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ANTIOXIDANT AND ANTIMICROBIAL EFFECTS OF ETHANOL EXTRACT OF *SCROPHULARIA STRIATA* PLANT ON QUALITY OF FILLET CHICKEN DURING REFRIGERATOR STORAGE

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ABSTRACT

Oxidative and pathogenic problems are some of the main challenges for fillet chicken meat products recently; however, now these products have been recommended as alternatives for processed red meat products. In this study, antibacterial and antioxidant effects of *Scrophularia striata* ethanol extract on fillet chicken during the 7-day refrigerator storage time have been investigated. Fillet chicken samples treated with 1, 3 and 5% *Scrophularia striata* ethanol extract were analyzed for microbiological and chemical analysis during the storage time. The antioxidant activity and antibacterial effect as well as against Staph. aureus of fillet chicken samples with 5% *Scrophularia striata* ethanol extract were demonstrated higher than other concentrations. Consequently, *Scrophularia striata* plant ethanol extract were recommended as antioxidant and antibacterial agents for treatment of fillet chicken as well as this result was corresponded with sensory evaluation of treated samples.

1. Introduction

Consumption of poultry meat products has been enhanced recently all over the world. These products are preferred by consumers instead of red meats for many nutritional reasons. Poultry meat products are reach in some nutritional values including vitamin E (tocopherols), minerals, essential amino acids, ascorbic acid and poly unsaturated fatty acids (PUFA); However, increasing level of PUFA in poultry meat leads vitamin E content of these products to be decreased (Valsta, Tapanainen, & Männistö, 2005). Tocopherol components have protection rule of PUFA from oxidative activities. Chicken meat is one of the most important poultry products with similar nutritional value but lower selenium and vitamin E content (Owens, 2010).

The quality properties of poultry meat products include texture, flavor, functionality and microbial quality influencing satisfactory of consumers. Flavor characteristics in simple and complex poultry products such as sausages, marinated, cooked and frozen fillets require special consideration as well as sensory properties influenced easily by oxidative reactions occurred usually in these products. Unsaturated fatty acids content in poultry meat products induce strong oxidative activity to produce oxidation final products leading to flavor undesirability (Fletcher, 2002). Also, unsaturated fatty acid oxidation process releases some carcinogenic components making oxidative stress to form DNA damages as well as safety condition for consumption of these products is not

acceptable. Another important factor in safety consumption of poultry meat products are microbiological properties as well as pathogens and toxins released by them (Kim, Warner, & Rosenvold, 2014). Some high risk pathogens are considerable in poultry meat simple and complex products including Listeria monocytogenes, Escherichia coli, Staphylococcus aureus, Salmonella and Campylobacter spp. These high risk pathogens enter the poultry meat products via initial and cross contamination. Activities against prevalence of these pathogens through poultry meat product manufacturing and distribution leads to assure safety consumption (Kozačinski, Hadžiosmanović, & Zdolec, 2006). Another aspect of microbiological characteristics of poultry meat products is the shelf life problem. Microbial counts of these products indicate the shelf life domain as well as higher microbial counting implying less shelf life (Mataragas, Skandamis, & Drosinos, 2008).

Application of plant extract as an antioxidant and antibacterial agent in food products have been suggested by many researchers recently. Extract of plants are obtained in aqueous and ethanol solutions naming aqueous and ethanol extracts, respectively. Phytochemical compounds including organic acids, essential oils, flavones, alkaloids, phenolics, tanins and terpenoids in plant extracts contribute to antibacterial and antioxidant properties. plant extracts are formulated with food and packaging materials for preservative purposes in food technology (Hsieh, Mau, & Huang, 2001). These extracts released from different parts of plant such as root, flower, leaves, seeds, fruits, bulb, herb and husk. Many plant extracts have suggested by researchers for employing in food technology for antimicrobial and antioxidant effects with family names including Arceae, Bixaceae, Lamiaceae, Liliaceae, Meliaceae, Myrtaceae, Poaceae and Rutaceae (Katalinic, Milos, Kulisic, & Jukic, 2006). Babuskin et al. in the year 2014 employed spice extract for shelf life extension raw chicken meat. They observed suitable antioxidant and antibacterial effect of spice extract for effective preservation of chicken meat. Phenolic constituents of spice extract were considered as strong antimicrobial and antioxidant agents to enhance microbial and oxidative quality of raw chicken meat during the storage preservation (Babuskin et al., 2014).

