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Original article

The Effects of Edible Coating, Echinacea Tea and Hawthorn Tea on Certain Microbial and Other Quality Parameters of Refrigerated Chicken Meat

Yenilebilir Kaplama, Ekinezya Çayı ve Alıç Çayının Soğutulmuş Tavuk Etlerinde Bazı Mikrobiyal ve Diğer Kalite Parametrelerine Etkisi

Seyhun Yurdugül [a, *]

[a] Department of Biology, Abant Izzet Baysal University, 14030, Bolu, Turkey

Abstract

The purpose of the current study is to evaluate the effect of edible fruit and vegetable coatings, the echinacea and the hawthorn tea solutions, solely or in combination of increasing the shelf-life of chicken meat by the inhibition of the microbial flora at the refrigeration temperature, without disrupting certain chemical and physical properties. Eight different combinations of treatment groups were formed as follows: echinacea, echinacea tea-hawthorn tea combination, Semperfresh®-echinacea tea combination, Semperfresh® -echinacea-hawthorn tea combination, hawthorn tea, Semperfresh®-hawthorn tea combination; the edible coating (Semperfresh®), including the control. The total mesophilic aerobic bacteria, yeast-mold, *Salmonella* spp., *Escherichia coli and Staphylococcus* species were determined. The pH, firmness, weight and total sugar content were analyzed. It was found that the sole hawthorn tea and triple combination of Echinacea-hawthorn tea-edible coating Semperfresh™ showed significant inhibitory effects on the mesophilic aerobic bacteria, *Staphylococci*, *Salmonella* and *E. coli*. Except for the Echinacea treatment group, the overall evaluation of taste panel showed a likeness of the remaining applications ($p \leq 0.05$). On the other hand, no significant effect was observed ($p > 0.05$) on the pH, the firmness, the total sugar content and the weight of chicken meat among the groups. Different combinations, especially the triple combination was found to be successful on chicken thighs, due to the microbiological, chemical and physical findings. It can be inferred from the study that echinacea, hawthorn, and Semperfresh™ showed a synergetic effect.

Keywords: CMAS, thermal barrier coating, perovskite, pyrochlore, glassy phase infiltration.

Özet

Bu çalışmanın amacı, tavuk etlerinin, birleşik ya da yalın olarak yenilebilir meyve ve sebze kaplama maddeleri, ekinezya ve alıç çayı çözeltilerine daldırılarak bazı kimyasal ve fiziksel özelliklere zarar vermeden, soğutma sıcaklığı olan +4°C'de mikrobiyal florayı inhibe ederek etkilerinin yorumlanmasıdır. Sekiz değişik bileşimdeki uygulama grupları şu şekildedir: (E): Ekinezya çayı, (E+M): Ekinezya çayı-Alıç çayı bileşimi, (E+S): Semperfresh®-Ekinezya çayı bileşimi, (E+M+S): Semperfresh® -Ekinezya çayı-Alıç çayı bileşimi, (M): Alıç çayı, (M+S): Semperfresh®-Alıç çayı bileşimi; (S): Kaplama maddesi (Semperfresh®), kontrol grubu. Toplam mezofilik aerobik bakteri(TMAB), maya-küf, *Salmonella spp.*, *E. coli ve Stafilokok* türleri tayin edilmiştir. pH, sertlik, ağırlık ve toplam şeker miktarı analiz edilmiştir. Yalnızca alıç çayı ve üçlü Ekinezya çayı-alıç çayı-kaplama maddesi Semperfresh™(E+H+S), mezofilik aerobik bakteriler (MAB), *Staphylococci*, *Salmonella* ve *Escherichia coli* üzerinde önemli inhibitör etki göstermiştir. Ekinezya grubu hariç, tad panelinin genel değerlendirmesi diğer uygulamalara benzerlik göstermiştir($p \leq 0.05$). Diğer yandan, pH, sertlik, toplam

* Corresponding author:

Seyhun Yurdugül, Department of Biology, Abant Izzet Baysal University, 14030, Bolu, Turkey.
Email: yurdugul_s@ibu.edu.tr

şeker miktarı ve ağırlık açısından tavuk etleri arasında önemli bir fark gözlenmemiştir ($p>0.05$). Farklı bileşimlerde, özellikle üçlü bileşim tavuk butları üzerinde, mikrobiyolojik, kimyasal ve fiziksel bulgulara göre başarılı olmuştur. Çalışmadan çıkan sonuca göre Ekinezya, alıç ve Semperfresh™ sinerjetik etki göstermektedir.

Anahtar Kelimeler: Yenilebilir kaplama, tavuk, alıç, ekinezya.

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INTRODUCTION

Chicken meat is an important commodity. The shelf life of chicken meat is restricted by the existence of microorganisms and enzymatic action, and the type of protection technology used to protect against those agents (Miyagusku *et al.*, 2007).

Hurdle technology combines the use of preservation agents to increase microbiological security, nutritional and sensory criteria of meat and meat products. Hurdle technology limits the proliferation of meat-contaminating bacteria (Leistner, 1999).

The preservation agents are always preferred getting rid of the contaminants of important food products. Edible coatings are termed as sustainable products that can be produced by using raw materials such as protein, polysaccharide and lipid (Çağrı *et al.*, 2004). In fruits and vegetables, edible coatings are reported to decrease moisture level, limit oxygen uptake, decrease respiration, delay ethylene formation, seal in flavor volatiles and bear extra functional components (e.g. antioxidant, anti-bacterial, anti-fungal products) that delay discoloration and microbial growth (Baldwin *et al.*,1995), prevent loss of flavour, texture and nutrients (Wiley,1994) and prolong the product acceptability (Maftoonazad *et al.*,2008). The edible coating used in this study, Semperfresh™, is a food grade one, derived from vegetable origin, preferred principally for composite coating materials made up of the Na^+ salt of carboxymethylcellulose (CMC) as the film forming agent, with sucrose mono and diglyceride fatty acid esters providing an important barrier for losing moisture (Baldwin *et al.*, 1995; Gonzalez-Aguilar *et al.*, 2008).

