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Article

Effect of Whey Protein Isolate Coating Enriched with Black Cumin Essential Oil and Lysozyme on the Shelf-life of Chicken Fillets during Refrigerated Storage

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Abstract: The objective of the present study was to investigate the effect of Whey Protein Isolate (WPI) edible coating enriched with black cumin essential oil and Lysozyme on the microbiological (total aerobic mesophilic bacteria, *Pseudomonas* spp., Enterobacteriaceae, Psychrotrophic bacteria) and sensory properties (taste , odor) of chicken meat during storage at 4 °C for 12 days. The results indicated that *cuminaldehyde* and *gamma-terpinene* stand as the two major groups of compounds with 21.51 and 18.49 percentages of the essential oil. Based on the results, it can be argued that coating by WPI containing black cumin essential oil and lysozyme successfully extend the shelf life of chicken fillet meat during storage in refrigeration. In present study, most reduced count of microbial population or samples was observed for samples coated with WPI containing1% (v/v) black cumin essential oil and 1% (w/v) lysozyme. Hence, it can be concluded that coating with WPI containing1% (v/v) black cumin essential oil and 1% (w/v) lysozyme have potential antimicrobial effect on food products (especially chicken meat) without any unfavorable organoleptic properties.

Keywords: Edible coating, Whey Protein Isolate, Chicken fillets, Lysozyme, black cumin.

1. Introduction

Chicken meat consider as a very popular food commodity in the world wide. Over past decades, consumers become more health conscious and leaner meats have become more demanding (Azlin-Hasim et al., 2015; Chouliara et al., 2007; Radha Krishnan et al., 2015). The shelf-life extension of this product, as a perishable food, has always been an important issue for meat industry. Considering to the high level of protein and moisture in chicken meat, spoilage and pathogenic microorganisms activity are main responsible of quality and safety reduction (Azlin-Hasim et al., 2015; Radha Krishnan et al., 2015; Chouliara et al., 2007).

In this way, developing methods for controlling the microbial growth and consequently increasing the shelf-life and quality of the chicken meat has been an ongoing efforts (Bazargani-Gilani et al., 2015; Khanjari et al., 2013; Rawdkuen et al., 2012). Among different preservation approaches, developing antimicrobial active systems incorporated with antimicrobial substances, which are applied directly to foods or to the coatings of foods, has been at the center of attention. This interest has root in raising concerns around chemical compounds in recent years. According to previous studies, edible coating are capable to increase the shelf-life of food products when they are integrated with antimicrobial agents such as essential oils, bacteriocins, antimicrobial enzymes and phenolic compounds (Appendini and Hotchkiss, 2002; Azlin-Hasim et al., 2015; Bazargani-Gilani et al., 2015; Fernández-Pan et al., 2014).

In terms of Essential oils (EOs), extensive studies have confirmed their antibacterial, antifungal, and antioxidant properties (Burt, 2004), some findings also demonstrated the potential of EOs in extending the shelf life of food products (Khanjari et al., 2013; Pavelková et al., 2014). In general terms, EOs are aromatic oily liquids which are extracted from different parts of plant (Burt, 2004). Regarding to Black Cumin (Zire-e-Irani, Zireh Koohi or *Bunium persicum*), its seed is used for edible and medical purposes for centuries (Cakir et al., 2016; Oroojalian et al., 2010) In the form of essential oil, Black cumin has been well documented as an antimicrobial and antifungal agent (Awad et al., 2013; Cakir et al., 2016; Ramadan, 2007).

Lysozyme (LYS) is one of the recognized antimicrobial enzymes obtained from hen egg white. This small lytic enzyme has suitable stability over a wide range of pH and temperature (Park et al., 2004). Its application in different bioactive films and coating for food systems showed its potential in inhibition of critical Gram-positive bacteria in refrigerated storage temperature (Boyaci et al., 2016).

The aim of present study was to investigate the effect of WPI coating containing Lysozyme and black cumin essential oil on the shelf-life extension of fresh chicken breast fillets stored at 4 ºC for a period of 12 days.

2. Materials and Methods

2.1. Plant Material

Black cumin seeds were collected from Rafsanjan (Kerman-Iran) in summer 2015. The taxonomic identification of Plant materials was dome by the Institute of Medicinal Plants, Medical University of Tehran, Iran.

