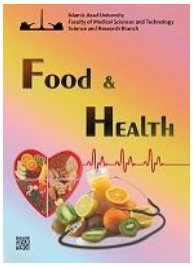

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Effect of chitosan coating contain Ajwain essential oil on the shelf-life of chicken breast meat during refrigerated condition

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ABSTRACT

Biodegradable active packaging containing essential oils with antimicrobial properties is one alternative strategy that can be considered by the food packing industry to reduce the use of environmentally harmful synthetic polymers. Chitosan is a safe, natural, no allergen, and biocompatible polymer with health benefits. This study was conducted to investigate the effects of chitosan coating contain Ajwain essential oil (AEO) on the shelf-life of chicken breast meat during refrigerated storage. AEO was extracted and its composition was analyzed by GC/MS, the AEO was added to chitosan solution in different concentrations (0, 0.5%, and 1% (v/v)) and chicken breast meat was coated by chitosan solution, The microbiological properties of chicken breast meats, such Total aerobic mesophilic bacteria (AMB), *Enterobacteriaceae*, total aerobic psychrotrophic bacteria (APB), and *Pseudomonas* spp. were determined during 13 days of storage. The results showed that using of AEO has significant effects on the reduction of all followed groups of microorganisms compared with the control group. In the present study, 1% v/v AEO treatment was the most effective in microbial groups throughout the storage period. This study showed that chitosan coating contains 1% AEO have good antimicrobial activity and can be used to extend the shelf-life of the meat products.

1. Introduction

Chicken meat is favored by consumers worldwide because of its desirable nutritional qualities, such as low-fat content and a relatively high concentration of polyunsaturated fatty acids (1). Poultry meat is a highly perishable food commodity providing an almost perfect medium for microbial growth including both spoilage and pathogenic microorganism (2). Decreasing microbial growth during storage can increase the shelf-life of meat (3). Furthermore, spoilage of fresh poultry meat is a financial burden to the producers and requires the development of new methods to extend the shelf-life and overall safety/quality of the meat, which is the main problem faced by the poultry processing industry (4, 5). Interest in edible films and coatings in recent years has been on the rise due to several advantages such as biodegradability, environmental friendliness, and extended shelf life. Coatings are a particular form of film designed for application directly onto the surface of target materials; although its removal may eventually be possible, they are normally regarded as part of the final product. Edible films and coatings are generally manufactured from proteins, polysaccharides, and lipids, used solely, or in combination with each other (6). Chitosan a cationic polysaccharide made from the alkaline N-deacetylation of chitin is commercially prepared from shellfish processing waste (2). produced by the deacetylation of chitin, is widely used in phenolic preservation of some fruits such as pomegranate, litchi, and raspberries (3). Chitosan is effective against both gram-positive and gram-negative bacteria and fungi (2–4). Essential oils are mixtures of compounds which was characterized by their capacity to generate flavor or aroma and are generally obtained from spices, aromatic herbs, fruits, and flowers (7). Ajwain (*Carum copticum* Benth. & Hook.) is a grassy, an annual herbaceous essential oil-bearing plant belonging to the *Apiaceae* family, which grows in India, Iran, and Egypt (8). Hydrodistillation of Ajwain fruits yields an

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essential oil consisting primarily of thymol, gamma-terpinene, and p-cymene as well as more than 20 trace compounds (predominately terpenoids) The Ajwain fruit oil has several therapeutic effects, including diuretic, carminative, analgesic, anti-dyspnoea and, anti-inflammatory compounds, carminative, anti-spasmodic, stimulant, anti-dyspnea, analgesic, anti-asthma, anti-vomiting, antimicrobial and antioxidant activity, anti-aflatoxigenic and antitermitic nature (8–12). To the best of our knowledge, there is a lack of data about using Ajwain essential oil in edible coatings. Hence, this study aimed to investigate the effect of chitosan coating containing with Ajwain essential oil on the shelf-life extension of fresh chicken breast meats stored at 4 ºC for 13 days.