Echinaceae was found to be effective against certain bacteria and yeast strains like *Listeria monocytogenes, Saccharomyces cerevisiae, Candida shehata, Candida kefyr, Candida albicans, Candida steatulytica* and *Candida tropicalis* by enhancing the resistance for the systemic infections (Steinmüller *et al.*, 1993; Binns *et al.*, 2000), by its ingredients such as echinacoside, polysaccharides, polyacetylenes, glycoproteins, cichoric acid, triglycoside, betain, sesquiterpenes and caryophylene. *Echinaceae purpurea* was used to strenghten the immune system and to fight common colds (Lindenmuth and Lindenmuth, 2000).

Hawthorn is the fruit of *Mespilus germanica* L. in the family of Rosaceae, growing poorly in frost free areas, on rocks and in poor soils (Baytop, 1999). It was reported that the fruits were eaten on an empty stomach to treat bloody diarrhea and throat infections (Steinmetz,1954), the syrup of the fruit was a popular remedy against enteritis (Baytop, 1999). The hawthorn fruit contains sugar, organic acid, amino acids, tannins and usnic acid in it was used as an antimicrobial agent (Řezanka *et al*., 2005). It has been of recent interest for its edible fruits and used as raw in salads in certain areas of the Mediterranean basin (Lentini and Venza, 2007).

In this study, the effects of an edible fruit and vegetable coating, Echinacea tea and Hawthorn tea on ready-to-eat chicken meat were examined in cold storage (+ 4˚C). It was aimed to evaluate the microbiological, chemical and sensory properties of chicken meat and the possibility of using biopreservative fruit and vegetable coatings in combination with herbal infusion teas to control certain pathogenic bacteria and increase the storage life of poultry meat in the commercial and household state. It is worth studying the effects of these antimicrobials and fruit/vegetable edible coatings since chicken meat is easily prone to contamination and with using their combination the removal of these microorganisms is expected to be enhanced.

MATERIALS AND METHODS

The chicken meat and the herbal teas

A 10 kilogram of chicken thighs, 20 bags of hawthorn tea and echinacea tea(each bag consists of five grams) were purchased from a local market (Nuhmar, Bolu, TURKEY). The skin of the chicken thighs were removed, they were washed and boiled for 45 minutes in tap water before the treatment. 30 g. of teas were infused in one liter boiled tap water and allowed to cool to room temperature. Boiled chicken meat is dipped into the cooled tea for one minute as treatment. The Semperfresh^{TM} treatment is similar to the tea treatment, one mL of Semperfresh^{TM} is dissolved in one liter of water and chicken meats are dipped into this solution for one minute duration.

Above three steps procedure is used to prepare eight different combination of treatments, chicken thighs are separated into eight pieces of 100 g. each, one bowl of hawthorn and one bowl of echinaceae tea are prepared. The first group of meats is the control group, so they are not processed, the remaining seven groups are treated as hawthorn tea only, echinacea tea only, Semperfresh^{TM} only, the double combinations and a single triple combination. All of the samples were wrapped with an aluminium foil and stored in a refrigerator at +4° C for 36 days.

Experimental methodology

Semperfresh® fruit coating application on chicken thighs

Semperfresh® edible fruit coating, composed of sucrose esters of fatty acids, sodium carboxymethyl cellulose and mono-diglycerides of fatty acids was obtained from ACC (Ankara, Turkey) in liquid concentrate form (50.0 % w/v). As no recommended concentration of Semperfresh® was defined for the chicken meat by the vendor, 0.05, 0.1, 0.5, 1 and 2 % (w/v) levels were subjected to trials with relevance to microbiological and chemical quality criteria. A previous taste panel carried out for this purpose, showed that 0.1% (w/v) can be preferred as a recommended concentration. All of the four samples, in the related treatment groups were immersed into the water solution containing Semperfresh®.

Echinaceae and Hawthorn teas

Treatment groups

Eight different combination of treatment groups were formed as follows: (E): echinacea tea, (E+M): echinacea tea-hawthorn tea combination, (E+S): Semperfresh®-Echinacea tea combination, (E+M+S): Semperfresh®-Echinacea tea-hawthorn tea combination, (M): hawthorn tea, (M+S): Semperfresh®-hawthorn tea combination; (S): the edible coating (Semperfresh®), including the control.

Experimental design

The pH, firmness, weight and sugar content of the samples were measured and bacterial, mold and yeast formation in refrigerated meats were investigated at 0^{th}, 20^{th} and 36^{th} days of the storage by spread plate technique. Meat was obtained from three different parts of each treatment group by a sterile tweezer as one gram, and put into the test tube which contained 9 mL of distilled water and mixed thoroughly by a vortex. Subsequently, 200 µL from this mixture was inoculated onto the XLD, EMB, nutrient and *Staphylococcus* agars for the initial enumeration (0^{th} day). After the inoculation the petri plates were placed for a period of 48 hours at 37° C in the incubator. The mesophilic aerobic bacteria (MAB) counts were carried out on the nutrient agar. XLD agar was used to obtain the *Salmonella* spp. counts. *E. coli* counts were carried out on the eosine methylene blue (EMB) agar. The *Staphylococcus* agar is used for the enumeration of *Staphylococcus* species. All the microbiological analysis were done in the appropriate agars in triplicates.

