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ORIGINAL ARTICLE

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The effect of chitosan coating incorporated with ethanolic extract of propolis on the quality of refrigerated chicken fillet

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Abstract

Chitosan is considered as a functional packaging component for maintaining the quality and increasing the shelf life of perishable foods include meat, poultry, fish, dairy products, and all cooked leftovers. The present study was conducted to evaluate edible coating of chitosan (2%) containing ethanolic extract of propolis (1% and 2%) on microbiological (mesophilic aerobic, psychrotrophic, lactic acid bacteria, coliforms, and *Staphylococcus aureus* counts), chemical (TBARS, TVN and Peroxide values) and sensory (odor, color, texture, taste, and overall acceptance) properties of chicken fillet. Microbial analysis showed that coating had a significant reducing effect on growth of bacteria during 12 days at 4 °C. Besides, the increase of TBARS, Total volatile nitrogen, and peroxide value of samples coated by chitosan and ethanolic extract of propolis was less than control group. According to our results, chitosan and propolis can be used to enhance the shelf life of fillet and maintain its quality.

Practical applications

Propolis is used for infections caused by bacteria, viruses, fungus, and by single-celled organisms called protozoans. Propolis is also used as an antioxidant and anti-inflammatory agent. Ethanol extract of propolis improve the properties of chitosan edible coating in chicken fillet preservation. The chitosan coating incorporated with ethanolic extract of propolis can improve the microbial, chemical, and sensory quality of food and enhance the shelf life of them by synergistic effects.

KEYWORDS

chitosan, fillet, propolis, shelf life

1 | INTRODUCTION

Fillet, fresh or frozen, is more widely used in the food industry in comparison with other parts of chicken, whereas it is more susceptible to spoiling. Hence, food industry focuses on finding modern methods and technologies to increase the shelf life of fillet. Susceptibility of poultry meat to spoiling is an economic problem for its producers, and various methods have been applied to enhance its shelf life (Petrou, Tsiraki, Giatrakou, & Savvaidis, 2012). Conversely, consumers' demand for healthy meals which are free of chemical preservatives, have been increased in respect to the past (Giatrakou & Savvaidis, 2012).

Therefore, using natural coatings and preservatives are solutions to increase the shelf life of perishable food like as meat products. Biodegradable biopolymers due to their degradation can be produced from wastes and thus help preserve natural resources. These natural biopolymers have favorable effects and may be used as a key ingredient for the new bioactive packaging. Today, various polysaccharides including cellulose, pectin, and starch derivatives are extensively used in coating film production. Chitin is one of the most important polysaccharides and is made from N-acetylglucosamine, which is the second most frequent biopolymer in the world. Chitosan is the only cationic polysaccharide, which is produced by acetylation of chitin in the concentrated alkaline environment. Chitosan is a biodegradable compound that used in foods and pharmaceuticals products and it has antioxidant and broad-spectrum antimicrobial activity against bacteria and fungi (Prashanth & Tharanathan, 2007). According to previous studies, edible chitosan coating on fruits and vegetables can reduce the growth of bacteria and fungi during storage and dispersal (Chien, Sheu, & Yang,

2007; Devlieghere, Vermeulen, & Debevere, 2004). Many applications of chitosan in different meat products have also been reported (Georgantelis, Blekas, Katikou, Ambrosiadis, & Fletouris, 2007; Giatrakou, Ntzimani, & Savvaidis, 2010; Kanatt, Chander, & Sharma, 2008; Roller et al., 2002; Yingyuad et al., 2006).

Propolis is a substance derived from plant resins, which is collected by bees and converted into the wax-like condensation product. The appearance and color of propolis change according to the resin sources and can be influenced by environmental and nutritional factors (Fernandes et al., 2007). Propolis due to its antibacterial, antiviral, anti-inflammatory and anesthetic properties is considered as a functional compound in food packaging (Koc, Silici, Ayangil, Ferahbaş, & Cankaya, 2005; Kujumgiev, Bankova, Igantova, & Popov, 1993; Paulino et al., 2006). Bodini, Sobral, Favaro-Trindade, and Carvalho (2013) used a gelatin-based film plasticized with sorbitol and ethanol–propolis extract (EPE) and reported the antimicrobial activity against *Staphylococcus aureus*.

Several studies on the beneficial effects of chitosan and its application in the food industry, either alone or in combination with other materials have been conducted. Roller et al. (2002) showed that chitosan-sulfite treatments prevented the growth of spoilage microorganisms and they were more acceptable than other treatments in sensory evaluation. Pranoto, Rakshit, and Salokhe (2005) indicated that using garlic oil (100 μg/lit), potassium sorbate (100 mg/g) and nisin in chitosan film prevented the growth of *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus*. A study suggested that the combination of chitosan film with extracts of anise, basil, and coriander had antibacterial effects against *L. monocytogenes* and *E. coli* (Zivanovic, Chi, & Draughon, 2005). Besides, Shahidi, Arachchi, and Jeon (1999) reported that using N-*carboxymethyl* chitosan (5000 ppm) in meat results in reduction of 93% thiobarbituric acid and 99% Hexanal production.

Improving antioxidant and antimicrobial activities of chitosan films as active food packaging by incorporating with propolis was reported by some researchers (Siripatrawan & Vitchayakitti, 2016; Torlak & Sert, 2013).

According to mentioned issues and microbial contamination of poultry meat as well as the emphasis on the application of biodegradable coating and packaging rather than synthetic ones, the main goal of the present study was to investigate preservative and antimicrobial effect of chitosan alone and in combination with ethanolic extract of propolis on chicken fillets in the fridge condition.