Scrophularia striata plant is classified to the family scrophulariaceae. The genus Scrophularia is the most important one between 300 species of scrophulariaceae family and usually grows in Turkey, Azerbaijan and Iran areas. There many phytochemical compounds detected in extract of this plant with expanded domain of functionality. Most important components known from Scrophularia extract with considerable antioxidant and antibacterial activity consist of quercetine, isorhamnetin-3-o-rutinoside, nepitrin, cinnamic acid and phenyl propanoid glycoside (Monsef-Esfahani, Hajiaghaee, Shahverdi, Khorramizadeh, & Amini, 2010). Sharafati-chaleshtori and Rafieian-kopaie (2014) observed antibacterial effect of Scrophularia striata plant extract against E. coli in vitro condition (Sharafati-Chaleshtori & Rafieian-Kopaei, 2014). Also, Bahrami and Ali in the year 2010, investigated the antimicrobial effect of Scrophularia striata plant ethanol extract and they found strong activity against S. aureus. Ethanolic extract of Scrophularia striata have been suggested as antioxidant and antibacterial additive for food and packaging formulation by many researchers (Bahrami & Ali, 2010). The aim of this study was to investigate effects of antibacterial and antioxidant activity of Scrophularia striata ethanol extract on

chemical and microbial quality properties of fillet chicken during the refrigerator storage.

2. Materials and methods

2.1. Bacterial culture

Lyophilized culture of Staphylococcus aureus NCTC 29213, a laboratory isolate was purchased from and confirmed by Pasteur institute of Paris, France. Before inoculation the bacterial culture to the samples, the cultures were incubation in nutrient broth then in saline overnight at 35 ˚C to reach 105 CFU mL-1 then isolated subcultures before the start of experiments. Obtained suspensions were diluted by saline solution as required in advance.

2.2. Sample preparation

Scrophularia striata plant was purchased from local market in Zanjan, Iran. Purchased plants were cleaned and washed then dried by sun. Fruits and leaves of the plant were separated and milled to produce powder form. For production of ethanol extract of plant, 100 grams of the powder form of Striata was added to 500 mL of 80% ethanol then mixed for 24 h at ambient temperature. After filtration through Whatman paper, ethanol was separated from mix extract by evaporation under vacuum condition at 50 ˚C (Bahrami & Ali, 2010). Finally, stoke obtained extract was preserved at 4 ˚C in dark place until sample preparation. Chicken fillet were purchased for experiments from local markets in Zanjan, Iran. 50-gram pieces of chicken fillet samples inoculated with Staphylococcus aureus were suspended in 0, 1, 3 and 5% Scrophularia striata plant ethanol extract for 5 min then stored at 4 ˚C then samples were taken at days 0, 2, 5 and 7 of refrigerator storage time for chemical and microbiological analysis. All treatments have been implemented in triplicate.

2.3. Chemical analysis

Lipid peroxidation give rise to produce Thiobarbituric Acid Reactive Substrate (TBARS) determined as an indicator of oxidation process of fat using the method suggested by Kannat et al. (2010). 4 grams of each samples were mixed with 16 mL of 5% trichloroacetic acid and BHT then filtered through Whatman paper. The filtrate in equal amount added to 0.02 M TBA, heated in a water bath for 30 min then cooled to the room temperature for absorbance measurement at 532 nm. The amount of TBARS was expressed as mg malonaldehyde per kg of fillet chicken meat. Peroxide value of oil extracted from fillet chicken meat samples was measured that is suggested by AOAC-965.33 (International, 2005). 3-5 grams of extracted oil was added to acetate-chloroform (2:3) with 0.5 mL potassium iodide. After shaking and addition of 30 mL distilled water, solution was titrated with 0.01 N sodium thiosulfate until the blue color of starch indicator was observed. The peroxide value of samples was calculated by the following equation (Eq. (1)):

$$PV = (V \times N \times 1000)/W \quad (1)$$

While PV is peroxide value, V is the volume of sodium thiosulfate and W is the weight of extracted oil. For measuring pH vale, 10 grams of fillet chicken meat sample mixed with 90 mL of distilled water then the pH value was determined by a digital pH-meter instrument (Metrohm 744, Netherland). All measurements were conducted in triplicate (International, 2005).