2. Materials and methods

2.1. Essential oil extraction

Ajwain seeds were purchased from Shiraz (Iran). In the preparation phase, 150 grams of powdered seeds were mixed in 1 liter of distilled water. The oil extraction Hydro distillation was done in 3h, using a Clevenger-type apparatus according to the method recommended by the European Pharmacopoeia (8). The EO obtained were separated from water and dried over anhydrous Na_2SO_4, and stored in dark glass bottles at 4˚C before use (13).

2.2. GC and GC/MS analyses

Gas chromatography analysis was performed using HP 6890 gas chromatograph equipped with an FID and DB1 fused silica column (60 m×0.25 mm i.d, film thickness 0.25 µm). The oven temperature was programmed at 250ºC at a rate of 4ºC/min. Injector and detector temperatures were 250ºC and 265ºC, respectively. The carrier gas, helium, was adjusted to a linear velocity of 30 cm/s and 1 µl of sample dissolved in CH_2Cl_2 was injected. Gas chromatography/mass spectrometry (GC-MS) analysis. GC conditions were the same as reported, and the same column was used. MS conditions were as follows: ionization voltage, 70 eV; ion source temperature was 260ºC scan rate, 1 scan/s. Identifications of components of the oil were based on retention indices relative to normal alkanes and computer matching with the Wiley 275 library, as well as by comparison of the fragmentation patterns of mass spectra with those reported in the literature (14).

2.3. Preparation of edible coatings

Chitosan (medium molecular weight, Sigma-Aldrich Chemical Co.) solution was prepared with 1.5% (w/v) chitosan in 1% (v/v) acetic acid. To achieve complete dispersion of chitosan, the solution was stirred at room temperature overnight. The solution in beakers was placed into a hotplate/magnetic stirrer and glycerol was added to chitosan at 0.75 mL/g concentration as a plasticizer and stirred for 10 min. The resultant chitosan coating solution was filtrated through a Whatman No. 3 filter paper to remove any undissolved particles. Then the AEO mixed with Tween 80 (Aldrich Chemical Co., Steinheim, Germany), and added to the chitosan solution. The final coating solution consisted of 1.5% chitosan, 1% acetic acid, 0.75% glycerol, 0.2% Tween 80 singly or incorporated with 0.5% and 1% AEO. The final coating forming solution was homogenized under aseptic conditions at 21600 rpm for 1 min. The control solution was prepared without the addition of AEO (15).

2.4. Preparation of chicken poultry

The fresh chicken was provided by local distribution centers 2 h after slaughter. They were placed in insulated polystyrene boxes within the ice and transferred to the laboratory. The chicken breast meat was aseptically removed and cut into 25 g by a sterile knife and used as described below. The chicken breast meat was covered in three steps: dipping, draining, and drying under sterilized conditions using a laminar flow hood. After preparing different treatments, chicken breast meat for 2 minutes in a solution prepared was immersed. Coating solutions (CS) excess was drained during 30 s and coatings were formed exposing medallions to a cool air stream during 45s on each side. The uncoated and coated samples (C- A0.5-A1) were packed in sterile polypropylene trays and stored at 4˚C and microbiological; sensory analyses were made after 0, 1, 3, 6, 9, and 13 during storage.

2.5. Microbiological analysis

Chicken meat (10 g) was mixed with 90 mL of 0.1% sterile peptone water (Merck, Darmstadt, Germany) in a sterile stomacher bag and stomached for 1 min. For microbial enumeration, 0.1 mL of serial dilutions of chicken homogenates was spread on the surface of agar plates (3). Microbiological characterization was focused on the main groups responsible for the spoilage of chicken breast meat: Total aerobic mesophilic bacteria (AMB), Enterobacteriaceae, total aerobic psychrotrophic bacteria (APB), and Pseudomonas spp. (3, 16). Total aerobic mesophilic bacteria (AMB) were determined using plate count agar (PCA, Merck, Darmstadt, Germany), after incubation for 2 days at 30˚C (3, 17). *Pseudomonas* spp. were determined using Pseudomonas agar (PAB-CM; Oxoid, England) after incubation for 24 and 48 h at 25˚C. *Enterobacteriaceae* were enumerated by the pour-overlay method using violet red bile glucose (VRBG) agar (Merck, Darmstadt, Germany). The plates were incubated for 24-48 h and 37°C (3, 17). Total aerobic psychrophilic bacteria (APB) were determined on plate count agar and the plates were incubated for 7 days and 7˚C. Microbiological data were reported as logarithms of the number of colony forming units (CFU/g) (16).

