Effect of chitosan and *Trachyspermum ammi* essential oil on microbial growth, proteolytic spoilage, lipid oxidation and sensory attributes of chicken fillet during refrigerated storage

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Abstract:

BACKGROUND: Chicken meat is susceptible to microbial and chemical contamination and using antimicrobial agents helps to prolong its shelf-life. **OBJECTIVES:** This study focuses on the effect of chitosan-based coating and *Trachyspermum ammi* essential oil on qualitative properties of refrigerated chicken fillet meat. **METHODS:** Chicken fillets treated with chitosan solution (2 %wv^{-1}) and *Trachyspermum ammi* essential oil (0, 1000, and 2000 ppm) were stored at 4 ^{o}C for 12 days. Microbial (lactic acid bacteria, coliform, and total mesophilic bacteria), chemical (peroxide value and total volatile nitrogen), and sensory (odour, texture, colour, and overall acceptance) analysis were done every 3 days until the end of storage. **RESULTS:** Total count, lactic acid bacteria, coliform and total mesophilic bacteria showed significant decrease in treatments compared to control. In chemical experiments, chitosan- *Trachyspermum ammi* essential oil treated fillets had lower peroxide and total volatile nitrogen during storage. Sensory analysis revealed that treatment containing 1000 ppm *Trachyspermum ammi* essential oil had higher overall acceptance than control and 2000 ppm treated group. **CONCLUSIONS:** Combination of chitosan and *Trachyspermum ammi* essential oil prolonged shelf-life of fillets. By considering sensory results and cost of analysis, chitosan containing 1000 ppm *Trachyspermum ammi* essential oil is recommended for industries.

Introduction

Food safety is already an important issue in the world despite various developments in food preparation and production and also animal slaugtering. It is estimated that 30% of people suffer from food borne diseases at least one time a year in industrialized countries (Scott, 2003). Therefore, foods should be stored properly for keeping them from spoilage and quality loss. The use of antimicrobial preseravtives is one of the safety

providing approaches. But, elevated doses of these chemicals can cause poisoning. In some cases, such as nitrate and nitrite, they pose carcinogenic effect if used more than maximum limit (Jahangir et al., 2011).

Today, people demand healthier foods with lower synthetic additives and longer shelf-life, and therefore, natural additives have become popular (Davidson and Zivanovic, 2003). Edible films prepared by natural biopolymers are interesting in experiments and practice. Various biopolymers including starch, cellulose derivatives, chitin, chitosan, hydrocolloids, proteins, and fats are used for coating of fresh and/or processed foods in order to increase their shelf-life. Edible films are popular because they are commonly biocompatible, nontoxic, and they have received considerable attention because of their advantages over synthetic films. They also preserve foods against physical, chemical and biological hazards by providing a barrier to moisture, oxygen and solute movement for the food (Kerry et al., 2006; Joerger, 2007; Vásconez et al., 2009). Chitosan is a cationic biopolymer derived from crustaceans' chitin through deacethylation process by chemical, enzymatic and microbiological processes. It has various applications in food industry and is the second natural biopolymer in frequency (cellulose is the first one). Chitosan has functional properties of antimicrobial, antifungal and antioxidant. It is a dietary fibre and its solution can form thin layers with high adhesiveness and absorbability, which are used in food industry, medicine, pharmacology, cosmetics, textile, and color industry (No et al., 2007; Vásconez et al., 2009). *Trachyspermum ammi* is fruit of Umbellifererae family which has 4-6% essential oil. It grows in grain farms in central Europe and Asia, especially India and Iran. In Iran, it mainly grows east of Sistan and Balouchestan. Main components of *Trachyspermum ammi* are thymol, cymene, and gamma terpinene. Some positive biological roles of *Trachyspermum ammi* including antiviral, anti-inflammatory, antifungal, antipyretic, antifilarial, analgesic, anti-nociceptive, and antioxidant have been approved (Gandomi et al., 2014).

Meat is a perishable food and is quickly subjected to chemical and microbial contamination. Its deterioration leads to organoleptic changes other than safety and quality loss. Edible films containing botanical extract and essential oils are used on fresh, frozen and processed meats surface to extend storage period and promote sensory attributes (Davidson and Zivanovic, 2003). There have been no reports of combination effects of chitosan and *Trachyspermum ammi* essential oil on chicken fillet. So, the aim of our research was studying the effect of chitosan and different concentrations of *Trachyspermum ammi* essential oil on storage time and quality of chicken fillet under refrigerated condition.