The pH

The pH was measured by using a portable pH meter (Isolab) at the three different points on the circumference of each treatment group, repeated in triplicates.

The firmness

A hardness tester (model FT011, Everwell, Japan) was used for the firmness measurement (kg) repeated three times (Yaman and Bayındırlı, 2002).

The weight determination

Weighs were determined by a digital balance (Precisa, model BJ210 C), repeated three times.

Total sugar content

The total sugar content of the control and the treated meats was determined by the spectrophotochemical dinitrosalicylic acid (DNS) method. An aliquot (mL) from each treatment group was mixed with 3 mL DNS reagent and 1 mL of distilled water. These groups were kept in water bath at 50° C for 30 min. incubation time (Nüve NB20, Turkey). The absorbance was determined by a spectrophotometer (CADAS 50S Dr. Bruno Lange GmbH-Berlin) at $O.D._{550}$ nm and the concentrations were determined against a standard curve, repeated three times.

Taste panel

The chicken meats were rated by twenty previously trained panellists according to texture, color, sweetness, sourness, bitterness, saltiness, odor, appearance, flavor, hardness and overall evaluation criteria, with a ten point scale per replication. A score of 10 represented the most liked, and one corresponded to the most disliked.

Statistical analysis

The method of analysis of variance (one way ANOVA) was applied to the data obtained from each treatment to detect significance of differences at 5% level of significance ($p< 0.05$).

RESULTS AND DISCUSSION

The effects of Echinacea, hawthorn, Semperfresh^{TM} and their combination on TMAB are determined at 0^{th} and 10^{th} day and the results are displayed in Figure 1. From the figure, the most important finding is the inhibitory effect of the hawthorn tea on TMAB population, as can be seen on the third column of the figure, both zeroth and the tenth day no TMAB was found when the samples were treated with hawthorn tea. It is interesting to point out that M+S treated samples have significant TMAB population at day 10 while they had no TMAB at day zero, which might be due to possible Semperfresh^{TM} providing anaerobic environment. The increase in the tenth day counts might be due to the nature of the edible coating, containing sucrose based esters which can be enzymatically metabolizable by certain species of the mesophilic aerobic bacteria into its minor components, glucose and fructose. The glucose formation might promote growth of microorganisms, together with the proteinaceous nature of the chicken meat. Moreover, as expected, no total mesophilic aerobic bacteria

was found in the tenth day in the triple combination group. From the same family, *Crataegus elbursensis* pulp and seed extract was found to show strong antioxidant and antibacterial activities, which were correlated with its high level of polyphenols (Salmanian *et al*., 2014) and the whole genus *Crataegus* spp. was shown to exhibit wide range of cytotoxic, gastroprotective, anti-inflammatory, anti-human immuno deficiency virus (HIV) and antimicrobial activities (Kumar *et al*., 2012) in line with our study.

According to Versino *et al*. (2016) natural and altered starch types are considered for biologically degradable film composition since they have a whole biological degradable characteristic, edible property, and cost-decreasing feature. Formation and definition of a starch-based film made by: (i) casting, (ii) blown extrusion and (iii) the thermo-compression moulding types are defined. The rheological characteristics of filmogenic suspensions, the barrier features, and the mechanic strength of the produced films are mentioned. The presence of special additive agents in the formulation change the functional characteristics of the film by transformation into active products. Diffusing properties of antibacterial substances like potassium sorbate from the active starch-based film, moreover their efficiency in milk and milk products is still under debate. Similarly, reinforcing compounds lead to composite products with increased mechanic resistivity. Starch-related compounds display high permeable characteristics to carbon dioxide than to oxygen, that is important to detect the respiring rate of fruits and vegetables. The use of active starch-contained coatings to strawberry and Brussel sprout in order to increase their cold shelf-life was investigated by Versino *et al*. (2016). A detailed consideration on the formula and performance of starch-containing films by using industrial and laboratory-scale methods, moreover the use of starch coating to increase food quality is shown, by the purpose of analyzing the possible development and utilization of these compounds. Chitosan coatings, containing 0.5% of oregano and thyme essential oils (EO) were tested onto instant peeled shrimp tails and kept under modified atmosphere packaging (MAP) conditions by Carrion-Granda (2016). The proliferation of natively existed spoiling microflora was defined for twelve days during cold storage (4° C). Coatings which contain thyme EO were more powerful during inhibition of the microorganisms tested, especially lactic strains and psychrotrophs. As carrying of EOs, chitosan was more powerful in inhibition of the microflora existed in peeled shrimps than the direct testing of an oil and water containing (O/W) emulsion. Consequently, results from sensory panel indicated that the sensory characteristics was changed by the chitosan-thyme coatings other than the properties like firmness and colour were not altered. The efficiency of chitosan added coatings, offers a good alternative to decrease the proliferation of spoiling and pathogenic microflora on peeled shrimps while refrigeration.

Another research group studied an edible coating (WPIG), which was made up of 1.5% (w/v) sorbitol + 5% (w/v) whey protein isolate (WPI) + 0.5% (w/v) alginate and 1.5% (v/v) ginger essential oil. For this purpose, kashar cheese samples were artificially inoculated with *E. coli* O157:H7 and *S. aureus* at an amount of 10^6 cfu/mL. After coating some of the Kashar samples, all samples were kept at

4° C for thirty days. Antibacterial action and selected physico-chemical characteristics were evaluated on the 1st, 7th, 15th and 30th days of storage. Whey-protein isolate was found to have good water barrier characteristics, which is increased by incorporation of 1.5% (v/v) ginger essential oil to the coating, and WPIG was found to show antibacterial effects. During storage, *E. coli* O157:H7 and *Staphylococcus aureus* counts elevated in the control group, while they are lowered in the coated ones (Kavas *et al*., 2016).