2.6. Statistical analyses

Each experiment was carried out in triplicate. Statistical analysis of data was performed using Microsoft Excel. Analysis of variance was calculated using the SPSS program

(SPSS 22, SPSS Inc., Woking, Surrey, UK). With a confidence level of 0.05, to find any significant difference between treatments. Duncan multiple range test (MRT) was used for mean separation at $p<0.05$ where the treatment effect was significant.

3. Results

3.1. The chemical composition of Ajwain essential oil

The results, derived from analysis of Ajwain essential oil of shiraz-Iran (2016), showed essential oil yield was 2.89 % (v/w). Among all individual constituents, which were identified by GC/MS, thymol (27.23%), followed by p-cymene (26.32%) and gamma-terpinene (22.64%) was the major compound of Ajwain EO with 98.6 percentage (Table 1).

Table 1. Main components of Ajwain essential oil.

Component	Retention Index	Content (%)
α-Thujene	922	1.17
α -Pinene	931	0.52
Sabinene	965	0.14
ß-Pinene	971	2.48
Myrcene	979	1.59
p-Cymene	1013	26.32
Sylvestrene	1023	2.31
γ-Terpinene	1051	22.64
meta Cymenene	1073	0.11
Terpinolene	1079	0.28
Terpinen-4-ol	1175	1.64
α-Terpineol	1189	0.42
Carvone	1246	1.73
Thymol	1281	27.23
Carvacrol	1292	9.12
Dill apoile	1591	0.9
Total		98.6

3.2. Bacteriological analysis

The effects of edible coatings were evaluated through the comparison of the development of the microbial population of AMB, Enterobacteriaceae, APB, and Pseudomonas spp. on the coated chicken breasts and on the uncoated ones, which had been stored under refrigeration and analyzed in days 0, 1, 3, 6, 9, and 13 of the storage period (Fig 1-4). Regarding the total aerobic mesophilic bacteria populations analyzed in this research, the initial (day 0) Total aerobic mesophilic bacteria of chicken meat was 3.07 log CFU/g (Fig.1). In particular, on day 13, the total aerobic mesophilic bacteria population reached 12.73 log CFU/g in the control sample, and 8.28 log CFU/g in the samples with the coating containing A1. TVC of the coated samples with the Ajwain essential oil (1% v/v) was below 7 log CFU/g until the ninth day. According to Senter et al. (18), chicken samples reached or exceeded the value of 7.00g CFU/g for total aerobic mesophilic bacteria, which was considered as the maximal acceptability limit for fresh meat. Researchers have reported similar findings indicating that various EOs could reduce the growth of the total aerobic mesophilic bacteria (AMB) population in refrigerated chicken meat (19, 20).

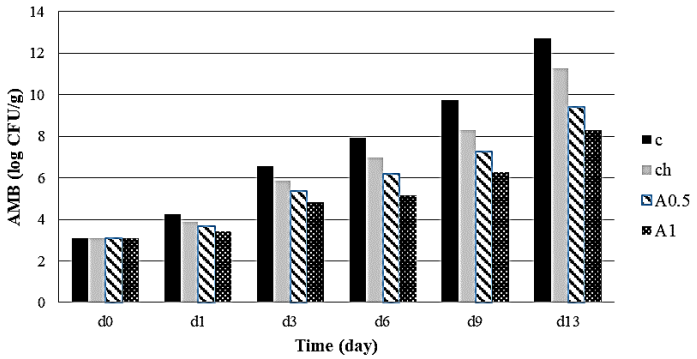

Fig.1. Changes in total aerobic mesophilic bacteria of chicken breast meat during storage at 4 °C.