2.4. Microbial analysis

Effect of Scrophularia striata plant ethanol extract on microbiological attributes of fillet chicken meat during refrigerator storage time including days 0, 2, 5 and 7 was evaluated. Microbiological analysis of

treated samples was carried out according to agar based plate counting method. Ten grams of each samples were taken and placed into the sterile plastic container then diluted with 90 mL buffered water. Diluted sample was homogenized in a stomacher (Stomacher 400, England) for 1 min then prepared in suitable decimal dilutions. For counting total aerobic mesophilic bacteria diluted samples was incubated into plate count agar (PCA, Merck, Germany) for 48 h at 37 ˚C. Total aerobic psychrophilic bacteria were measured by incubation of samples in plate count agar (PCA, Merck, Germany) for 7 days at 7 ˚C. Staphylococcus aureus was counted using Bird Parker Agar (BPA, Merck, Germany) incubated for 24 h at 37 ˚C. All measurements in plate counting were carried out in triplicate (Muhammad et al., 2013).

2.5. Sensory evaluation

Each sample was grilled and cooked ready for sensory evaluation by panelists. A panel team consist of 30 persons semi-experienced in evaluation of meat products sensory attributes was used. Each panelist previously had become familiar with sensory properties of cooked fillet chicken meat. All panelists were asked to evaluate taste, color, texture and total acceptability of samples. Sensory characteristics were evaluated using 5 point hedonics scale (0-5). The scale points include: very poor (1), poor (2), acceptable (3), good (4) and very good (5) (Feng et al., 2016).

2.6. Statistical procedure

All analysis and measurements in this study were carried out in triplicate. Analysis of Variance (ANOVA) was used to determine significantly ($p<0.05$) between treatments and the contrast between means (Duncan`s multiple range test for chemical, microbiological and sensory analysis) were used to assess the differences between the variables. Statistical analyses were conducted using SPSS ver. 22 for windows (Chicago, USA).

3. Results and discussions

3.1.Chemical analysis

Fig. 1 provides pH value changes during the 7-day storage time of fillet chicken samples different ethanol extract of Scrophularia striata plant. As can be seen in Fig. 1, pH values of all samples increase gradually during the storage time final pH value of sample with 5% extract (the highest concentration) is the lowest one (pH = 6.13) in comparison with the other similar treated samples with lower plant extract. Variations of PV through refrigerator storage time are demonstrated in Fig. 2. As it can be observed from Fig. 2, PV increasing of samples with higher plant extract were less than others; consequently, samples with 5% plant extract demonstrated the lowest PV (1.91 meq O2/Kg lipid) after 7 days indicating the best oxidative condition. Oxidative reactions of fatty acids give rise to enhancement of pH value and PV due to creating some compounds explained these phenomena. These observations concluded that Scrophularia striata plant extract has the antioxidant effect and prevents oxidative reactions specially in food systems. Azadmehr et al. (2009), Monsef-esfahani et al. (2010), Ghoran et al. (2012) and Mahboubi et al. (2013) reported antioxidant activity of Scrophularia striata plant aqueous and ethanol extracts (Azadmehr et al., 2009; Ghoran, Safavi, Meighani, & Ebrahimi, 2012; Mahboubi, Kazempour, & Nazar, 2013; Monsef-Esfahani et al., 2010) . Pasdaran et al. (2012) also showed high antioxidant activity of essential oil of Scrophularia striata plant (Pasdaran, Delazar, Nazemiyeh, Nahar, & Sarker, 2012).

TBA value of treated samples with different Scrophularia striata plant ethanol extract are

shown in Fig. 3. Considering the Fig. 3, it can be concluded that TBA value of samples with high concentration of plant extract have lower enhancement than others; however, this result had been seen for pH value and PV previously. Lipid oxidation can be monitored by TBARs test as higher TBA value indicates better oxidative condition and low levels of oxidation. Antioxidant activity and characteristic of Scrophularia striata plant ethanol extract prevent oxidation in treated fillet chicken samples. Phenolic compounds, flavonoids, cinnamic acid and phenyl propanoid in Scrophularia striata plant lead to higher antioxidant activity. Azadmehr et al. in the year 2013, also showed the antioxidant and neuroprotective activity of Scrophularia striata plant extract usable in medical and food technology (Azadmehr et al., 2009).