Various coating material (chitosan-beeswax, sodium caseinate-beeswax and Semperfresh(TM)) were applied to green asparagus and evaluated for alteration in fresh weight, sensory criteria, tiprot disorder and texture after keeping them at fourteen days at 4°C in a research done by Fuchs *et al*. (2008). It was pointed out that chitosan combined with beeswax significantly decreased fresh weight losing of asparagus with relevant to untreated groups. Semperfresh(TM) and sodium caseinate coatings resulted in the low loss of expected green color in spears. Coatings have no effect on significant retardation of the development of tiprot disorder with relevant to untreated spears. No antibacterial action were detected when Semperfresh™ is used. Coatings did not significantly delay undesirable changes to spear texture. The availability of such coatings for utilization on asparagus is related to not only its improved formula, but also its application technique. Another study was performed by testing the edible coatings in combined form by anti browning compounds on fresh-cut 'Bravo de Esmolfe' apple (Guerreiro et al., 2017). Four different edible coating types were used: sodium alginate (AL) at 2% (w/v) with either eugenol (Eug) 0.1% (w/v) or Eug 0.1% + citral (Cit) 0.15% (w/v) and pectin (PE) 2% (w/v) with Eug 0.2% or Cit 0.15%. Three anti browning substances were preferred: ascorbic and citric acids at 1% (w/v) and sodium chloride at 0.05% (w/v). Fresh cut apples were put into those solutions and then kept at four degrees C. On days 0, 2, 4, 6 and 8, samples were obtained to analyze physico-chemical and biochemical criteria (browning index, firmness, weight loss, microbial growth, taste panels, total phenols, flavonoids, sugar content, anti-oxidant activity and ethylene production). The AL edible coatings were found to show a performance with relevant to pectin in decreasing the browning index. These coatings increased the shelf life of fresh cut apples by decreasing microbiological proliferation without significantly changing sensory and nutritional criteria. The anti browning compounds further elevated this capacity, where ascorbic acid showed the highest performance. If generalized and sensorial quality properties were considered, fresh cut apples were found to be better protected with AL 2% + Eug 0.1% plus putting in ascorbic acid 0.1%. In our study also, E+M+S combination causes an approximately six log cycle reduction in TMAB at the end of tenth day. The effectiveness of E, S, E+M and E+S treatments in TMAB reduction is low compared to that of M and E+M+S.

Hawthorn was reported to contain glucose, fructose and sucrose, and the antimicrobial components of the hawthorn such as malic acid, succinic acid, quinic acid, oxalic acid and citric acid which might provide an inhibition (Řezanka et al., 2005). In a study performed by Behidj-Benyounes

et al. in 2013 on *Crateagus oxyacantha,* belonging to the family Rosaceae with a subsequent finding of the evaluation of the antibacterial action of ethanol extract on bacterial pathogens to determine their effect on these microorganisms and it was detected as effective on *S. aureus, Klebsiella pneumoniae, Bacillus subtilis,* and *Pseudomonas aeruginosa* as well as yeasts (*Candida albicans* and *Saccharomyces cerevisiae*) but not on *E. coli*. It should be noted that these agents are characterized by a high frequency of contamination and pathogenicity. Phytochemical tests on this extract carried out by Behidj-Benyounes *et al*. (2013) showed that the plant has a wealth of flavonoid. Another study conducted by the same group on antibacterial action of the extracts containing flavonoids arising from *Crateagus oxyacatha* fruits in 2 bio-climatic phases (Dellys and Bainem) in Algeria pointed out that *E. coli* was found to be slightly sensitive to the extract of *C. oxyacantha* of Bainem district. However, *S. aureus* and *B. subtilis* have an increased sensitivity to this extract. The other microorgansims, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were found to be fairly sensitive. If the action on yeast was discussed, *S. cerevisiae* was found to be slightly resistant to the extract and it was found to show an inhibitory action on *C. albicans.*

If another extract of *C. oxyacantha* of Dellys district was considered, *E. coli* was found to show a lowered sensitivity to the extract of *C. oxyacantha*. On the other hand *Staphylococcus aureus* and *Bacillus subtilis* displayed a high sensitivity to this extract, but the other microorganisms studied are fairly sensitive. When the action on yeast was considered, *S. cerevisiae* was found to be slightly resistant to the extract. This extract has a powerful inhibition capacity on *C. albicans.* By this study, the sensitivity of microorganisms was found to be more in G(+) compared to the Gram(-) bacteria for the two areas.