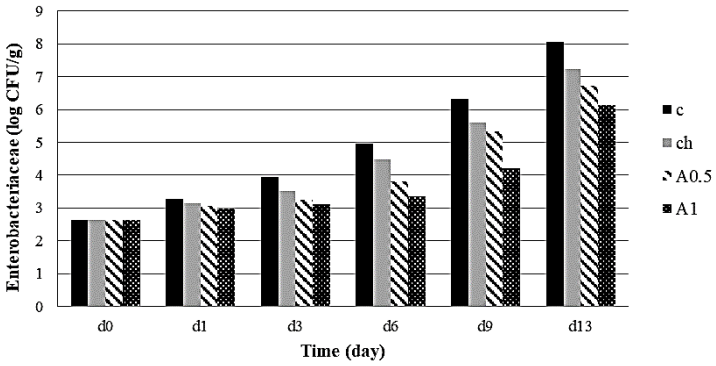

Fig.2. Changes in *Enterobacteriaceae* of chicken breast meat during storage at 4 °C.

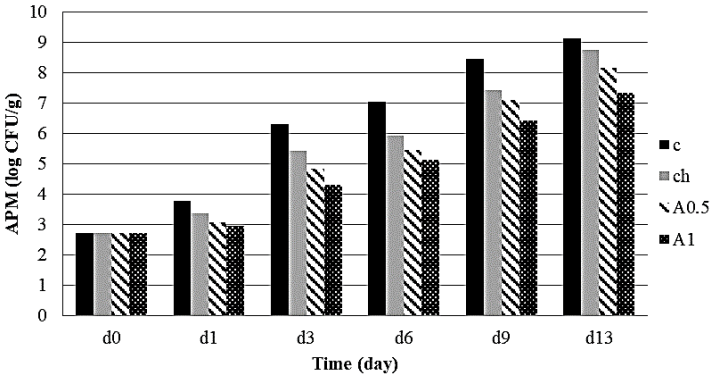

Fig.3. Changes in total aerobic psychrophilic bacteria of chicken breast meat during storage at 4 °C.

In our study, *Enterobacteriaceae*, a facultative anaerobic bacterial group, formed a substantial part of the chicken meat microbial flora and reached final counts of 8.05 logs (control samples) on day-13 (Fig. 2). In our study, the initial count of fresh meat chicken sample was 2.64 log CFU/g and its final population decreased significantly ($p<0.05$) as compared to the control samples (day13). In particular, on day 13, the

Enterobacteriaceae population reached 8.05 log CFU/g in the control sample, and 6.13 log CFU/g in the samples with the coating containing A1. Researchers have reported similar findings indicating that various EOs could inhibit the growth of *Enterobacteriaceae* population in refrigerated chicken meat (2, 19, 20). In our study, the initial Total aerobic psychrophilic bacteria (day 0) of samples ranged from 2.73 log CFU/g to 9.12 log CFU/g in controls (Fig. 3). The total aerobic psychrophilic bacteria population in all treatments was significantly ($p<0.05$) lower than control, A1 treatment was the most effective in psychrotrophic bacteria (PTC) throughout the storage period and extended the shelf life of chicken meat to at least 9 days. These results are consistent with other studies reporting a reduction in psychrotrophic bacteria (PTC) with the addition of a mixture of various EO in meat chicken during storage at refrigeration condition (2, 19, 20).

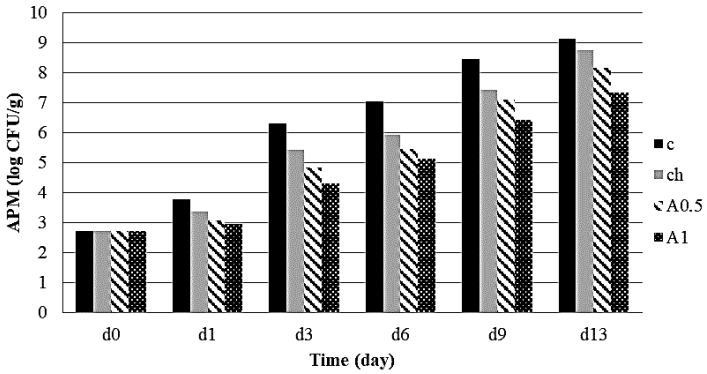

Fig.4. Changes in Pseudomonas spp. of chicken breast meat during storage at 4 °C.

