of pathogens and the presence of Citrox, chitosan, and Citrox plus chitosan; and Experiment B, in the presence of both artificially inoculated pathogens and the aforementioned antimicrobials.

In Experiment A (effect of added antimicrobials in turkey meat, absence of inoculated pathogens), turkey meat was subdivided into four lots: T (control, absence of antimicrobials), C with added Citrox (2 mL/kg), H with added chitosan (2% w/v), and CH with added Citrox (2 mL/kg) and chitosan (2% w/v). In Experiment B (effect of added antimicrobials on the survival of pathogens, inoculated in the turkey meat), eight treatments in total (four for each strain) were tested as follows: E (*E. coli*, with no added antimicrobials), EC (*E. coli* with Citrox), EH (*E. coli* with chitosan), ECH (*E. coli* with Citrox and chitosan), S (*S. enterica*, with no added antimicrobials), SC (*S. enterica* with Citrox), SH (*S. enterica* with chitosan), and SCH (*S. enterica* with Citrox and chitosan). In Experiment B, the antimicrobials were added at the concentrations described for experiment A.

2.5. Antimicrobial activity in TSB

As previously described, inoculation was carried out with 10^4 log cfu/g bacteria in TSB. Solutions of Citrox (2 mL/kg), chitosan (2% w/v), and Citrox (2 mL/kg) with chitosan (2% w/v) were added to sterile TSB (40 mL) in screw-capped glass bottles. Following inoculation with the pathogens, broth samples of all examined treatments were incubated at 37 °C for 32 h. Sampling for microbiological analysis of inoculated broth was performed after 0, 4, 8, 12, 16, 24, and 32 h of incubation.

2.6. Microbiological analyses

Turkey meat samples (10 g) were aseptically transferred to 90 mL of 0.1% peptone water (Merck, Darmstadt, Germany) in a stomacher bag (Seward Ltd, London, UK), and the mixture homogenized for 1 min at room temperature. For microbial enumeration, 1- or 0.1-mL samples of the appropriate dilutions were pour or spread plated on the following media (APHA, 2015): de-Man-Rogosa-Sharp (MRS) medium (CM 0361, Oxoid, UK) for LAB, adjusted to pH 6.2 and supplemented with 0.05% (w/v) cycloheximide (inhibition of yeasts/molds) overlaid with the same medium (to create anaerobic conditions) at 30 °C for 48–72 h; plate count agar (PCA; Merck) for mesophilic TVC at 25 °C for 72 h; selective Sorbitol McConkey agar SMAC (Oxoid) supplemented with cefixime-tellurite (Oxoid) for *E. coli* at 37 °C for 48 h; or selective xylose lysine deoxycholate (XLD) agar (CM 0469; Oxoid) for *S. enterica* after incubation at 37 °C for 18–24 h. When *E. coli* O157:H7 or *S. enterica* cells could not be enumerated by direct plating (detection limit 1 log cfu/g), surviving cells were detected by enrichment according to ISO 16654:2001 for *E. coli* O157:H7 detection and ISO 6759:2002/Cor.1:2004 for *Salmonella* spp. detection.

2.7. Sensory analysis

Turkey samples were cooked in a microwave oven at high power (700 W) for 10 min. A panel of seven judges experienced (laboratory-trained, mostly staff and postgraduate students) in poultry evaluation was used for sensory evaluation (Experiment A). All panelists who evaluated the sensory attributes of cooked turkey had previously participated in training sessions to become familiar with the sensory characteristics of cooked turkey. Panelists were asked to evaluate the taste, odor, and appearance of the cooked fillets. Acceptability as a composite of odor and taste was estimated using a scale ranging from 0 to 9. The scale points were as follows: excellent, 9; very good, 8; quite good, 7; good, 6; acceptable; 5; poor (first off-odor, off-taste development) < 5. A score of 5 was taken as the lower limit of acceptability. The product was defined as being unacceptable after development of first off-odor or off-taste, judged by the average of seven panelists.