3.2. Microbiological properties

As can be seen from Figs 4 and 5, total aerobic bacteria and total psychrophilic bacteria increase gradually during the 7-day refrigerator storage time in fillet chicken treated samples with Scrophularia striata ethanol extract. The final point, after 7 days storage, was observed the lowest counting for both total anaerobic bacteria and total psychrophilic bacteria with 5% Scrophularia striata ethanol extract treatment. As reported by Mahboubi et al. (2013), Scrophularia striata extract have significant antimicrobial effect on gram positive and negative bacteria in comparison with antibiotic treatment. However, methanol and ethanol extract of Scrophularia striata plant observed more effective for antibacterial characteristic. The phenolic and flavonoid compound in Scrophularia striata extract were recommended to be the cause of antibacterial effect of this plant extract (Mahboubi et al., 2013). Abbasi et al. (2007) also observed antimicrobial properties of Scrophularia striata extract on various range of bacteria (Abbasi, Azizi Jalilian, Abdi, & Saifmanesh, 2007). Antibacterial effect of Scrophularia striata extract against E. coli also was demonstrated by Sharafati-chaleshtori et al. (2014) (Sharafati-Chaleshtori & Rafieian-Kopaei, 2014).

Fig. 6 provides effect of Scrophularia striata ethanol extract on Staph. aureus inoculated into the fillet chicken samples during the storage time. Trends in figure indicated significant increase in samples treated with 1% Scrophularia striata ethanol extract and control; but, decrease then increase observed for 3 and 5% extract treatments overall leads to considering no change in Staph. aureus counting in samples after 7 days storage time significantly. Abbasi et al. in the year (2007) showed antibacterial effect of Scrophularia striata extract against Staph. aureus. They reported phenolic and flavonoid compound in Scrophularia striata plant extract are causes of antibacterial effect against Staph. aureus in this extract. Also, this plant extract is recommended useful against pathogenic bacteria in food product formulation (Abbasi et al., 2007). Bahrami and Ali in the year 2010 also demonstrated antibacterial effect of Scrophularia striata leaves ethanol extract against Staph. aureus significantly (Bahrami & Ali, 2010).

3.3. Sensory evaluation

The results of sensory evaluation including color, odor, flavor, texture and total acceptability of fillet chicken treated with 1, 3 and 5% Scrophularia striata ethanol extract were demonstrated in Fig. 7. As it can be seen in data from figure 7; color, odor, texture, flavor and total acceptability were observed high score for sensory evaluation. As a result, Scrophularia striata ethanol extract without any sensory preventive effect can be used as antibacterial and antioxidant effect in fillet chicken products.