2.2. EO Extraction

In the preparation phase, 150 g of powdered seeds were mixed in 1.5 liter of distilled water. The essential oil extraction was performed via 'Clevenger apparatus', during 3 hours. The extracted essential oil was dehydrated by Sodium Sulfate and then stored in dark glass at $4^{\circ C}$ for further analysis (Oroojalian et al., 2010).

2.3. Chemical Analysis

Quantitative and qualitative analysis of the black cumin essential oil was performed by GC/MS (GC: HP 6890, MS: HP 5973), a HP5-MS column (60 m 0.25 mm fused silica capillary column, film thickness 0.32 μm). The temperature programmed from 60 °C (3 min) to 230 °C (3 min) with an increase rate of 7 °C/min (injection temperature 250 °C, carrier gas: helium with purity of 99.999%). The detector temperature was 150 °C. The ionization energy in mass was 70 eV, with the mass range of 10–300 amu. The scan time was 1 second. The MS spectra was used to identify the constituents. For this purpose, the retention indices were compared with data in the literature or those authentic compounds (Adams, 1997).

2.4. Preparation of Edible Coatings

Coating solutions were prepared by an aqueous solution of 100 g of WPI (Davisco Food International, USA) by 1000 ml distilled water, 50 ml of glycerol was added as a plasticizer. The FFS were kept in a thermostatic bath at 90 ºC for 30 min (Completely dissolving) WPI. After cooling, different concentration of EO combination (*Buninum persicum*) (B) (0, 0.5% and 1% (v/v)) and Lysozyme (L) from Merck (Darmstadt, Germany), were added to 100 ml of coating solution at the levels of 0, 0.5% and 1% (w/v). Homogenization of FFS was done by using of Homogenizer (Heidolph Instruments GmbH and CoKG, Germany) at 24, 000 rpm for 2 min (Fernández-Pan et al., 2014).

2.5. Preparation of Chicken Meat

Fresh chicken meat were provided by a local poultry processing plant, 2 hour after slaughter. They were put in insulated polystyrene boxes on ice and transferred to the laboratory. Chicken fillets were then aseptically removed and cut into 25 g pieces by sterile knife. Breast fillets were immersed in

a prepared solution for 2 minutes. The excess of solution was then drained lasting in 30s. The uncoated and coated samples were then packed in sterile polypropylene bags and stored at 4 ° C. Microbiological and sensory properties were analysed on day 0, 1, 3, 6, 9, and 12 of the storage time.

2.6. Microbiological Analysis

Microbiological properties (total aerobic mesophilic bacteria (AMB), Enterobacteriaceae, total aerobic psychrotrophic bacteria (APB), and Pseudomonas spp.) Of samples (10 g) were homogenized with 90 mL of 0.1% sterile peptone water solution in a stomacher (Bagmixer® 400 W, Interscience, St Nom, France) for 2 min, then serial decimal dilutions were prepared in 9 ml volumes of 0.1% peptone water. The amount of 0.1 ml of these serial dilutions of chicken homogenates was spread on the surface of agar plates. Total aerobic mesophilic bacteria were determined by using Plate Count Agar (PCA, Merck, Darmstadt, Germany) and after incubation for 48 h at 37 °C. *Pseudomonas* spp. were enumerated on cetrimide fusidin cephaloridine agar (CFC, Fluka, Germany) and incubated for 24-48 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 at 37 $^{\circ}C$ for 24-48 h (ISO, 1979). APB were determined on Plate Count Agar and the plates were incubated at 7 °C for 10 days. Microbiological data were transformed into logarithms of the number of colony forming units (cfu/g) (Fernández-Pan et al., 2014).

2.7. Sensory Evaluation

After sampling, fillets were frozen at -20°C keeping for sensory analyses. Samples cooked according to the American Meat Science Association methodology (AMSA, 1995) at 170 °C in a convection oven (Tefal, France) until reaching an internal temperature of 71 °C after defrosting, measured the geometrical center of each sample by a thermometer. A panel of ten judges experienced in chicken evaluation was used for sensory analysis. Panelists were asked to evaluate odor and taste intensities of cooked samples. Acceptability of odor and taste was estimated according to an acceptability scale ranging from 5 to 0 that 5 was corresponding to the most liked sample and 0 corresponding to the least liked sample. A score of 3.0 was taken as the lower limit of sensory acceptability (Chouliara et al., 2007; Khanjari et al., 2013).