2.8. Statistical analysis

Experiments were replicated twice (n = 2) on different occasions with different turkey samples. Analyses were run in triplicate for each replicate. Results are reported as mean values ± standard deviations (SDs). Data were subjected to analysis of variance (ANOVA). The least significant difference (LSD) procedure was used to test for differences between means ($P < 0.05$). Microbiological counts were converted to log cfu/g and were subjected to ANOVA using Stat Graphics software (Statistical Graphics Corp., Rockville, MD, USA).

3. Results

3.1. Experiment A (effect of added antimicrobials on turkey meat, absence of inoculated pathogens)

3.1.1. Effect of citrus extract and chitosan on turkey meat mesophilic and lactic flora

The initial population density of the mesophilic TVC in untreated turkey (T) samples was 4.2; this value increased incrementally with storage time ($P < 0.05$), reaching final averages of 8.65 and 9.3 log cfu/g after 21 days of storage at 4 and 10 °C, respectively (Fig. 1a,c). Interestingly, Citrox-treated (C) samples showed a similar pattern ($P > 0.05$) of increasing populations with time, reaching final averages of 8.3 and 8.7 log cfu/g, respectively, at 4 and 10 °C (Fig. 1a,c). The mesophilic TVC populations both in the chitosan-treated (H) and Citrox/chitosan-treated (CH) samples were significantly lower ($P < 0.05$), with final densities of approximately 4.7 or 4.0 and 4.5–3.2 log cfu/g at 4 and 10 °C, respectively (Fig. 1a,c), as compared with those in the T and C samples.

ANOVA showed that the lactic acid flora (LAB population on MRS agar; Fig. 1b,d) followed a pattern similar to that observed for the mesophilic TVCs. Interestingly, as previously noted for TVC data, LAB populations in the H and CH samples were significantly lower ($P < 0.05$), with final densities of approximately 3.7 or 3.2 and 4.1 or 2.5 log cfu/g at 4 and 10 °C, respectively (Fig. 1b, d), as compared with those in the T and C samples.

3.1.2. Effects of Citrox and chitosan on sensory changes

The appearance and odor sensory scores for the untreated (T) and Citrox-treated (C) samples showed similar patterns of decreasing acceptability with time. At 4 °C, scores of 5 (the lowest limit of acceptability) were recorded for T and C samples on days 15 and 19, respectively, for appearance and on days 13 and 17, respectively, for odor (Fig. 2a). At 10 °C, the lower limit for appearance and odor acceptability was reached approximately on days 9 and 13 for T and C samples, respectively (Fig. 2b). In contrast, both the appearance and odor scores for the chitosan-treated (H) and Citrox/chitosan-treated (CH) turkey, irrespective of temperature, during the entire storage period never dropped below 5, with scores being significantly higher ($P < 0.05$) as compared with those for the T and C samples (Fig. 2a,b).

Taste sensory scores for the H and CH turkey during the entire storage period at 4 °C were on average more than 7 at 4 °C. Major decreases were observed at 10 °C ($P < 0.05$), and values remained in the acceptable region up to day 13. Based on microbiological (mesophilic TVC limit value of 7 log cfu/g) and sensory data (odor attribute, a score of 5, taken as the sensory limit value of acceptability), shelf-lives of approximately 13 (T), 17 (C), and more than 21 days (H and CH) were obtained at 4 °C. At 10 °C, the shelf-lives of T, C and H, CH turkey samples were 9, 13, and greater than 21 days, respectively. Our results (TVC and sensory data) showed that the shelf-life of VP turkey meat may be extended by more than 8 or 12 days at 4 or 10 °C, respectively.

3.2. Experiment B (effects of added antimicrobials on the survival of pathogens, inoculated in the turkey meat)

3.2.1. Antimicrobial activity in TSB

The antimicrobial effects of Citrox, chitosan, and Citrox plus chitosan on *E. coli* and *S. enterica* growth in TSB were studied (results not shown). The populations of the pathogens were significantly increased ($P < 0.05$) with incubation time; final populations ranged from 9 to 10 log cfu/g for *E. coli* and *S. enterica*. Compared with the untreated control, the addition of Citrox, chitosan, or Citrox plus chitosan in TSB significantly reduced *E. coli* and *S.*