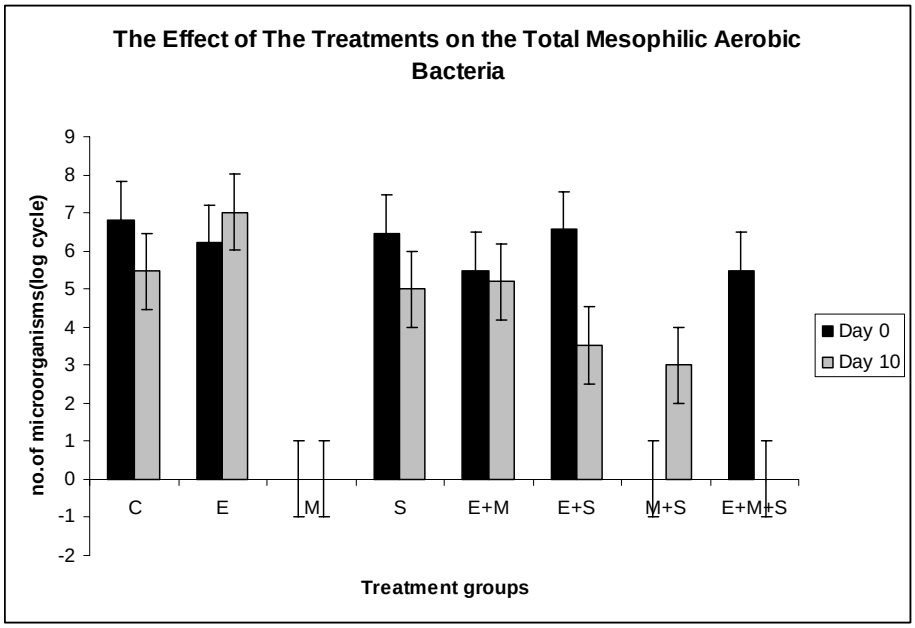

Figure 1. The total MAB counts of all of the treatment groups.

Surprisingly, all treatments show a very sharp inhibitory effect on the *Staphylococcus* species. While the *Staphylococcus* population increases in the control group at day 10 compared to the day 0, it is unobservable for all treatment groups at day 10.

When the *Staphylococcus* species were considered, except the control, no growth was recorded on the other applications, in the tenth day counts. At the beginning, although a high number of *Staphylococcus* was observed in the group of E and E+S, the tenth day values indicated none. From the figure, it was clearly observed that all treatments showed profound effects on the microbial growth. Antibacterial effects of the treatments on the *Staphylococcus* species were analyzed similarly and the results are displayed in Figure 2. Antibacterial activity of *Echinacea purpurea* was reported by Hudson (2012) not only on *Streptococcus pyogenes*, *Hemophilus influenzae*, *Legionella pneumophila*, but also little effect was observed on methicillin resistant *S. aureus* and methicillin sensitive *S. aureus*.

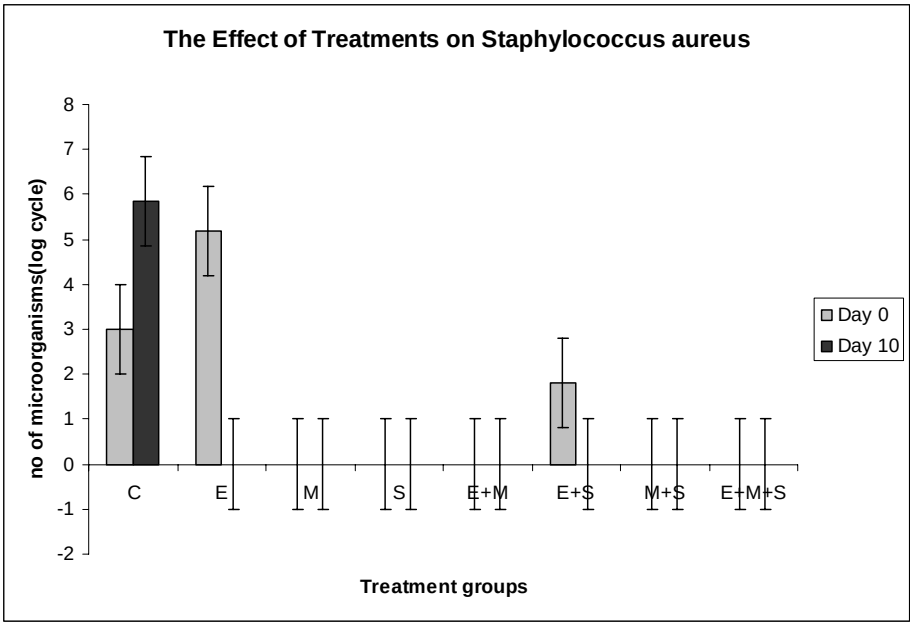

Figure 2. *Staphylococcus aureus* counts of all of the treatment groups.

Moreover when *E.coli* was enumerated on the eosine methylene blue agar, the control group showed the highest increase on 10^{th} day. However, no bacteria is observed in all of the groups on 10^{th} day except control (Figure 3).

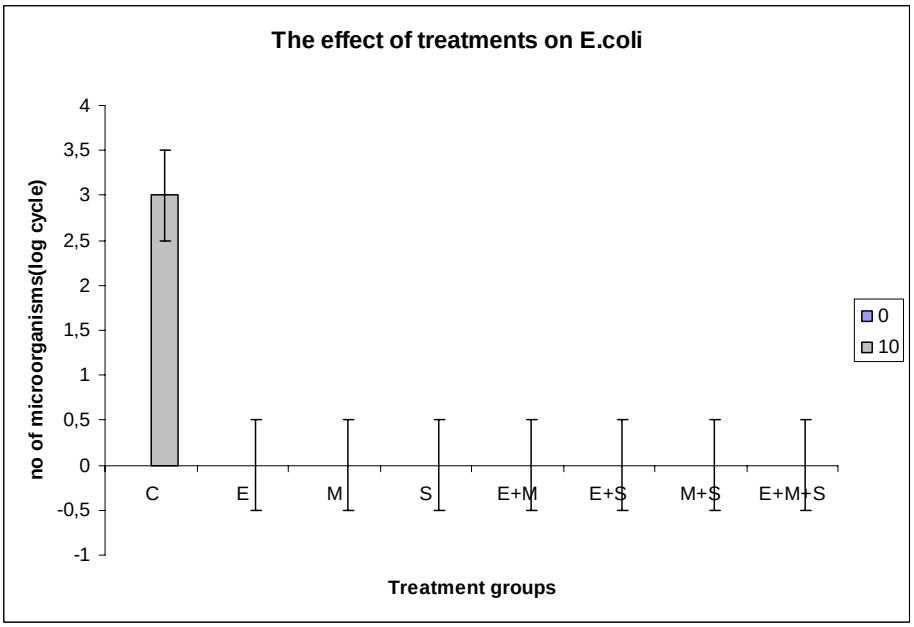

Figure 3. *E.coli* counts of all of the treatment groups.