2.8. Statistical Analyses

Each parameter was tested in triplicate. Conventional statistical methods were used to calculate means and standard deviations. Analysis of variance (ANOVA) was used to determine significant differences ($P<0.05$) between treatments. To assess differences between the levels of the main factor, contrasts between means (Duncan test) were used. For sample characterization, ANOVAs with two

factors were applied for each parameter. The Kruskal-Wallis one-way analysis of variance test was used for sensory evaluation. The Statistical analyses were done using SPSS statistical package (SPSS 22, SPSS Inc., Woking, Surrey, UK).

3. Results and Discussion

3.1. Chemical Composition of Black Cumin Essential Oil

The results, derived from analysis of black cumin essential oil of Kerman-Iran (2015), showed essential oil yield at 6% (v/w). Among all individual constituents that were identified by GC/MS, *cuminaldehyde* and *gamma-terpinene* stand as the two major groups of compounds with 21.51% and 18.49% percentages respectively (Figure 1 and Table 1). The results are in line with findings were reported by Soleimani, Sattari and Daneshmandi, (2011) and Oroojalian *et al.*, (2010), who noted that the main components of EO of *B. persicum* were *gamma-terpinene* and *cuminaldehyde*. Some researchers justified that the season of harvesting, geographic location and soil conditions and type of essential oil extraction are possible factors that could affect the quality and quantity of a certain species of essential oils (Kalemba and Kunicka, 2003; Kizil et al., 2010). Antimicrobial activity of black cumin EO was attributed to Monoterpenes compounds such as *cuminaldehyde* and *gamma-terpinene*.

Table 1.Main components of black cumin essential oil

Compounds	**The amount (%)**	**Retention Time***
Cuminaldehyde	21.51	1280
gamma-terpinene	18.49	1080
1,2-Ethanediol,1-phenyl	18.19	1336
Piridine,3,5-dimethyl	10.91	1342
Para Cymene	9.62	1037
Limonene	7.53	1049
beta-Pinene	3.12	890
alpha-Pinene	2.08	946
anti-7-Isopropylbicyclo[2.2.1] hepta	1.71	1220
beta-Myrcene	1.71	1000
Terpinene-4-OL	1.70	1211
Sabinene	1.57	984
alpha-Thujene	0.94	984
alpha-Terpinolene	0.90	936
total	99.98	

* Retention time on HP5-MS column, experimentally determined using homologous series of C8–C28 alkanes.

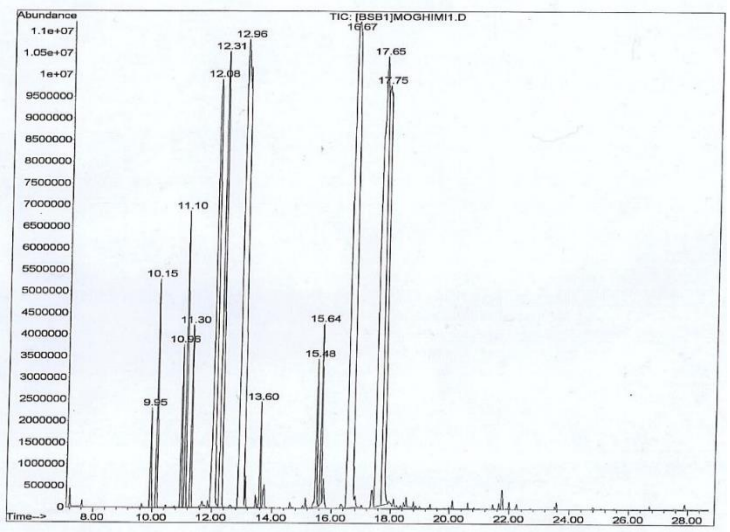

Figure 1. GC–MS of Black cumin Essential oil.