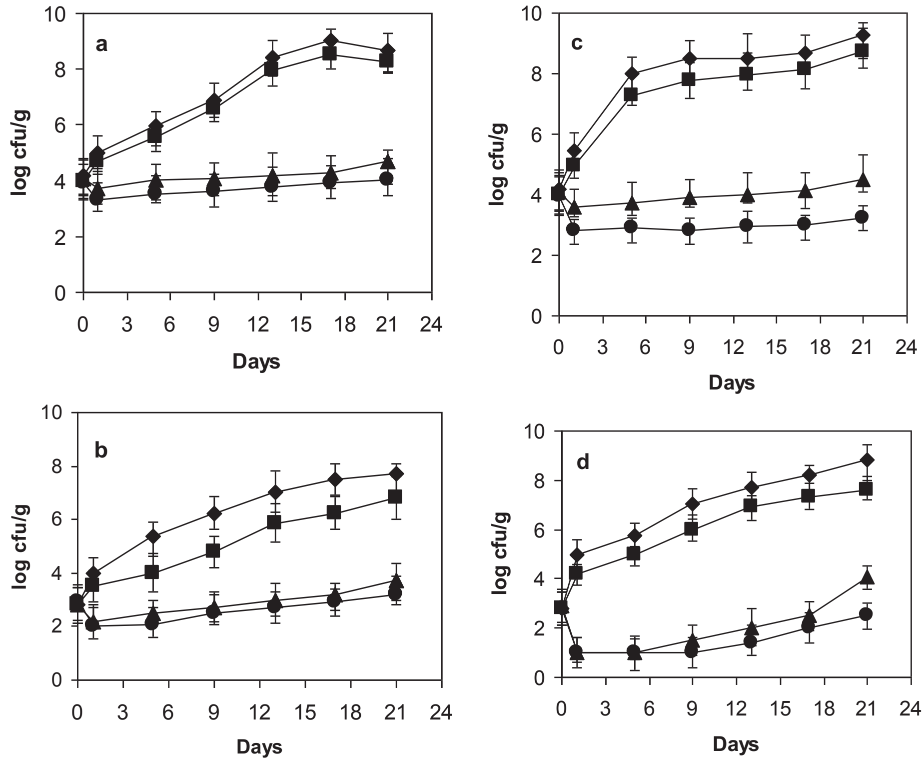

Fig. 1. Changes in the mesophilic TVC and LAB (log cfu/g) in fresh turkey meat stored under vacuum at 4 °C (a, b) and 10 °C (c, d) in the absence of antimicrobials (◆) with added citrus extract (2 mL/kg, ■), with added chitosan (2% w/v, ▲) with added citrus extract and chitosan (●). Data shown represent the average of two independent experiments (n = 2 × 3 = 6) plus the standard deviation (error bars).

enterica populations throughout the incubation period ($P < 0.05$). Interestingly, all the antimicrobial treatments examined had a high inhibitory effect against both *E. coli* and *S. enterica*.

3.2.2. Effects of chitosan and Citrox treatments on E. coli O157:H7 and S. enterica

The presence of *E. coli* and *S. enterica* in turkey fillets prior to inoculation was tested through direct plating technique, and the pathogens were not detected. Initial populations of inoculated *E. coli* (E) and *S. enterica* (S) on the turkey samples (approximately 3.2 and 2.7 log cfu/g, respectively; Figs. 3 and 4) did not increase significantly ($P < 0.05$) during storage at 4 °C, reaching average final values of 3.7 and 3.2 log cfu/g, respectively. In contrast, at 10 °C, steady growth was observed for the respective *E. coli* (E) and *S. enterica* (S) populations (Figs. 3b and 4b, respectively; $P < 0.05$), reaching average final values of 6.25 and 7.1 log cfu/g, respectively. The addition of citrus extract was more effective against *S. enterica* in turkey (SC) than against *E. coli* (EC), causing reductions of more than 0.5 and 2 log cfu/g at 4 and 10 °C, respectively, after 21 days of storage (Fig. 3a,b). Interestingly, the addition of chitosan had a significant inhibitory effect on *E. coli* (EH) at 4 °C (Fig. 4a) and *S. enterica* (SH) at 10 °C, compared with the inoculated E and S samples, resulting in dramatic reductions in *E. coli* (EH, 2 log) and *S. enterica* (SH, 5 log) cell counts on day 21 (Fig. 4a).