Materials and Methods

Essential oil preparation: 100 g of *Trachyspermum ammi* plant were milled and essential oil was extracted by water distillation using a Clevenger apparatus. Extracted essential oil was dehydrated with anhydrous sodium sulphate and stored in refrigerator for further analysis. Essential oil was analysed by Gas Chromatography (Agilent 6890, USA)-Mass Spectroscopy (Agilent 5973, USA). GC-MS was equipped with HP-5 MS capillary column (30 m×0.25mm×0.75mm). Carrier gas of

helium was used at flow rate of 0.8 ml/min. Initial temperature of column was set at 50 °C by further increasing to 240 °C at the rate of 3 °C/min. Then, temperature was raised to 300 °C at 15 °C/min and was held at final temperature for 3 min. Ion source and GC injector had the temperature of 220 and 290 °C, respectively (Jemings and Shibamoto, 1980).

Peaks were identified based on comparing their retention indices with literature values and their mass spectral data with those from the MS data system (Willey-229 lib., Nist-62 lib., and Nist-12 lib) (Adams, 1995).

Chitosan edible coating: Chitosan powders (190-310 kDa molecular-weight, Sigma, USA) with a de-acetylation degree of 75-85% were used in this study. 3 g chitosan was added to 100 ml of acetic acid (1% vv^{-1}) under stirring at 40 °C. Then, 0.75 ml of glycerol per 1 g of chitosan and 0.02 % wv^{-1} of polysorbate were added as plasticizer and emulsifier, respectively. The mixture was stirred at 30 °C for 30 min. Final solution was divided into three parts containing 0, 1000 and 2000 ppm *Trachyspermum ammi* essential oil. Chitosan-essential oil solutions were stirred for an additional 2 min (Ojagh et al., 2010).

Chicken fillet coating: Fillets were obtained from a local commercial poultry processing plant. They were taken from production line and transported under refrigeration to the laboratory within a few hours. They were cut into 10-g pieces and submerged in solutions for 30 sec. Submerging was repeated 2 times with 2 min interval. Control samples had no coating. Finally, fillets were packed in stomacher bags (Interscience, Germany) and stored at 4 °C. Experiments were done every 3 days (Ojagh et al., 2010).

Chemical analysis (Proximate composition): Moisture, protein and fat were analysed by AOAC methods (AOAC, 2005b). Moisture content was determined by drying the samples overnight at 105 °c. The protein content was determined by the Kjeldahl method and the lipid content was determined by the Soxhlet method.

Peroxide value: Peroxide test was done acording to the AOAC method (AOAC, 2005a). In this regard, lipid sample (1.0 g) was treated with 25 ml of organic solvent mixture (chloroform/acetic acid, 2/3: v/v). The mixture was shaken vigorously, followed by addition of 1 ml of saturated potassium iodide (Merck) solution. The mixture was kept in the dark for 5 min before adding 75 ml of distilled water. 0.5 ml of starch solution (1%, w/v) was added to the mixture, as an indicator. The PV was determined by titrating the iodine liberated from potassium iodide with standardised 0.01 N sodium thiosulfate solution and was expressed as milliequivalents (meq) of peroxide per kg of lipid. For fat extracion, 50 g of fillet was mixed with 150 ml of Chloroform:methanol (2:1) for 1 min. After filteration, it was added to 50 ml of pottasium choride solution (0.88%). Then, the permeate (water phase) was removed and the supernatent was mixed with 100 ml of methanol:pottasium chloride 0.88% (1:1) in 2 steps. Final solution was dried with rotary evaporation (IKA, Germany) at 35 °C and residual solvent was removed under nitrogen gas (Jebelli Javan et al., 2013).

Total volatile nitrogen (TVN): This factor was analysed by Kjeldal method (Mihaljev et al., 2015). The result was reported as mg of nitrogen in 10 g of fillets.

Microbial experiments: Serial dilutions of chicken samples were prepared in peptone water (0.1 % w/v in distilled water) and

bacteria were cultured in media as below:

MRS agar for lactic acid bcterai (Ojagh et al., 2010), VRBA for Coliform (Ojagh et al., 2010), and Brain Heart Infusion Agar for total count (Ojagh et al., 2010). All media were obtained from Merck Company (Germany).