Initial *Pseudomonas* spp. count was 2.98 log CFU/g (Fig.4), increasing during storage to reach the final population of 11.94 log CFU/g (control samples). *Pseudomonas* spp. population in all treatments was significantly ($p<0.05$) lower than control. Samples containing A1 were the most effective treatments for the inhibition of *Pseudomonas* spp. These results are consistent with other studies reporting a reduction in Pseudomonas spp. with the addition of a mixture of various EO in meat chicken during storage at refrigeration condition (2, 19, 20).

4. Discussion

The results, derived from analysis of Ajwain essential oil of Shiraz-Iran, showed essential oil yield was 2.89% (v/w). Among all individual constituents, which were identified by GC/MS. The main constituents were *Thymol* (27.23%), *p-Cymene* (26.32%) and *γ-Terpinene* (22.64 %) stand as the major groups of compounds. In most of the earlier reports with Ajwain essential oil, *thymol* was identified as the chief component (8, 21-23). Khajeh et al. (21) showed that the major components of Ajwain EO were *thymol* (49.0%), *γ-terpinene* (30.8%), *p-cymene* (15.7%). Rasooli et al. (22) showed that has the main compounds including *thymol* (37.2%), *p-cymene* (32.3%), *γ-terpinene* (27.3%). Oroojalian et al. (13) showed that Ajwain EO contained main compounds, including *thymol* (48.4%), *p-cymene* (21.8%), *γ-terpinene* (21.3%), and *β-pinene* (2.6%). Goudarzi et al. (8) reported that Ajwain EO contained main compounds, including *thymol* (36.7%), *p-cymene* (21.1%), *γ-terpinene* (36.5%). Moazeni et al. (23) reported *Thymol* (50.07%), *γ-terpinene* (23.92%), and *p-cymene* (22.9%) were found to be the major Ajwain essential oil. An important characteristic of EOs and their components is their hydrophobicity, which enables them to partition in the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable. Leakage of ions and other cell contents can then occur (7). *Carvacrol* and *thymol* are able to disintegrate the outer membrane of gram-negative bacteria, releasing lipopolysaccharides (LPS) and increasing the permeability of the cytoplasmic membrane to ATP. The presence of magnesium chloride has been shown to have no influence on this action, suggesting a mechanism other than chelation of cations in the outer membrane (7). *p-Cymene* is not an effective antibacterial when used alone but when combined with *carvacrol*, synergism has been observed (7). In general, compared to control samples, A1 treatment was the most effective in total aerobic mesophilic bacteria, *Enterobacteriaceae*, total aerobic psychrotrophic bacteria, and *Pseudomonas* spp. ($p<0.05$) throughout the storage period and extended the shelf life of chicken meat to at least 9 days. In this regard, other studies reported the same results (2,19,20,24). Many authors' researchers have applied antimicrobial essential oil and edible films and coatings of meat products. Yingyuad et al. (25), found that the combined use of vacuum packaging (VP) and chitosan solutions (2% and 2.5% w/v) kept the TPC count of grilled Thai-style pork meat below 4 log cfu/g for more than 28 days, achieving a shelf-life extension of more than 14 days, as compared to the control samples. Georgantelis et al. (24), reported that the combined use of rosemary extract and chitosan on the preservation of fresh pork sausages led to a reduction of the TPC count (ca. by 1–2 log cfu/g), extending their shelf-life at 4 °C. Economou *et al.* (26) evaluated effect of nisin and EDTA treatments on the shelf-life of fresh chicken meat stored under modified atmosphere packaging at 4ºC, and reported that the use of MAP in combination with nisin–EDTA antimicrobial treatments resulted in an organoleptic extension of refrigerated, fresh chicken meat by approximately 1–2 days (N2), 3–4 days (N3 and N4), 7–8 days (N5), 9–10 (N7) and by 13–14 days (N6). The chicken was better preserved under treatments N6 and N7, maintaining acceptable odour attributes even up to 24 and 20 days of storage, respectively. Ojagh et al. (15) evaluated the effects of chitosan coating enriched with cinnamon oil (Ch+C) on the quality of rainbow trout (Oncorhynchus mykiss) during refrigerated storage (4±1˚C) were examined over 16 days. The results indicated that the effect of the Ch + C coating on the fish samples was to enable the good quality characteristics to be retained longer and to extend the shelf life during the refrigerated storage. Khanjari