The results of the treatments on the *E.coli* populations at day 0 and day 10 are displayed in Figure 3 and similar to the *Staphylococcus* spp. All treatments are found to be effective in prohibiting the *E.coli* growth.

No *Salmonella* spp. were observed on XLD agar counts except the control, which showed an approximate of three log cycle values on the tenth day. In general, the inhibitory compounds in the hawthorn and the echinacea tea infusions might have a strong inhibitory effect towards *Staphylococcus*, *E.coli* and *Salmonella* spp, as well as the total mesophilic aerobic counts. As since the edible coating can provide an anaerobic medium to the chicken thighs, this medium does not probably allow the growth of these pathogenic microorganisms at the refrigerated storage conditions.

The chicken meat is an important food commodity. On the other hand, the shelf life of chicken meat is very short due to the presence of pathogenic microorganisms. Therefore consumers are greatly concerned about the issue of food and health because of additives which are used for extending the shelf life of the food so the food industry has to search for novel innovative solutions. On the other hand, *Salmonella* which is a Gram(-) bacteria, infect an estimated number of 1.4 million people in one year in the United States according to the reports from the Centers for Disease Control and Prevention. It is a common poultry associated pathogen. The mesophilic aerobic bacteria counts of the groups M and M+E+S indicated that the hawthorn application, together with the triple combination was proven to be effective in TMAB reduction in this study.

When the effect of *Echinacea purpura* on yeast and mold was considered, its extract, independent of the extraction technique applied, showed a considerable growth inhibition on *C. albicans* and *S.*

cerevisiae, while no growth inhibition zones were observed for *Aspergillus niger* (Stanisavljevic et al.,2009) Another study conducted by Binns et al. (2000) pointed out that hexane extracts of Echinacea variably inhibit growth of yeast strains of *S. cerevisiae*, *C. shehata*, *C. kefir*, *C. albicans*, *C. steatulytica* and *C. tropicalis* under near UV irradiation (phototoxicity) and to a lower extent without irradiation.

The hawthorn and the echinacea teas contain some acidic substances, therefore this can eliminate the effectiveness of the edible coating by disrupting its film nature in due combinations. This may cause an increase in the total mesophilic aerobic counts. No *Staphylococci* were recorded in the counts of the application groups when compared with the control. No *E. coli* was recorded, except the control.

The sole and the combined applications of E, M and the S indicated that these applications were mostly effective against the total mesophilic aerobic bacteria counts, *Salmonella* spp. and *Staphylococci* spp. and as well as on *E.coli*. These were in line with the findings of Rahimi *et al*. (2011) in which the combination effect of thyme-coneflower-garlic extract was studied on intestinal selected bacterial population in broiler chickens, where the colony forming units of *E. coli* in digesta of ileocecum in the combination group of thyme-coneflower-garlic extract showed a significantly lower number compared with control. 5 g/kg diet containing EP was found to be leading the highest antibody titers against Newcastle virus as well in a study conducted by Landy *et al.*(2011) but no effect on coccidiosis, a parasitic disease caused by *Eimeria acervulina* on Ross male broilers (Orengo *et al*.,2012). All studies implied an immune fortification of the broilers by the use of coneflower extracts. The taste panel showed that the tea applications are the preferred ones when compared with the edible coating and the control.

According to the total sugar content data presented in Figure 4; the sugar content increases on the tenth day in most of the groups. However, in M group there was a sharp decrease in total sugar content between the zeroth and tenth days. Although it has been reported to contain certain sugars, the organic acids in hawthorn have a decomposing effect on sugar molecules, and it is in use against the *diabetes mellitus* and the hyperglycaemia, when consumed as a fresh fruit or in vinegar form, as folk medicine in Bolu and Düzce environs of Turkey. The total sugar values of the thighs, as given in mg per one gram, implied that during the ten days' period the sugar content of the thighs increased except the M and E+M treatments. Together with Semperfresh^{TM}, hawthorn showed an increase of sugar content. Conversely, a study carried out by Vishwasrao and Ananthanarayan(2017) used a coating composed of methyl cellulose and palm oil and it was delayed the increase in total soluble solids and total reducing sugars as compared to control group of sapota fruits. Another study carried on guava by Soares *et al*.(2011) through applying the coating of cassava starch, the coating of cassava starch added with acetic acid, the coating of cassava starch added with 1.0% and 1.5% chitosan enhanced postharvest fruit sugar contents, providing that the sugar content was preserved until the 8th day. A study carried out by Maqbool *et al.* (2011) indicated that total carbohydrates and reducing sugars were found to be 59% and 40% higher than the control, in bananas with chitosan coating respectively.

The increase might be mostly due to the metabolization of the sugars by the intensive growth of aerobic bacteria on the thighs (Figure 4).