Sensory evaluation: Six trained penalists of laboratory staff assessed the samples by 9 points hedonic method. Scales were considered as dislike extremely for 1 and like extremely for 9 (Fan et al., 2009).

Statistical analysis: Data were analyzed by SPSS software (version 16.0). Normality of data was tested with Kolmogorov-Smirnov method and One-Way ANOVA was used for comparison of means. Significant differences of means were determined by Tukey method. All experiments were done in triplicate. Significant level was 0.05. Sensory results were analysed by Kruskal Wallis and Mann-Whitney methods.

Results

Chemical analysis: Essential oil analysis is presented in Table 1. The results of gas chromatography-mass spectrometry analysis showed eight compounds consisting of more than 98% of the hydrodistilled essential oil. The major components were thymol (62.42%), p-cymene (18.01%) and γ-terpinene (15.89%).

Proximate composition: Moisture, protein and fat were 76.2±1.3%, 20.5±0.5%, and 1.8±0.3%, respectively.

Peroxide Value (PV): Results of peroxide values are reported in Table 2. It can be seen that oxidative spoilage in essential oil containing treatments was significantly lower than control ($p<0.05$). Results showed that concentrations of essential oil had no significant effect on peroxide value until day 6. Furthermore, there were no significant differences between essential oil-free chitosan coating and chitosan-essential oil samples until day 3 ($p>0.05$) which was due to the anti-oxidative effect of chitosan.

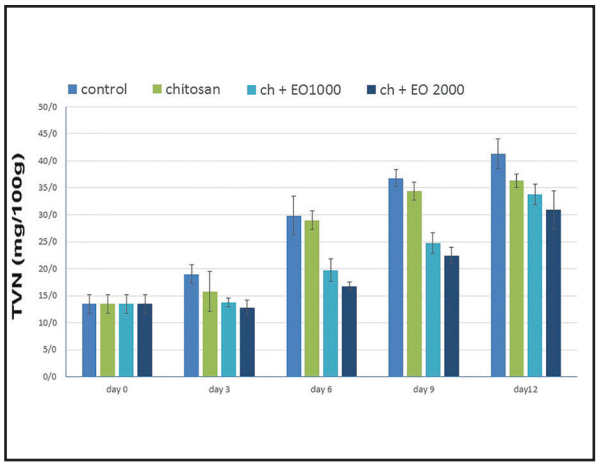

Figure 1. Total volatile nitrogen (mg $100g^{-1}$) in chicken fillet coated by chitosan and different *Trachyspermum ammi* essential oil concentrations during 12 days at 4°C.

Table 1. *Trachyspermum ammi* composition.

Components	Retention index	Percent
α-thujene	931	0.07
α-pinene	939	0.09
Sabinen	976	0.44
β-pinene	980	0.84
ρ-cymene	1018	18.01
γ-terpinen	1061	15.89
Thymol	1278	62.42
Carvacrol	1292	0.33
Total		98.09

Total volatile nitrogen: Figure 1 shows volatile nitrogen changes in control and treatments. The maximum desirable amount of TVN in chicken meat is considered as 27 mg /100 g^{-1} of meat (Fallah et al., 2016).

Control group showed increasing trend over time and their TVN reached to 29.8 on day 6. Although essential oil-free chitosan group had lower TVN compared to control, the differences were not significant ($p>0.05$). Adding essential oil in both concentrations reduced TVN significantly

Table 2. Peroxide value (meq kg-1) in chicken fillet coated by chitosan and different *Trachyspermum ammi* essential oil concentrations during 12 days at 4°C. * Different letters at each column show significant differences (p≤0.05). ** n=3.

	Day				
	0	3	6	9	12
Control	4.7±0.6[a]	6.7±0.6[a]	10.5±1.3[a]	12.9±0.6[a]	14.4±0.9[a]
Chitosan	4.7±0.6[a]	5.5±1.3[ab]	10.2±0.6[a]	12.0±0.6[a]	13.2±0.3[a]
Chitosan+EO 1000	4.7±0.6[a]	4.8±0.3[b]	6.9±0.7[b]	9.9±0.4[b]	12.0±0.4[b]
Chitosan+EO 2000	4.7±0.6[a]	4.5±0.5[b]	5.9±0.3[b]	7.9±0.5[c]	9.2±1.0[c]

Table 3. LAB (Log CFU g-1) in chicken fillet coated by chitosan and different *Trachyspermum ammi* essential oil concentrations during 12 days at 4°C. * Different letters at each column show significant differences (p≤0.05). ** n=3.