3.2. Bacteriological Analysis

In general, compared to control samples, all coatings in this experiment showed significant antibacterial effects against total aerobic mesophilic bacteria (AMB), Enterobacteriaceae, total aerobic psychrotrophic bacteria (APB), and Pseudomonas spp. ($P<0.05$) (represented Figures 2, 3,4,5).

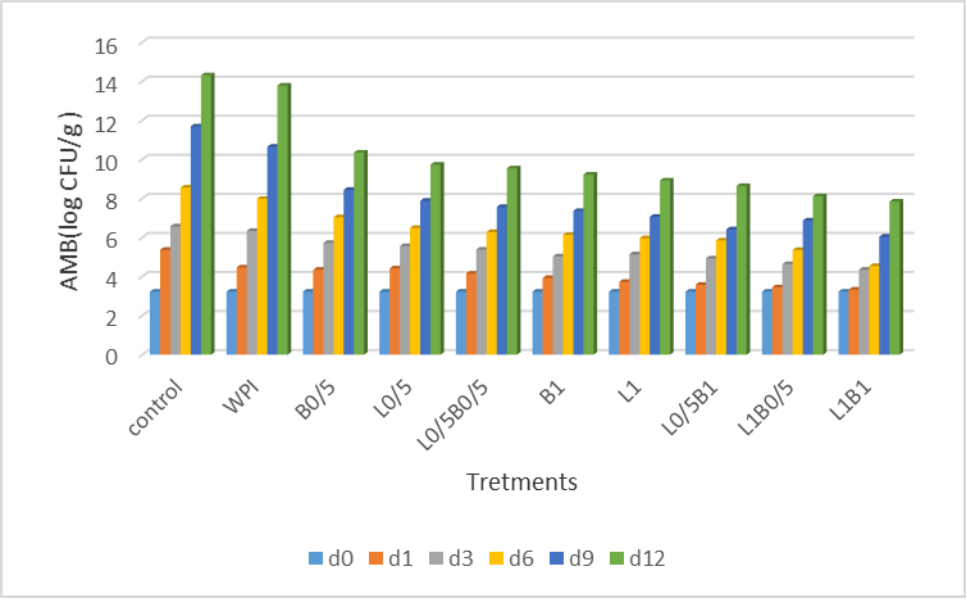

Figure 2. Changes in Total aerobic mesophilic bacteria of chicken fillet meat during storage at 4°C.

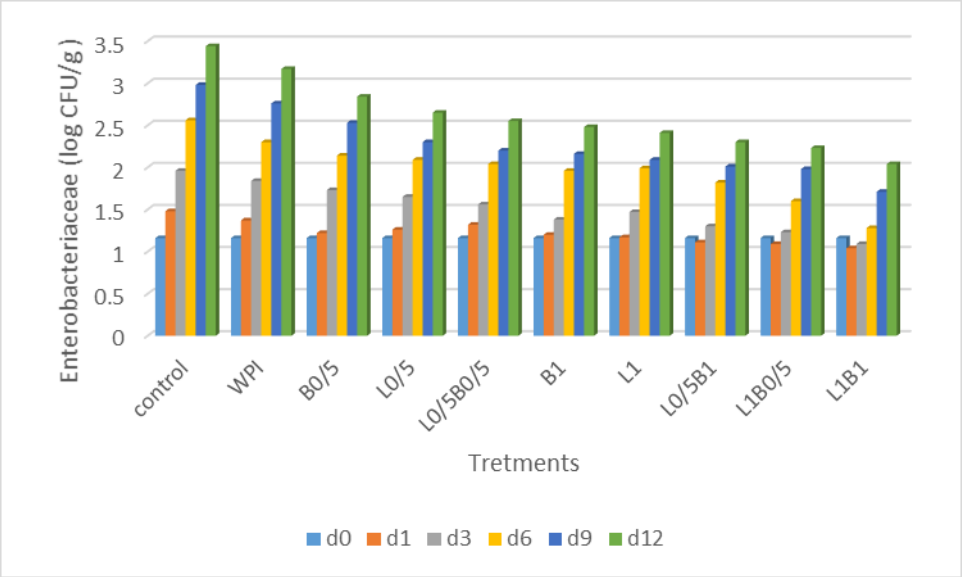

Figure 3. Changes in Enterobacteriaceae of chicken fillet meat during storage at 4 °C.