et al. (2) investigated the combined effect of 1 g/100 ml N, O-carboxymethyl chitosan (NOCC) and 1% oregano essential oil (OEO) dip shelf life extension of chicken breast meats. Results showed that total viable count (TVC) exceeded 7 log CFU/g after day 6 and 10 for control samples and samples treated with OEO respectively. Samples treated with either NOCC or OEO plus NOCC never reached 7 log CFU/g throughout storage. Fernández-Pan et al. (16) developed antimicrobial edible coatings based on WPI incorporating oregano or clove essential oils in order to extend the shelf-life of refrigerated chicken breast. Coatings with 20 g/kg of oregano EO showed their efficacy by doubling the storage time of chicken breast, keeping most of the microbiological groups below the recommended limits for distribution and consumption of chicken breast. Bazargani-Gilani et al. (3) developed antimicrobial edible coatings based on chitosan enriched with *Zataria multiflora* Boiss essential oil on the shelf-life of chicken meat during refrigerated storage 4˚C for 20 days and analyzed at 5-day intervals. All of the treatments significantly decreased microbial groups compared control during the storage period. In the present study, A1 treatment (1% (v/v) Ajwain essential oil) had lowest microbial counts throughout the storage period.

5. Conclusion

Results of the present study demonstrate that the combined use of chitosan and Ajwain essential oil during refrigerated storage could inhibit the growth of microbial spoilage flora of fresh chicken meat.