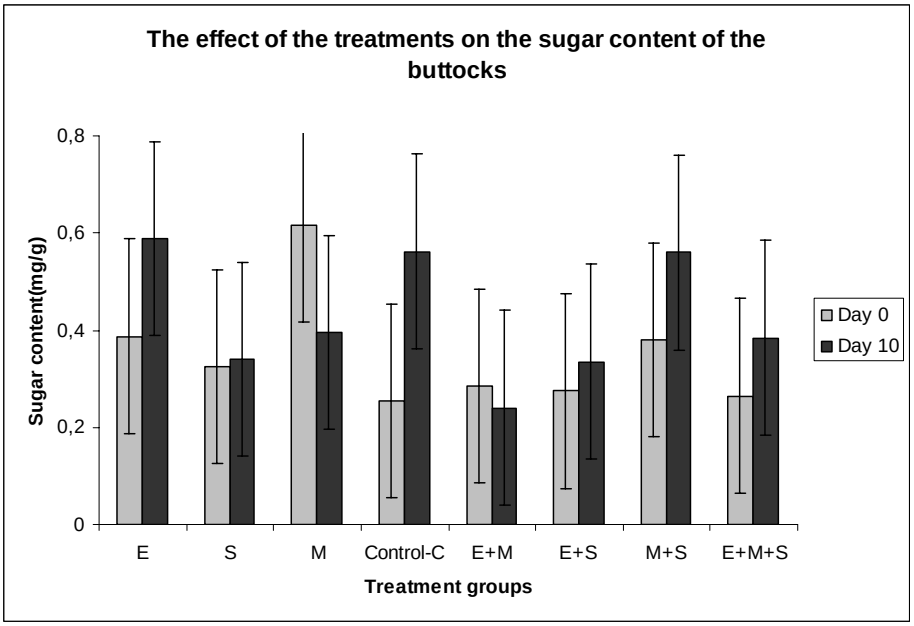

Figure 4. The total sugar content levels of all of the treatment groups.

The firmness values of the chicken thighs indicated an increase between the zeroth and 10th days, except the application groups of M+S (Figure 5). It was in line with the findings of Vishwasrao and Ananthanarayan (2017), implying a decrease in fruit firmness losses. whereas fruit firmness was found to be 31% higher than the control in bananas with chitosan coating in the study of Maqbool *et al.*(2011).

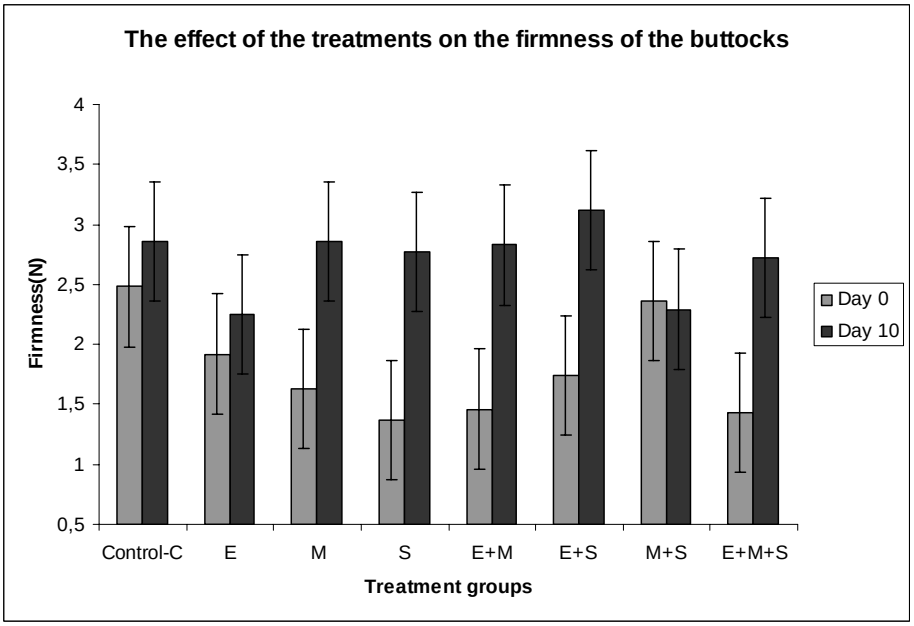

Figure 5. The firmness levels of all of the treatment groups.

The effect on the pH of the meat at 0^{th} and 10^{th} day is shown in Figure 6. This is possibly due to the acidic compounds present in the hawthorn, lowering the pH. In all treatment groups, pH was found to increase except the hawthorn treated samples. In Semperfresh^{TM} and Semperfresh^{TM}-cold storage treated groups a slight increase was recorded in quince fruits (Yurdugul, 2005), as well as in breadfruit (Worrell *et al.*,2002) which was also in line with this study, mainly owing to the formation of an anaerobic environment for microorganisms by the help of edible coatings.

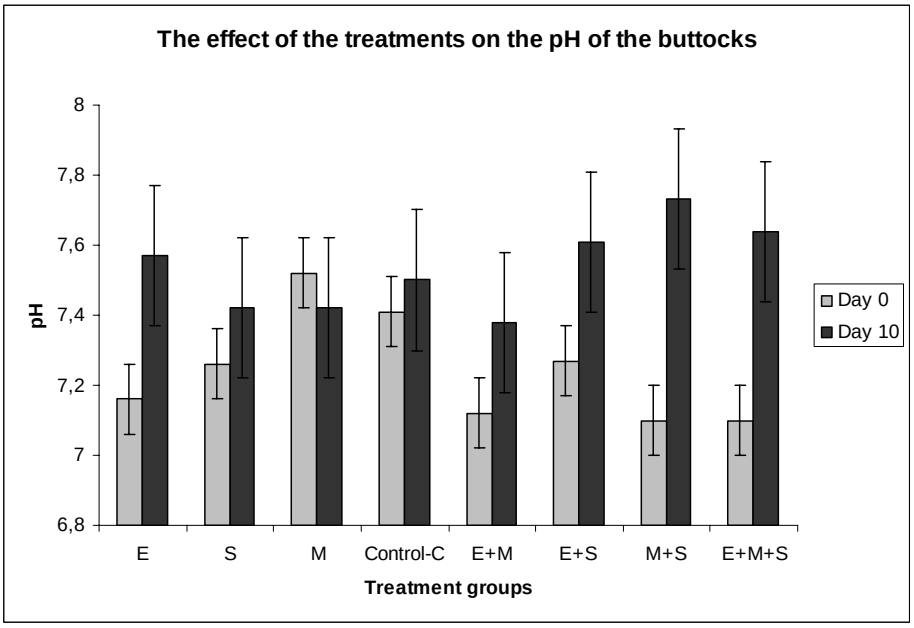

Figure 6. The pH levels of all of the treatment groups.