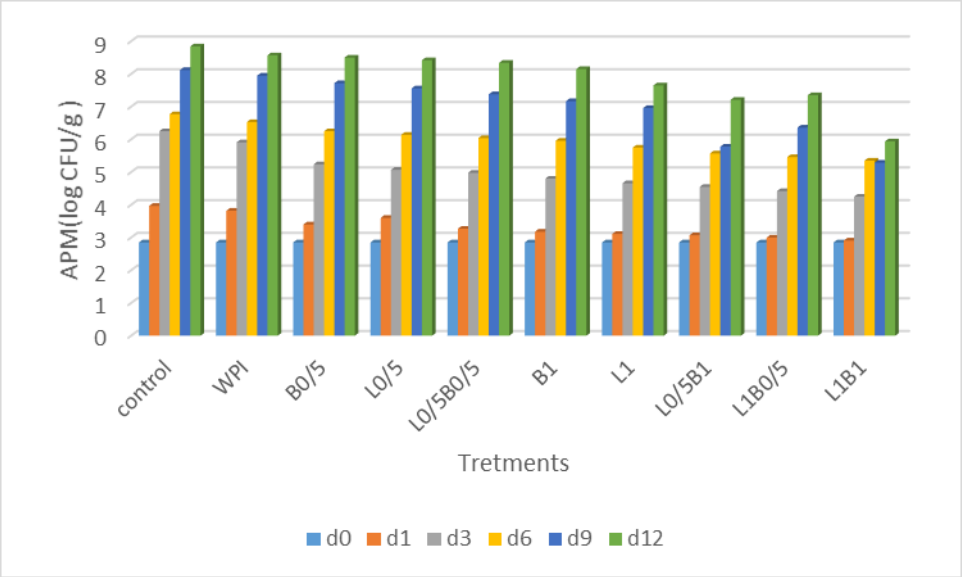

Figure 4. Changes in Total aerobic Psychrotrophic bacteria of chicken fillet meat during storage at 4°C.

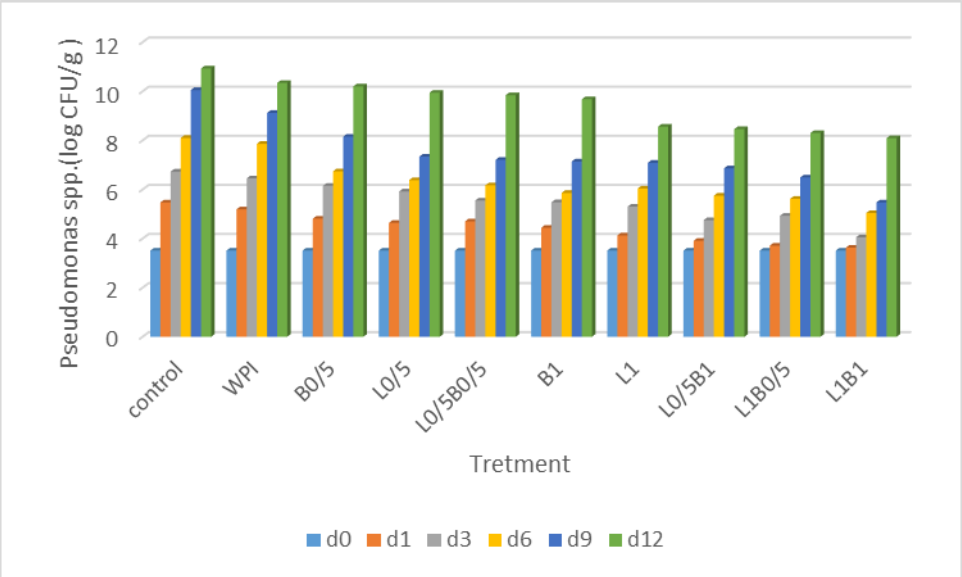

Figure 5. Changes in Pseudomonas spp. of chicken fillet meat during storage at 4 °C.

The initial (day 0) total aerobic mesophilic bacteria of chicken meat were 3.23 log cfu/g. Total aerobic mesophilic bacteria for control sample reached 8.35 log CFU/g after 6 days of storage and gradually increased to 14.31 log CFU/g at the end of storage. TVCs of the coated samples with the EO and lysozyme was below 7log CFU/g until the ninth day. According to Senter, Arnold and Chew, (2000), 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.