References

1. Patsias A, Badeka A V., Savvaidis IN, Kontominas MG. Combined effect of freeze chilling and MAP on quality parameters of raw chicken fillets. *Food Microbiology*. 2008;25(4):575-81.
2. Khanjari A, Karabagias IK, Kontominas MG. Combined effect of N, O-carboxymethyl chitosan and oregano essential oil to extend shelf life and control *Listeria monocytogenes* in raw chicken meat fillets. *LWT - Food Science and Technology*. 2016 ;25;53(1):94-9.
3. Bazargani-Gilani B, Aliakbarlu J, Tajik H. Effect of pomegranate juice dipping and chitosan coating enriched with *Zataria multiflora* Boiss essential oil on the shelf-life of chicken meat during refrigerated storage. *Innovative Food Science and Emerging Technologies*. 2015;29:280-7.
4. Petrou S, Tsiraki M, Giatrakou V, Savvaidis IN. Chitosan dipping or oregano oil treatments, singly or combined on modified atmosphere packaged chicken breast meat. *International Journal of Food Microbiology*. 2012;156(3):264-71.
5. Zhang H, Wu J, Guo X. Effects of antimicrobial and antioxidant activities of spice extracts on raw chicken meat quality. *Food Science and Human Wellness*. 2016;5(1):39-48.
6. Ramos OL, Silva SI, Soares JC, Fernandes JC, Poças MF, Pintado ME, et al. Features and performance of edible films, obtained from whey protein isolate formulated with antimicrobial compounds. *Food Research International*. 2012;45(1):351-61.
7. Burt S. Essential oils: their antibacterial properties and potential applications in foods-a review. *International Journal of Food Microbiology*. 2004;94(3):223-53.
8. Goudarzi GR, Saharkhiz MJ, Sattari M, Zomorodian K. Antibacterial activity and chemical composition of Ajowan (*Carum copticum* Benth. & Hook) essential oil. *Journal of Agricultural Science and Technology*. 2011;13(2):203-8.
9. Bekhechi C, Boti JB, Bekkara FA, Abdelouahida DE, Casanova J, Tomi F. Isothymol in Ajowan essential oil. *Natural Product Communications*. 2010;5(7):1107-10.
10. Malhotra SKK, Vijay OPP. Ajowan. Handbook of herbs and spices. 2004;2:107-16.
11. Murthy PS, Borse BB, Khanum H, Srinivas P. Inhibitory effects of Ajowan (*Trachyspermum ammi*) ethanolic extract on *A. ochraceus* growth and ochratoxin production. *Turkish Journal of Biology*. 2009;33(3):211-7.
12. Nagalakshmi S, Shankaracharya NB, Naik JP, Rao LJM. Studies on chemical and technological aspects of ajowan (*Trachyspermum ammi* (L.) Syn. Carum copticum Hiern) seeds. *Journal of Food Science and Technology*. 2000;37(3):277-81.
13. Oroojalian F, Kasra-Kermanshahi R, Azizi M, Bassami MRR. Phytochemical composition of the essential oils from three *Apiaceae* species and their antibacterial effects on food-borne pathogens. *Food Chemistry*. 2010;120(3):765-70.
14. Adams RP. Identification of essential oil components by gas chromatography/ mass spectroscopy. Allured Pub. Corporation; 2007. 807 p.
15. Ojagh SM, Rezaei M, Razavi SH, Hosseini SMH. Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. Food Chemistry. 2010;120(1):193-8.
16. Fernández-Pan I, Carrión-Granda X, Maté JI. Antimicrobial efficiency of edible coatings on the preservation of chicken breast fillets. *Food Control*. 2014;36(1):69-75.
17. Chouliara E, Karatapanis A, Savvaidis IN, Kontominas MG. Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4 °C. *Food Microbiology*. 2007;24(6):607-17.
18. Senter SD, Arnold JW, Chew V. APC values and volatile compounds formed in commercially processed, raw chicken parts during storage at 4 and 13°C and under simulated temperature abuse conditions. *Journal of the Science of Food and Agriculture*. 2000;80(10):1559–64.
19. Moroney NC, O'Grady MN, O'Doherty J V., Kerry JP. Addition of seaweed (*Laminaria digitata*) extracts containing laminarin and fucoidan to porcine diets: Influence on the quality and shelf-life of fresh pork. *Meat Science*. 2012;92(4):423-9.
20. Govaris A, Solomakos N, Pexara A, Chatzopoulou PS. The antimicrobial effect of oregano essential oil, nisin and their combination against Salmonella Enteritidis in minced sheep meat during refrigerated storage. *International Journal of Food Microbiology*. 2010;137(2–3):175-80.
21. Khajeh M, Yamini Y, Sefidkon F, Bahramifar N. Comparison of essential oil composition of *Carum copticum* obtained by supercritical carbon dioxide extraction and hydrodistillation methods. *Food Chemistry*. 2004;86(4):587-91.
22. Rasooli I, Fakoor MH, Yadegarinia D, Gachkar L, Allameh A, Rezaei MB. Antimycotoxigenic characteristics of *Rosmarinus officinalis* and *Trachyspermum copticum* L. essential oils. *International Journal of Food Microbiology*. 2008;122(1-2):135-9.
23. Moazeni M, Saharkhiz MJ, Hosseini AA. In vitro lethal effect of Ajowan (*Trachyspermum ammi* L.) essential oil on hydatid cyst protoscoleces. *Veterinary Parasitology*. 2012;187(1-2):203-8.
24. Georgantelis D, Ambrosiadis I, Katikou P, Blekas G, Georgakis SA. Effect of rosemary extract, chitosan and α-tocopherol on microbiological parameters and lipid oxidation of fresh pork sausages stored at 4 °C. *Meat Science*. 2007;76(1):172-81.
25. Yingyuad S, Ruamsin S, Reekprkhon D, Douglas S, Pongamphai S, Siripatrawan U. Effect of chitosan coating and vacuum packaging on the quality of refrigerated grilled pork. *Packaging Technology and Science* 2006;19(3):149-57.
26. Economou T, Pournis N, Ntzimani A, Savvaidis IN. Nisin-EDTA treatments and modified atmosphere packaging to increase fresh chicken meat shelf-life. *Food Chemistry*. 2009;114(4):1470–6.