	0	3	6	9	12
Control	4.2±0.29[a]	4.8±0.4[a]	6.1±0.33[a]	7.1±0.37[a]	7.2±0.39[a]
Chitosan	4.2±0.29[a]	4.3±0.43[a]	6.0±0.47[a]	7.0±0.36[a]	7.1±0.32[a]
Chitosan + EO 1000	4.2±0.29[a]	2.8±0.42[b]	4.6±0.47[b]	5.5±0.49[b]	5.9±0.32[b]
Chitosan+ EO 2000	4.2±0.29[a]	2.0±0.05[c]	3.6±0.46[c]	4.7±0.47[b]	5.2±0.47[b]

Table 4. Coliform (Log CFU g-1) in chicken fillet coated by chitosan and different *Trachyspermum ammi* essential oil concentrations during 12 days at 4°C. * Different letters at each column show significant differences (p≤0.05). ** n=3.

	0	3	6	9	12
Control	2.3±0.22[a]	3.2±0.11[a]	5±0.14[a]	5.5±0.06[a]	6.6±0.13[a]
Chitosan	2.3±0.22[a]	2.9±0.11[b]	4.8±0.12[a]	5.2±0.25[a]	6.5±0.07[a]
chitosan +EO 1000	2.3±0.22[a]	2.6±0.1[c]	3±0.08[b]	3.9±0.1[b]	5.10±13[b]
chitosan+ EO 2000	2.3±0.22[a]	2.1±0.09[d]	2.3±0.2[c]	3±0.13[c]	4.1±0.14[c]

($p<0.05$) and also no significant difference was observed between essence treated samples ($p>0.05$).

Microbial analysis (Lactic acid bacteria): According to Table 3, chitosan had no inhibitory effect on LAB so no significant differences were observed between control and chitosan treated sample throughout the study ($p>0.05$). In comparison, in essential oil treated samples (1000 and 2000 ppm), the number of LAB was lower than 6 log CFU g^{-1} at day 12. Our results showed that there was no significant difference in 1000 ppm and 2000 ppm essential oil treated samples from day 6 to the end of study ($p>0.05$) while they both were significantly different from control ($p<0.05$).

Coliform data are shown in Table 4. It can be seen that number of Coliform in control changed from 2.3 log CFU g^{-1} at day 0 to 6.6 log CFU g^{-1} at day 12. A similar trend was observed in chitosan treated sample with the exception of day 3, when chitosan had a significant inhibitory effect compared to control. Using 1000 and 2000 ppm essential oil resulted in 1.5 and 2.5 log CFU g^{-1} reduction at day 12 compared to control which was more significant in Coliform than LAB. The most significant inhibitory effect was observed in 2000 ppm essential oil treated sample.

Total mesophilic bacteria: According to Table 5, the number of mesophilic bacteria in control increased continuously. In chitosan group, mesophilic bacteria showed no significant change compared to control during the study ($p>0.05$). Adding 1000 ppm essential oil led to significant changes compared to both control and chitosan samples so that bacteria were counted lower

Table 5. Total mesophilic bacteria (log CFU ml-1) in chicken fillet coated by chitosan and different *Trachyspermum ammi* essential oil concentrations during 12 days at 4°C. * Different letters at each column show significant differences (p≤0.05).** n=3.

	0	3	6	9	12
Control	4.5±0.21[a]	5.1±0.11[a]	7.0±0.31[a]	7.6±0.21[a]	7.6±0.18[a]
Chitosan	4.5±0.21[a]	5.0±0.26[a]	6.8±0.18[a]	7.6±0.11[a]	7.6±0.21[a]
Chitosan+EO 1000	4.5±0.21[a]	2.9±0.23[b]	5.1±0.15[b]	5.9±0.10[b]	7.1±0.08[a]
Chitosan+ EO 2000	4.5±0.21[a]	2.2±0.11[c]	4.1±0.21[c]	5.0±0.11[c]	6.8±0.07[b]

Table 6. Scores of sensory evaluation at day 3. * Different letters at each row show significant differences (p≤0.05).