Concerning Total aerobic mesophilic bacteria, L1B1 formulation was the most effective throughout the storage period. At day 12, final total aerobic mesophilic bacteria population in samples coated with 1% *buninium persicum* essential oil and 1 % lysozyme decreased significantly ($P<0.05$) (approximately 3 log CFU/g) as compared with the uncoated samples (Fernández-Pan et al., 2014).

In this study, Enterobacteriaceae, a facultative anaerobic bacterial group, formed a substantial part of the chicken meat microbial flora and reached final counts of 3.44logs (control samples) on day 12 (Figure 3). The initial count of fresh meat chicken sample was 1.16 log CFU/g and its final population decreased significantly ($P<0.05$) (approximately 1-2 log CFU/g) as compared to the control samples (day-12).

The Enterobacteriaceae family is commonly used as an indicator of faecal contamination during food microbiological analysis, and includes important zoonotic bacteria such as *Salmonella* spp., *Yersinia* spp. and *Escherichia coli*. Enterobacteriaceae are the main causes of serious infections, and many of the most important members of this family are becoming increasingly resistant to present available antimicrobials (Paterson, 2006). In this regards, many members of the Enterobacteriaceae

have been detected on raw beef, lamb, pork, and poultry products, as well as on offal meats (GarciaLopez and Prieto, 1998).

In particular, on day 12, the Enterobacteriaceae population reached 3.44 log CFU/g in control sample, and 2.04 log CFU/g in the samples with the coating containing L1B1. Similar studies concede this finding indicating that various EOs could inhibit the growth of Enterobacteriaceae population in refrigerated chicken meat.

The Gram-negative Psychrotrophic bacteria (PTC) are the major group of microorganisms responsible for spoilage of aerobically stored fresh meat at chilled temperatures (Ibrahim Sallam, 2007). Accordingly, the initial total aerobic Psychrotrophic bacteria (day 0) of samples ranged from 2.84 log CFU/g to 8.84 log CFU/g in controls (Figure 4). Total aerobic Psychrotrophic bacteria population in all treatments was significantly ($P < 0.05$) lower than control, L1B1 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 same studies reporting a reduction in psychrotrophic bacteria (PTC) is parallel with the addition of a various mixture of EO in meat chicken during storage at refrigerated condition.

It is well documented that *Pseudomonas* spp. may form a significant part of the spoilage microflora of chicken meat stored under refrigeration (Jay, 2012). *Pseudomonas* spp. is known to compete other bacterial groups (Gram-positive or Gram-negative) for nutrients by forming siderophores that may inhibit growth of both spoilage microorganisms and pathogens (Wei et al., 2006). Proteolysis is an important phenomenon involved in the meat spoilage. The microflora of chicken meat particularly pseudomonads are responsible for proteolysis and the subsequent slime production. This event starts when the bacterial counts reach to 10^7–10^8 CFU/g and the contents of glucose and gluconate are exhausted (Nychas et al., 1988; Nychas and Tassou, 1997).

According to findings, initial *Pseudomonas* spp. count was 3.50 log CFU/g (Figure 5), increasing during storage to reach final population of 10.93 log CFU/g (control samples). *Pseudomonas* spp. population in all treatments was significantly ($P < 0.05$) lower than control. Samples containing L1B1 were the most effective treatments for the inhibition of *Pseudomonas* spp., probably due to the combined antimicrobial actions of lysozyme and BEO. These results are consistent with same studies in this ground.

This result could be attributed to the inhibitory effect of the combined antimicrobials, suppressing the growth of Gram-positive, as well as Gram-negative spoilage organisms.

3.4. Sensory Evaluation

Sensory properties (taste and odor) of cooked chicken fillets meat for all different treatments as a function of storage time are shown in Figure 6 and 7. During 12 days of storage taste, odor of the L1B1 treatment (1% (v/v) black cumin essential oil and 1% (w/v) lysozyme) were superior.