When the weight of the treatment groups was considered (Figure 7), the weight of the thighs was kept as almost constant in all of the treatment groups, in the zeroth and the tenth days of the experiment. Although the edible coating acts as a barrier which limits the water loss by film coating property in the fruits and the vegetables, as since the meat were cooked prior to the treatments, the groups including the coating material showed the same properties with the other applications. The study conducted by Vishwasrao and Ananthanarayan (2017) showed significant ($P < 0.05$) delay in physiological weight loss and this was found to be in line with our findings. The low mass loss was reported in the study of Soares *et al.* (2011).

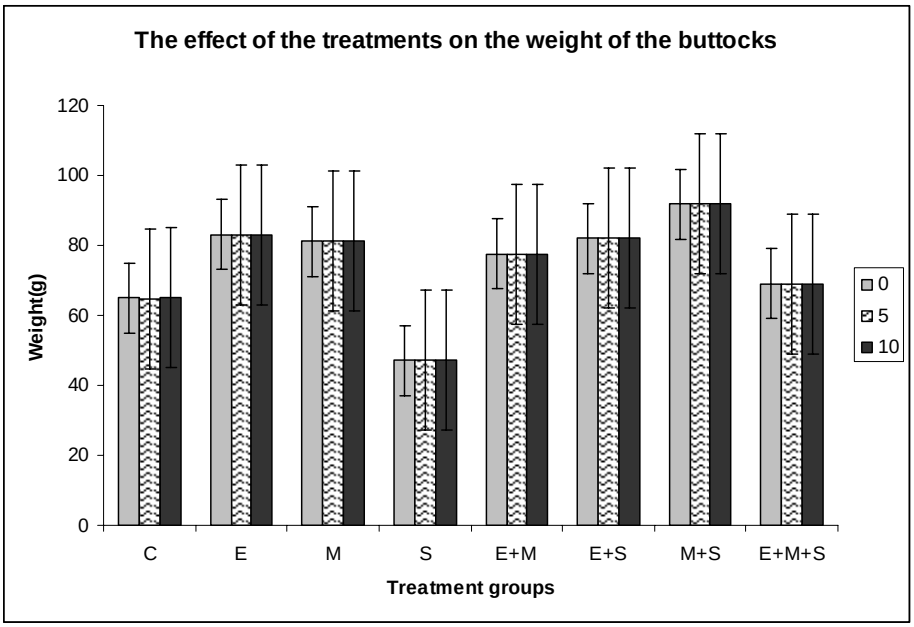

Figure 7. The changes in the weight retention of all of the treatment groups.

According to the ANOVA analysis, there were no significant differences in the pH, the firmness, the weight and the total sugar content of chicken meat ($p > 0.05$) between all of the application groups (Table 2,3,4 and 5). Ready-to-eat chicken thighs which was coated with (E) was disliked by people at taste panel. ANOVA analysis showed a significant difference between the (E) and the control ($p < 0.05$). Interestingly, the most liked group was controlled (Table 6).

Table 1. The codes of the application groups

C	Control
E	Echinacea tea
M	Hawthorn tea
S	Semperfresh™
E+M	Echinacea tea – Hawthorn tea combination
E+S	Echinacea tea – Semperfresh™ combination
M+S	Hawthorn tea – Semperfresh™
E+M+S	Echinacea tea – Hawthorn tea – Semperfresh™

Table 2. The statistical analysis of the pH of all groups.

Variance Source	SS	df	MS	F	P-value	F criteria
Between the groups	0,059663	7	0,008523	0,191928	0,982924	2,657197
Within the groups	0,710533	16	0,044408			
Total	0,770196	23				

Table 3. The statistical analysis of the firmness of all groups

Variance Source	SS	df	MS	F	P-value	F criteria
Between the groups	0,620933	7	0,088705	0,159677	0,989942	2,657197
Within the groups	8,8884	16	0,555525			
Total	9,509333	23				

Table 4. The statistical analysis of the weight of all of the treatment groups

Variance Source	SS	df	MS	F	P-value	F criteria
Between the groups	0,093685	7	0,013384	0,497053	0,823216	2,657197
Within the groups	0,430815	16	0,026926			
Total	0,5245	23				

Table 5. The statistical analysis of the total sugar content of all groups

Variance Source	SS	Df	MS	F	P-value	F-criteria
Between the groups	11,76471	1	11,76471	10,35599	0,002953	4,149097
Within the groups	36,35294	32	1,136029			
Total	48,11765	33				

Table 6. The statistical comparison of the control and the Echinacea treatment in the taste panel by ANOVA.

Source of variance	*SS*	*df*	*MS*	*F*	*P-value*	*F criteria*
Between the groups	16,93382	7	2,419118	1,402985	0,209601	2,081872
Within the groups	220,7059	128	1,724265			
Total	237,6397	135				

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