	Control	Chitosan	Ch+ EO 1000	Ch+ EO 2000
Color	7.8[a]	8[a]	7.75[a]	7.5[a]
Odor	8.25[a]	8[a]	7[b]	5.75[c]
Taste	8[a]	8[a]	6.75[b]	5.5[c]
Texture	7[a]	7[a]	7[a]	7[a]
Overall Acceptance	7.5[a]	7.25[a]	6.25[b]	5.75[c]

than 7 log CFU g^{-1} until day 9. The more inhibitory effect was observed when 2000 ppm was added to the coatings and bacteria were lower than 7 log CFU g^{-1} until day 12. Significant changes were observed between 1000 and 2000 ppm groups until day 12 ($p<0.05$).

Sensory evaluation: Result of sensory evaluation on day 3 is presented in Table 6. Chicken fillets are appropriate for human consumption when sensory score is more than 6 (Goulas and Kontominas, 2005).

Control and chitosan groups had no significant changes over time. In essenial oil contining groups, no significant changes were observed in color and texture, while they were differently scored in odor, taste and overall acceptance. Changes were more significant in 2000 ppm group so that the sensory scores were lower than critical level (score 6).

In 1000 ppm group, the lower score was also observed compared to control and chitosan but they were within the acceptable limit.

Discussion

Essential Oil Compositions: As shown in Table 1, thymol, p-cymene and γ-terpinene were found as the major components.

Some previous studies have reported that thymol is the major component of this oil (Gandomi et al., 2014). Although in our study (Iranian species) thymol represents 62.42% of the total oil, in Indian species (Paul et al., 2011), it appears to represent 49.64 %, which may reflect variations due to time of plant growing, preparation process, cultivar differences and geographical location from which the plants were collected (Jebelli Javan et al., 2013).

Peroxide Value (PV): Combination of chitosan and essential oil could increasingly delay oxidation of fillets compared to control during storage. Peroxide value in control and chitosan samples exceeded the maximum level (10 meq/kg) (Jebelli Javan et al., 2015) on day 6 and treated samples with chitosan+1000ppm essential oil and chitosan+2000ppm essential oil passed the upper limit on days 9 and 12, respectively. Other than anti-oxidative effect of *Trachyspermum ammi* essential oil which is due to some components such as thymol, ρ-cymene and γ-terpinen (Burt, 2004), chitosan can increase this effect by its chelating activity (Wang et al., 2003).

Total volatile nitrogen: It is reported that meat and chicken usually contain 30 mg

TVN per 100 g product at the beginning of deterioration (Fallah et al., 2016). Adding essential oil in both concentrations reduced TVN significantly so that essential oil treated samples were within TVN range until day 9, indicating a fast reduction of bacterial population or limited potential of microorganisms for deamination of non-protein nitrogen compounds as a result of essential oil addition in treated samples (Fan et al., 2008). Lopez et al. also observed the protective role of chitosan and gelatin in inhibition of TVN increasing and further spoilage of fish patties (López-Caballero et al., 2005).

Microbial analysis: The results indicated that chicken fillets treated with chitosan and *Trachyspermum ammi* essential oil at 1000 and 2000 ppm can reduce the growth rates of LAB, Coliform and mesophilic bacteria in the treated chicken breast samples. This is in line with previous studies on in-vitro antimicrobial activity of *Trachyspermum ammi* essential oil (Gandomi et al., 2014). In this regard, inhibitory effect of *Trachyspermum ammi* essential oil against some of the bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, *E coli* and *Klebsiella pneumoniae* has been shown (Usha et al. 2012; Hassanshahian et al., 2014). Chemical composition of *Trachyspermum ammi* essential oil exhibited the presence of thymol, γ- terpinene and p-cymene without carvacrol as the main components of the oil. Essential oils rich in carvacrol, thymol, γ-terpinene and p-cymene have recently gained great importance for their considerable antimicrobial activity (Jebelli Javan et al., 2013). The antimicrobial effect of thymol and carvacrol is due to damage in cell membrane integrity with change in pH hemostasis and ion exchange equilibrium. p-cymene does not have antimicrobial activity but it can increase the antimicrobial activity of thymol or carvacrol by synergistic effect (Hassanshahian et al., 2014). It has been proved that essential oils have more inhibitory effect on gram positive bacteria (Bassolé and Juliani, 2012). This is due to the outer membrane around gram negative bacteria which act as an external barrier (Singh et al., 2007). In our study, using chitosan enhanced the effect of essential oil against gram negative bacteria (coliform compared to LAB). It can be related to the chelation of metals which play a crucial role in stabilizing the cell wall by its amine group which is bound to hydroxyl and carboxyl moieties (Wang et al., 2003; Zimoch-Korzycka et al., 2016). The higher inhibition owing to the higher amount of esential oil in our result was in agreement with the study of Chouliara et al (2007) which studied the inhibitory effect of essential oil and modified atmosphere on mesophilic bacteria (Chouliara et al., 2007). In general, combination of chitosan and *Trachyspermum ammi* essential oil had more inhibitory effect on mesophilic bacteria than mentioned study. Tagarida et al (2000) also observed 2-3 log CFU g^{-1} reduciton in Listeria monocytogenes by using 2.8% oregano, but the essential oil was used in more than 10 times concentration compared to the current study (Tsigarida et al., 2000).