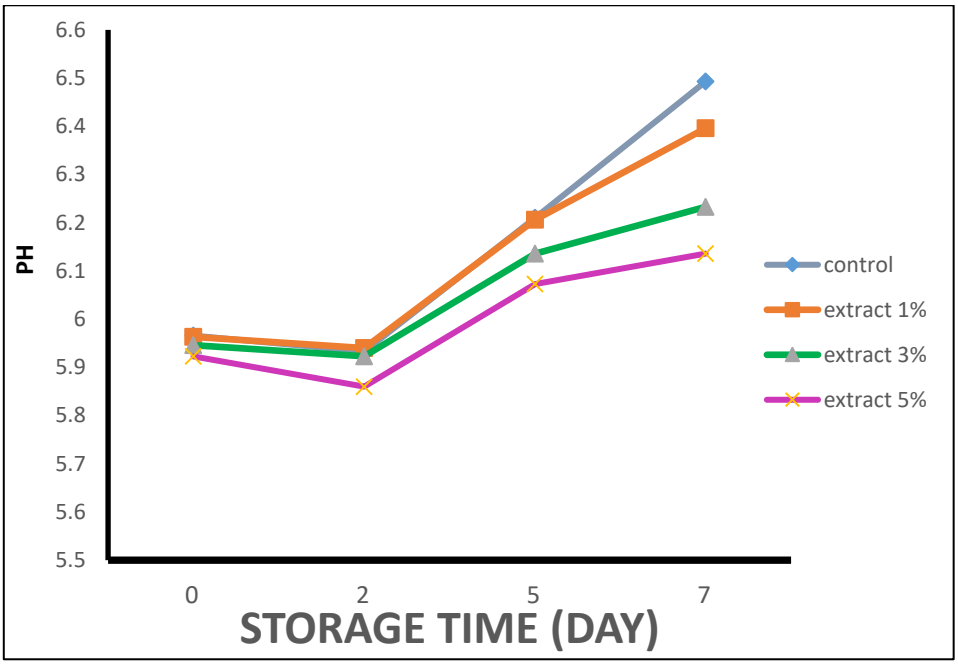

Figure 1. pH changes during the refrigerator storage time of fillet chicken samples treated with Scrophularia striata ethanol extract

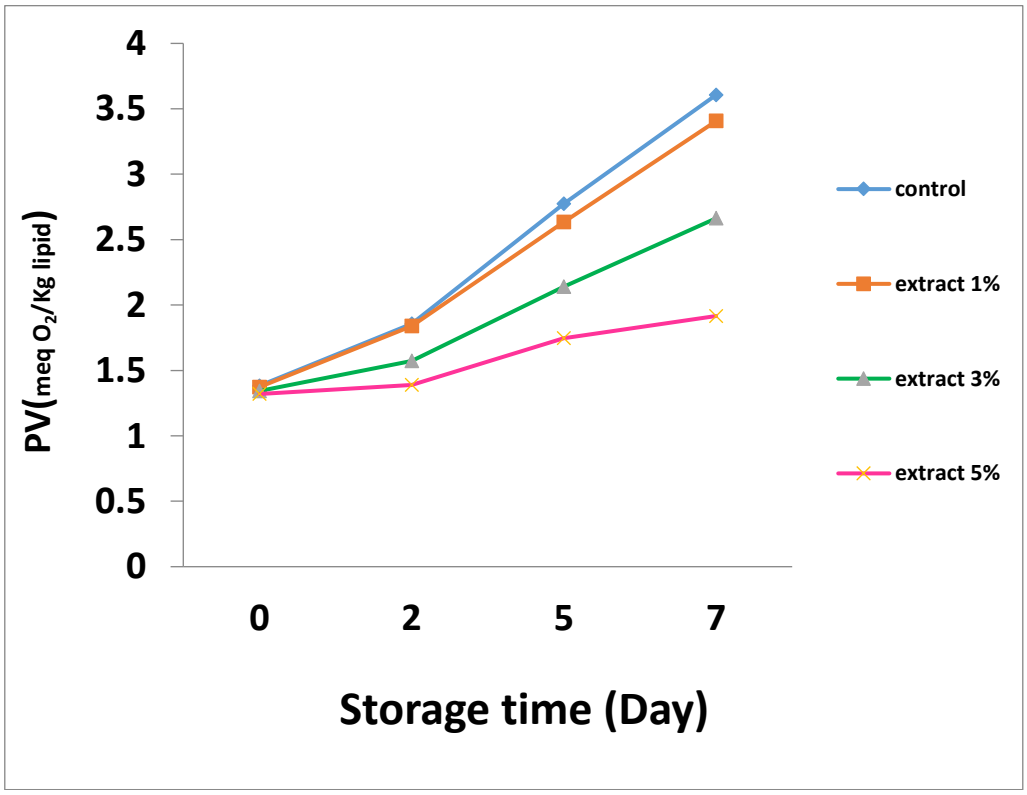

Figure 2. PV variations during the refrigerator storage time of fillet chicken samples treated with Scrophularia striata ethanol extract