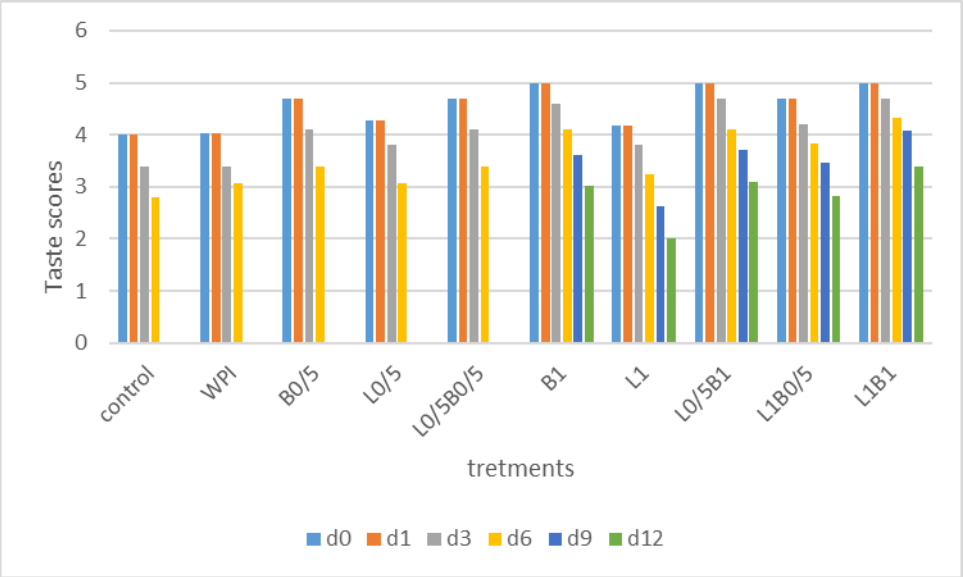

Figure 6. Taste scores of cooked breast chicken meat as a function of specific treatments and storage time.

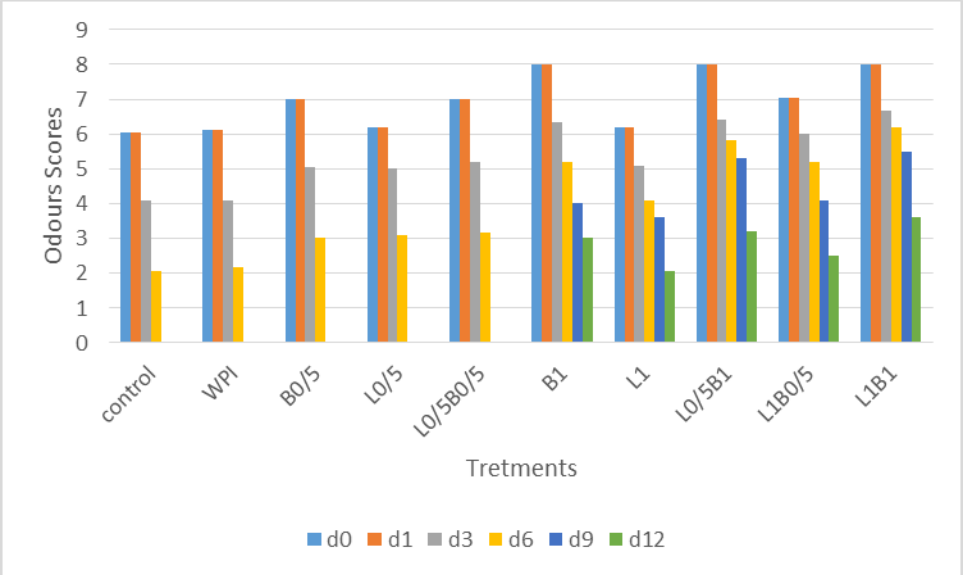

Figure 7. Odors scores of cooked breast chicken meat as a function of specific treatments and storage time.

4. Conclusions

The present study showed that edible coating based on different biopolymers that contained similar or higher amounts of EOs increased the shelf life of different meat products. Current study showed that black cumin essential oil incorporated with lysozyme has the ability to delay microbial changes and extend the shelf life of chicken fillets. Whereas it could exhibit desirable sensory attributes including taste and odor so, it could be suitable natural antimicrobial agents that could be integrated with food coatings and films.

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