Sensory evaluation: In the current study, effect of lower concentration of essential oil and chitosan on sensory attributes was acceptable. This result was contrary to Chouliara et al's (2007) study by using 1% oregano on sensory parameters of fresh chicken breast meat (Chouliara et al., 2007).

Conclusion: Using *Trachyspermum ammi* essential oil and chitosan could inhibit bacterial growth on the surface of chicken

fillets and prolong the shelf-life over 3 days. Due to the unacceptable sensory scores of 2000 ppm groups, we suggest the treatment containing 1000 ppm essential oil because it showed no significant differences with 2000 ppm in hydrolytic and oxidative spoilages until the 9th and 6th days, respectively and could suppress microbial growth significantly.

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مطالعه اثر پوششی کیتوزان حاوی اسانس زنیان بر میزان رشد میکروبی، فساد پروتئولیتیک، اکسیداسیون چربی‌ها و تغییرات حسی فیله‌های مرغ در طی نگهداری در شرایط یخچالی

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چکیده

زمینه مطالعه: گوشت مرغ مستعد آلودگی میکروبی و شیمیایی است، بنابراین استفاده از نگهدارنده‌هایی با خصوصیت ضد میکروبی ضروری می‌باشد. **هدف:** این مطالعه به منظور بررسی اثر پوشش خوراکی کیتوزان و اسانس زنیان بر روی کیفیت و زمان ماندگاری فیله‌های گوشت مرغ در دمای یخچال بررسی گردید. **روش کار:** در این مطالعه فیله‌های مرغ توسط محلول کیتوزان ۲٪ حاوی غلظت‌های ppm ۰ و ۱۰۰۰ و ۲۰۰۰ اسانس زنیان تیمار و به مدت دوازده روز در چهار درجه سلسیوس نگهداری شد. آزمون‌های میکروبی (شمارش کلی باکتری‌های اسید لاکتیک، کلیفرم‌ها و شمارش کلی میکروارگانیسم‌های مزوفیل) و آزمون‌های شیمیایی (تعیین عدد پراکسید و باز های ازته فرار) و خصوصیات حسی (بو، بافت، رنگ، پذیرش کلی) در بازه زمانی سه روز یک بار انجام گرفت. **نتایج:** نتایج نشان داد که شمارش باکتری‌های اسید لاکتیک ، باکتری‌های کلیفرم و شمارش باکتری‌های مزوفیل در گروه‌های تیمار نسبت به گروه کنترل کاهش معنی‌داری را نشان داده است. بررسی نتایج مربوط به آزمون عدد پراکسید نشان داد که تیمار کیتوزان همراه با اسانس زنیان به صورت معنی‌دار توانست پرکسیداسیون چربی را در فیله‌های مرغ به تأخیر بیندازد همچنین استفاده از پوشش کیتوزان و اسانس زنیان باعث کاهش میزان بازهای ازته فرار نسبت به گروه کنترل گردید. از نظر خصوصیات حسی گروه پوشش کیتوزان با ppm ۱۰۰۰ اسانس بیشتر مورد تأیید قرار گرفت. **نتیجه گیری نهایی:** در مجموع استفاده توام اسانس زنیان و کیتوزان باعث افزایش مدت زمان ماندگاری فیله مرغ گردید و با توجه به آزمون‌های حسی و مسائل اقتصادی پوشش کیتوزان و غلظت ppm ۱۰۰۰ اسانس زنیان جهت افزایش مدت زمان نگهداری فیله مرغ در صنعت گوشت وطیور توصیه می‌گردد.

واژه های کلیدی: فیله مرغ، پوشش خوراکی کیتوزان، اسانس، زنیان، زمان ماندگاری

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