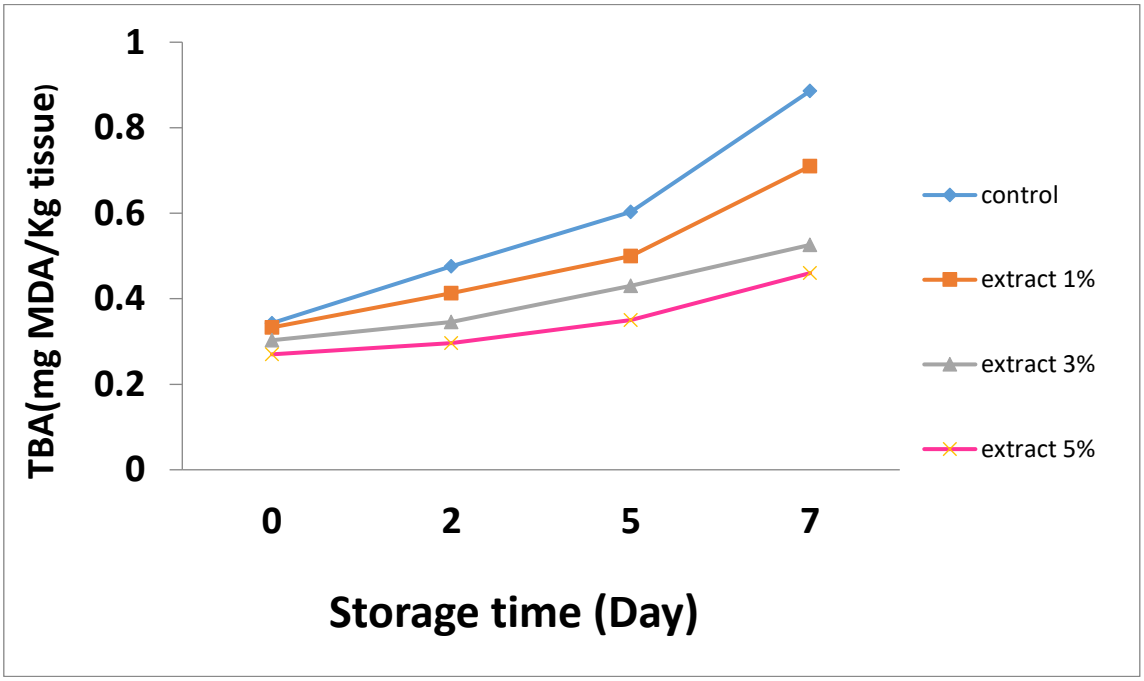

Figure 3.TBA value during the refrigerator storage time of fillet chicken samples treated with Scrophularia striata ethanol extract

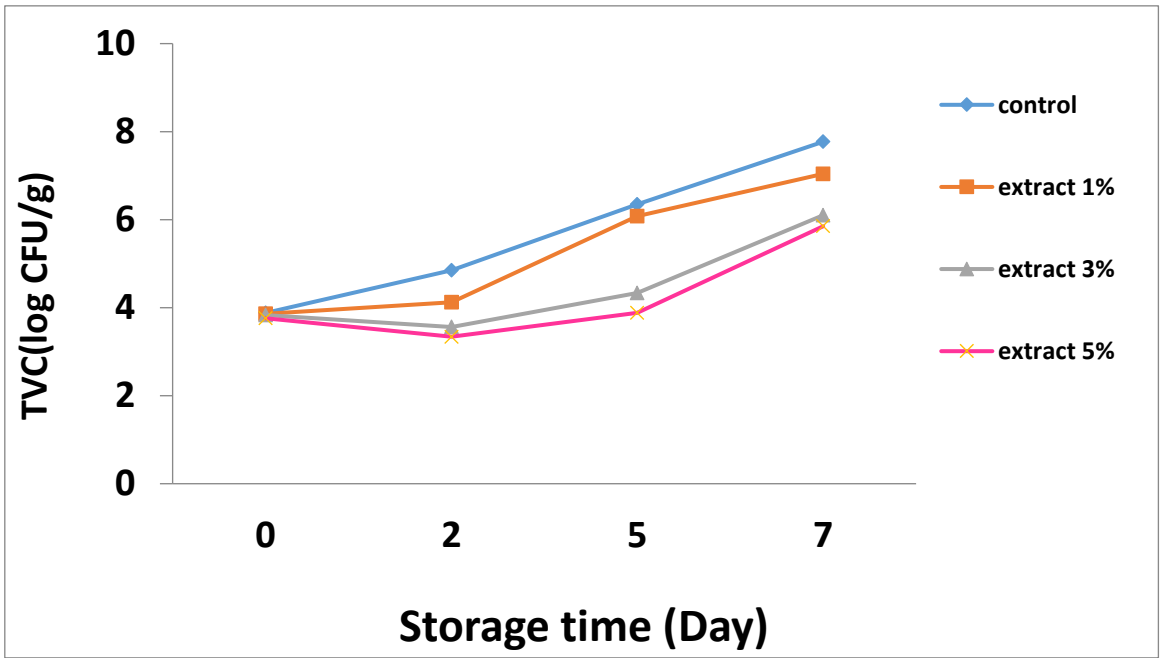

Figure 4. Total aerobic count during the refrigerator storage time of fillet chicken samples treated with Scrophularia striata ethanol extract