In this study, of all the treatments examined, Citrox in combination with chitosan (CH) showed an additive inhibitory effect against both pathogens, reducing *E. coli* (ECH) and *S. enterica* (SCH) populations by approximately 2.7 or 4.5 and 2.2 or 5.6 log cfu/g, respectively, at 4 and 10 °C on day 21 of storage (Figs. 3a,b and 4a,4b).

Both *E. coli* O157:H7 and *S. enterica* survived in the untreated, inoculated turkey samples during the entire storage period at 4 and 10 °C; however, the survival profiles at these temperatures were different. Our data showed that *E. coli* (a mesophilic organism) and *S. enterica* (susceptible to low temperatures) did not grow ($P > 0.05$) in the control turkey (E and S) samples at 4 °C. In contrast to the results at 4 °C, population densities at 10 °C for both bacteria were increased to their maximum levels on day 21 of storage.

4. Discussion

In this study, we examined the effects of Citrox and chitosan, alone or in combination, on the growth of pathogens on turkey meat samples. Our results demonstrated that all treatments blocked the growth of pathogens and extended the shelf-life of the samples, with the most dramatic effects observed for the combined treatment. These findings suggest that combined Citrox plus chitosan may provide an effective alternative for maintaining quality of meats.

In the present study, chitosan (H) alone and chitosan combined with Citrox (CH) were the most effective treatments inhibiting the growth of mesophilic TVCs and LAB at 4 and 10 °C throughout the storage period. LAB are the main spoilage organisms under vacuum or low oxygen, and their high numbers can lead to spoilage and discoloration of meat. In this study, chitosan alone or in combination with citrus extract showed an inhibitory effect on the growth of LAB in turkey meat (uninoculated samples), whereas Citrox did not cause a major reduction in the numbers of LAB. LAB, i.e., *Lactobacillus* and *Leuconostoc* spp., are known to be acid tolerant, similar to other bacterial species (acetic acid, *Acetobacter*; Lancefield N streptococci, etc.) and resistant to modifications by organic acids (Mozzi, 2016). It is speculated that there is a mechanism providing tolerance and resistance of LAB to the action of citrus extract, similar to that of organic acids (Arnold et al., 2001).