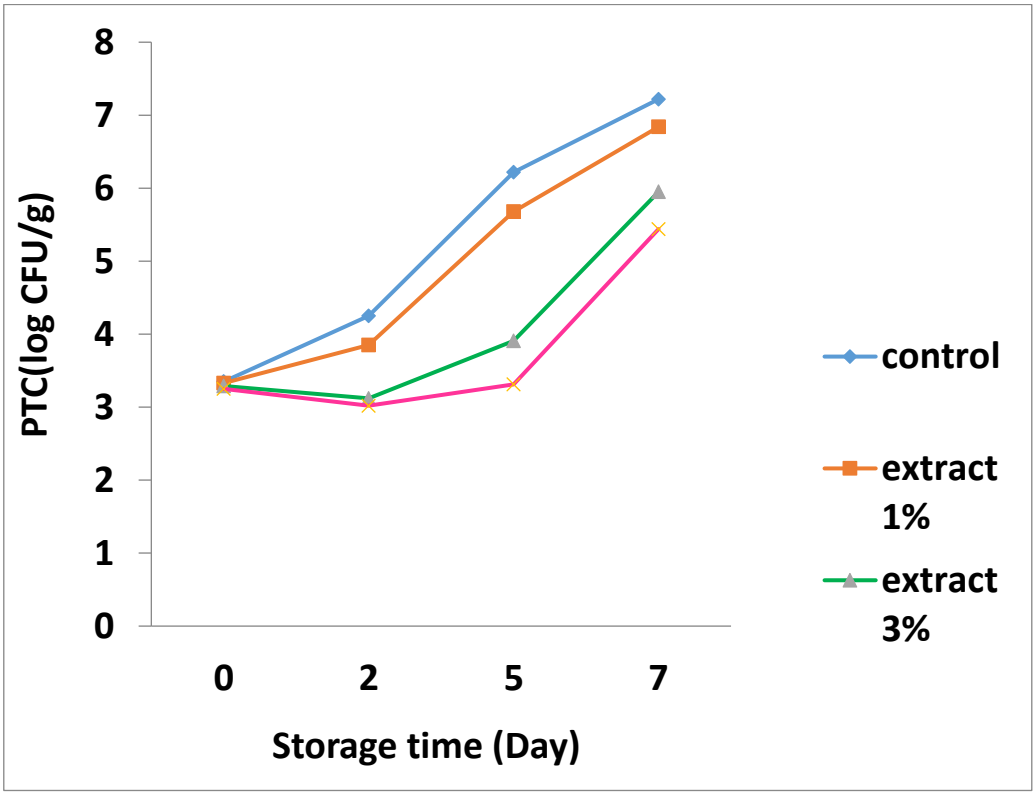

Figure 5. Total psychrophilic count during the refrigerator storage time of fillet chicken samples treated with Scrophularia striata ethanol extract