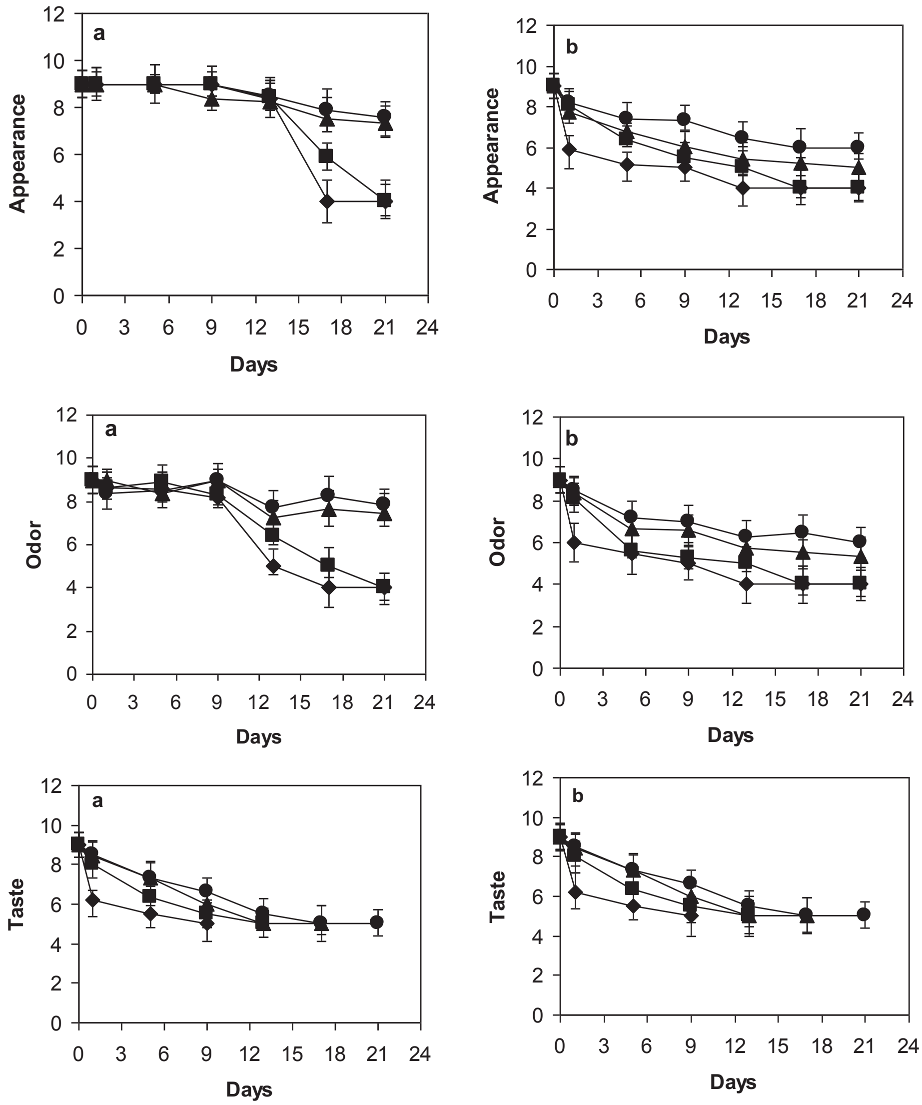

Fig. 2. Changes in appearance, odour and taste in fresh turkey meat stored under vacuum at 4 °C (a) and 10 °C (b) in the absence of antimicrobials (◆) with added citrus extract (2 mL/kg, ■), with added chitosan (2% w/v, ▲) with added citrus extract and chitosan (●). Data shown represent the average of two independent experiments (n = 2 × 3 = 6) plus the standard deviation (error bars).

However, this hypothesis was not investigated in the present study. In a recent study (Nair et al., 2015), MAP with 1% carvacrol was shown to cause a reduction of 3.0 log cfu/g LAB on day 21 as compared with aerobic packaging in turkey breast cutlets stored under MAP (95%CO_2/5%O_2).

Chitosan is believed to act on the cells of spoilage microorganisms and pathogens by changing the permeability of the cytoplasmatic membrane, leading to cell death (Prashanth and Tharanathan, 2007). In other studies, the combined use of oregano EO with chitosan applied on fresh chicken breast (Petrou et al., 2012) and cured chicken meat (Shekarforoush et al., 2015) or the use of rosemary EO with chitosan applied on turkey meat (Vasilatos and Savvaidis, 2013) were also shown to inhibit the growth of spoilage bacteria. Additionally, citrus extract (0.1 mL/100 g) used singly or in combination with an oxygen absorber reduced the TVC of aerobically packaged ground chicken meat by 0.5 and 1.5 log cfu/g, respectively (Mexis et al., 2012). The addition of citrus extract and chitosan (CH) to the turkey meat improved its taste and odor, in agreement with the findings of Petrou et al. (2012), who reported that chitosan, applied either singly or in combination with oregano EO, did not negatively influence the taste of chicken breast meat.

Both chitosan and Citrox were sensorially acceptable when added to turkey samples, with chitosan characterized by spicy, fruity, and oriental flavors and Citrox characterized by a citrus-like flavor. Moreover the addition of both citrus extract and chitosan may provide the possibility of new flavors and options for poultry products; however, further sensory tests are needed to examine this possibility.

Our results regarding the growth of pathogens on untreated turkey samples were in agreement with the findings of Lahmer et al. (2012) and Nair et al. (2015), who demonstrated the survival of *E. coli* O157:H7 in chicken juice and *Salmonella* in turkey breast cutlets. Additionally, the reduced effectiveness of VP on *E. coli* and *S. enterica* at 4 °C may be due to the facultative anaerobic nature of these organisms, possessing enzyme-mediated protective mechanisms to survive under stressful conditions (Yamamoto and Droffner, 1985).

To date, few studies have examined the ability of plant EOs (Bajpai et al., 2012) or CEOs, including citrus extracts, to control

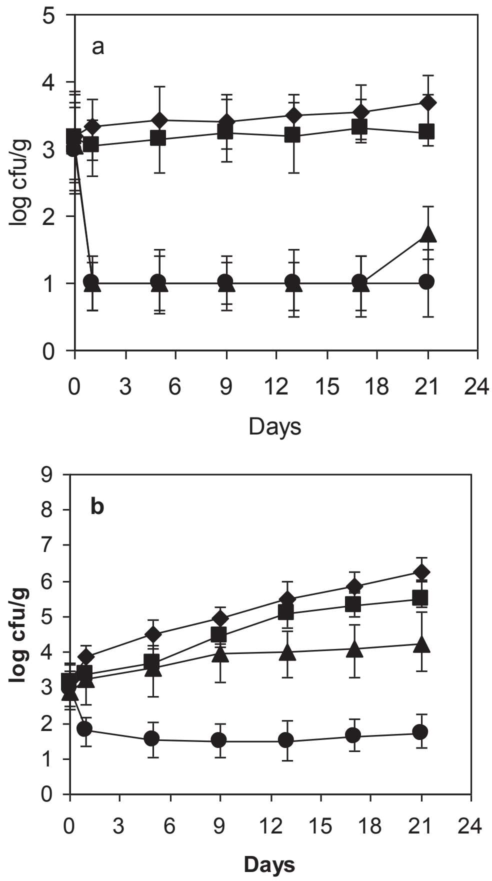

Fig. 3. Survival of *Escherichia coli* O157:H7 in fresh turkey meat stored under vacuum at 4 °C (a) and 10 °C (b) in the absence of antimicrobials (◆) with added citrus extract (2 mL/kg, ■), with added chitosan (2% w/v, ▲) with added citrus extract and chitosan (●). Data shown represent the average of two independent experiments (n = 2 × 3 = 6) plus the standard deviation (error bars).

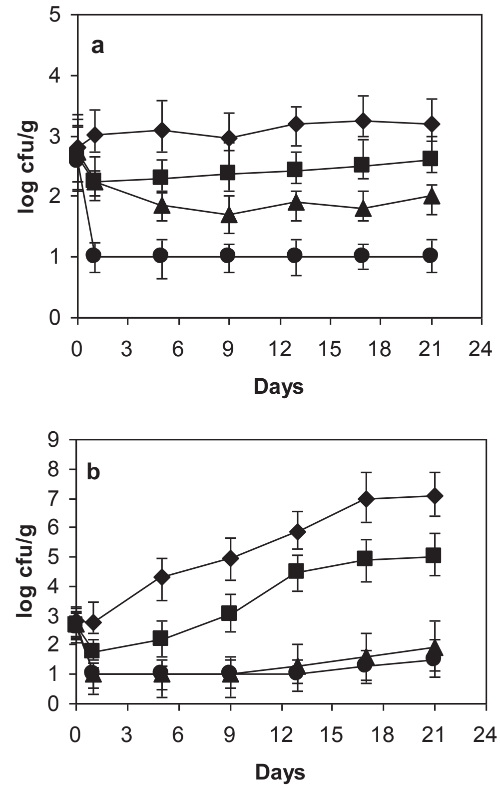

Fig. 4. Survival of *Salmonella enterica* in fresh turkey meat stored under vacuum at 4 °C (a) and 10 °C (b) in the absence of antimicrobials (◆) with added citrus extract (1 mL/kg, ■), with added chitosan (2 mL/kg, ▲) with added citrus extract and chitosan (●). Data shown represent the average of two independent experiments (n = 2 × 3 = 6) plus the standard deviation (error bars).