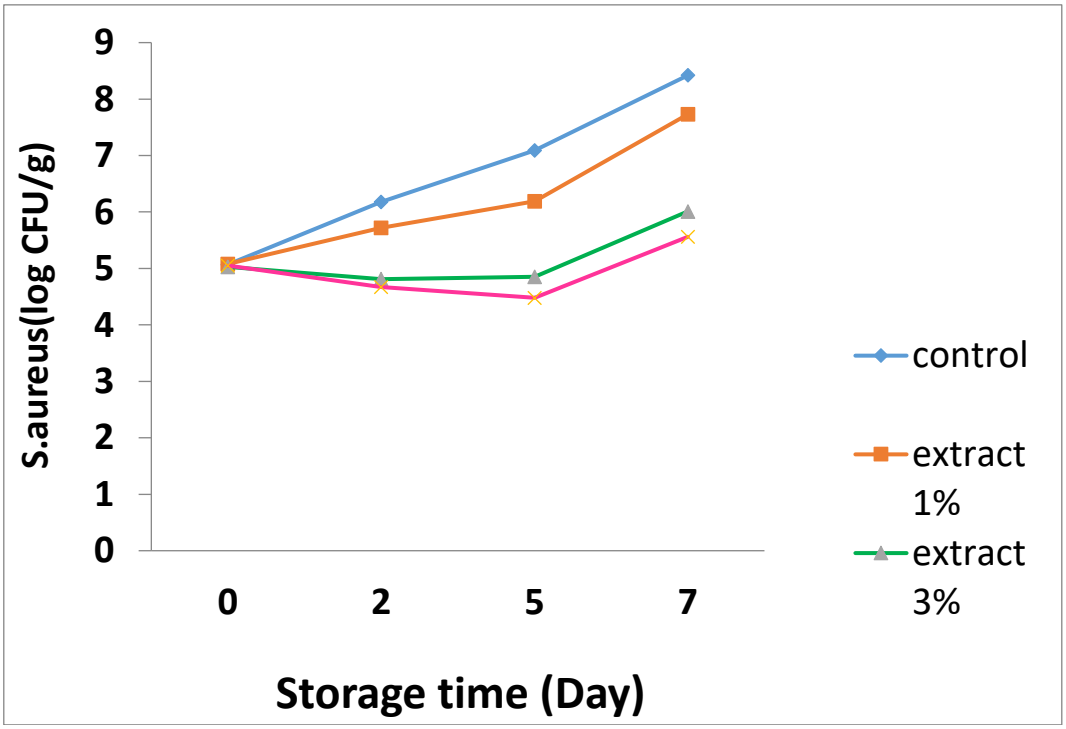

Figure 6. Total count of Staph. aureus during the refrigerator storage time of fillet chicken samples treated with Scrophularia striata ethanol extract

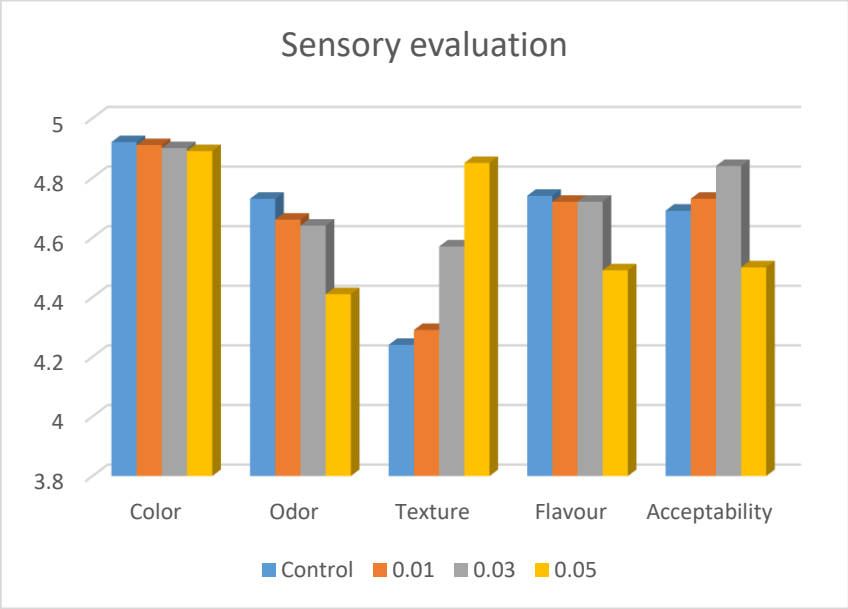

Figure7. Sensory evaluation of fillet chicken samples treated with Scrophularia striata ethanol extract after 7-day refrigerator storage time

4. Conclusions

As conclusion, antibacterial and antioxidant effects of Scrophularia striata ethanol extract on fillet chicken during the 7-day refrigerator storage time were observed significantly. Fillet chicken samples treated with 1, 3 and 5% Scrophularia striata ethanol extract were analyzed for microbiological and chemical analysis during the storage time. The antioxidant activity and antibacterial effect against Staph. aureus of fillet chicken samples with 5% Scrophularia striata ethanol extract were demonstrated higher than other concentrations. As a result, in this study Scrophularia striata plant ethanol extract were recommended as antioxidant and antibacterial agents for treatment of fillet chicken as this was corresponded with sensory evaluation of treated samples.

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