either *E. coli* or *S. enterica* in poultry meat. Citrus extract is a natural food additive that has antimicrobial effects and has recently been demonstrated to inhibit the growth of spoilage organisms (yeasts and molds) and the growth of artificially inoculated food pathogens, i.e., *S. enterica* and *Bacillus cereus*, in the Greek deli-type yogurt/garlic emulsion-type salad appetizer "Tzatziki" (Tsiraki and Savvaidis, 2014, 2016). In the current study, we demonstrated that Citrox applied alone was more effective against *S. enterica* than *E. coli* (inoculated) in turkey meat stored under VP at 4 and 10 °C, whereas when combined with chitosan, Citrox caused dramatic reductions in both *E. coli* and *S. enterica* populations, irrespective of the temperature. Work by Callaway et al. (2008) showed that citrus products can inhibit the growth of *E. coli* O157:H7 and *Salmonella* spp.; however, these researchers added orange pulp and orange peel (the source of the CEOs) to pure cultures of *E. coli* and *S.* Typhimurium. It is widely known that experiments involving pure cultures in broth are not consistent with those in real food systems because of the effects of different matrices and native microflora. Indeed, in our study, we found that treatment of pure cultures of *E. coli* O157:H7 and *S. enterica* in TSB broth with Citrox, chitosan, and their combination on resulted in significant reductions in the numbers of bacteria, inconsistent with our current experimental *in vitro* data.

In the present study, we showed the antimicrobial effects of chitosan against both *E. coli* and *S. enterica* in turkey samples stored at 4 and 10 °C. Of the two antimicrobials examined, the use of chitosan was more effective than that of Citrox. Consistent with this, the presence of nutrients, such as proteins and salt, as well as the pH may influence the efficacy of CEOs in foods (Gutierrez et al., 2008).

Interestingly, treatment with chitosan alone showed inhibitory effects against *E. coli* at 4 °C and *S. enterica* at 10 °C. Chitosan exerts antimicrobial effects against gram-negative bacteria (Fernandez-Saiz et al., 2008). Additionally, the behavior of *E. coli* in presence of chitosan may be attributed to the low storage temperature (4 °C) as this microorganism can grow well at temperatures of at least 7 °C or higher. Additionally, Tsai et al. (2006) demonstrated that chitosan is more effective at lower temperatures. In contrast, in turkey samples inoculated with *S. enterica*, chitosan was not as effective at 4 °C as compared with that at 10 °C; the reason for this observation is not clear as this organism would be expected to show better growth at the higher temperature. In contrast to our results, Shekarforoush et al. (2015) recently reported that chitosan was not effective against *E. coli* O157:H7 inoculated in ready-to-eat chicken meat stored at 3, 8, or 20 °C. Bacterial strain, incubation temperature, and food substrate are the main variables that greatly determine the activity of chitosan (Fernandez-Saiz et al., 2010).

In the current study, we demonstrated the additive antibacterial effects of Citrox and chitosan in turkey meat on the growth of *E. coli* and *S. enterica*, and this could be explained by the disintegration of the protective outer membrane by Citrox, which could enhance the sensitivity of the bacteria to chitosan. Due to the stabilizing and emulsifying nature of chitosan, increases in the efficacy of CEOs are observed when used synergistically. In other studies, Solomakos et al. (2008) showed that when thyme EO (0.6%) was used in combination with nisin (500 and 1000 IU/g), an additive effect was observed against *E. coli* O157:H7 in minced beef. Shekarforoush et al. (2015) recently reported findings demonstrating an important antimicrobial combination of chitosan and oregano EO against *E. coli* O157:H7 and *Listeria monocytogenes* 4b in ready-to-eat chicken. Moreover, Nair et al. (2015) showed that the combination of carvacrol with MAP was effective in reducing *Salmonella* in turkey breast cutlets.

5. Conclusion

The results of this study indicate that Citrox plus chitosan could be used as an antimicrobial treatment against *E. coli* O157:H7 and *S. enterica* in VP turkey meat stored at refrigeration (4 °C) or mild temperatures (10 °C), maintaining populations of these pathogens at low levels. Chitosan added alone was capable of controlling the growth of *E. coli* at 4 °C and *S. enterica* at 10 °C. Citrus extract was more effective at reducing *S. enterica* on turkey meat at either 4 or 10 °C than against *E. coli*. However, further studies are needed to support and reinforce our findings for different food products.

Acknowledgments

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for funding this research though Research Group Project No. RG-1435-016. Additionally, a Hellenic Scholarship Foundation (IKY, Athens, Greece) grant to Ms. V. D. Vardaka is greatly appreciated.

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