2 | MATERIALS AND METHODS

2.1 | Preparation of ethanolic extract of propolis

Propolis samples were collected from different locations of Tehran Province in May 2016 and pooled. Hand-collected propolis samples were kept in the dark up to their processing and stored at −20 °C. Then, 20 g of the ground powder was extracted with 100 ml of 80% ethanol with continuous stirring at room temperature for 48 h. The suspension was filtered by Whatman ≠3 filter paper and separated by centrifugation at 600 ×g for 20 min (Isla, Nieva Moreno, Sampietro, & Vattuone, 2001). The supernatant was then concentrated in a rotary evaporator under reduced pressure at 40 °C and the propolis powder was obtained by freeze-drying.

2.2 | Preparing the chitosan coating containing propolis extract

High purity, low-molecular-weight chitosan (≥75% deacetylation, mushroom-derived) was purchased from Pars Sigma Company, Tehran Iran. To preparation of solution, 2 gr of chitosan was added to 100 ml of acetic acid solution (1% v/v) and gently mixed at 40 °C on a magnetic stirrer. Subsequently, 0.75 ml/g of glycerol was added as the plasticizer and 0.2% of Tween 80 added as the emulsifier. The pH was then adjusted to 5.7–6 by adding 1 mol/L NaOH, then the solution was steered at 30 °C for 30 min. The prepared solution then filtrated through Whatman ≠3 filter papers and autoclaved for 15 min at 121 °C (Ojagh, Rezaei, Razavi, & Hosseini, 2010). The freeze dried ethanolic extract of propolis was added to the coating at 1% and 2% concentrations and homogenized for 10 min by magnetic stirrer.

2.3 | Fillet coating

Chicken fillets were prepared from a production line and transferred to the laboratory in cold conditions. 50 g of the fillet samples were prepared in a sterile condition and 40 fillets were considered for each treatment. To create coating, fillets were soaked in coating solution containing 0, 1 and 2% propolis extract for 30 s, then they were taken out and after 2 min, they were soaked in the solution for 30 s again (Ojagh et al., 2010). The coated samples were allowed to drain for 5 minutes under a biological safety cabinet. The samples were placed in sterile bags and kept at 4 °C and microbiological, chemical and sensory evaluation of fillets were conducted at intervals of three days. The controls were treated similarly in water solution lacking coating materials.

2.4 | Bacteriological analysis

Bacteriological counts were determined by placing a 10 g of prepared samples with 90 ml of peptone water (0.1%), mixing in a sterile bag, and homogenizing with Stomacher (BagMixer 400, Interscience, France) at 200 rpm/min for 1 minute. Then other decimal dilutions were prepared from this dilution in tubes containing peptone water. Bacterial counts were performed by method using de man-rogosa-sharpe Agar (MRS) for lactic acid, plate count agar (PCA) for psychrotrophic and mesophilic aerobic bacteria, Baird–Parker agar for *Staphylococcus aureus*, and violet red bile agar (VRBA) for coliforms. The inoculated plates were incubated at 37 °C for 2 days for total viable counts, *S. aureus*, and coliforms. The incubation condition was 7 °C for 10 days for psychrotrophic counts and 30 °C for 2 days for lactic acid bacteria (Muhlisin, Utama, Lee, Choi, & Lee, 2016; Ojagh et al., 2010). All counts were expressed as $\log_{10}$ CFU/g and performed in duplicate.

2.5 | Determinations of thiobarbituric acid reactive substances (TBARS)

The TBARS was determined colorimetrically as described by Botsoglou et al. (1994). A portion (10 g of sample) and 30 ml of 4% perchloric acid was added to a 50 ml centrifuge tube as well as 1 ml of 0.5% BHT in ethanol and homogenized. Then the mixture was filtered through Whatman ≠4 filter paper. After that, 5 ml of the filtrate with 5 ml of solution TBA (0.02 M) were mixed and then placed in a boiling water bath for 20 minutes. After cooling the samples, they were read at wavelength of 532 nm. The TBA was measured based on malondialdehyde (MDA) mg/kg of standard sample.

2.6 | Measuring total volatile nitrogen (TVN)

To measure TVN, 10 g of sample, plus 2 g MgO and 500 ml distilled water were mixed in the balloon and eventually TVN was collected in a solution containing boric acid (2%) and methyl red as an indicator. Titration was performed with sulfuric acid described as TVN mg/100 g of chicken fillet (Goulas & Kontominas, 2005). TVN was calculated as follow:

$$\%\text{TVN} = \text{sulfuric acid} \times 14$$

2.7 | Measuring peroxide value

Twenty grams of fillet with 100 ml of chloroform/methanol solution was mixed at a portion of 2:1 and blended for 1 minute. After dewatering by potassium chloride, the aqueous phase (lower phase) was collected and used for titration by sodium thiosulfate.

2.8 | Sensory evaluation

A panel of six trained panelists was selected among the staff of the University of Tehran on the basis of their experience in the sensory analysis. The uncoated/coated fillets after cooking in microwave at 185 °C were evaluated based on taste, odor, color, texture, and overall acceptability attributes. The results were expressed on a 9-point hedonic scale. The sensory scores were 9, like extremely; 8, like very much; 7, like moderately; 6, like slightly; 5, neither like nor dislike; 4, dislike slightly; 3, dislike moderately; 2, dislike very much; 1, dislike extremely (Kao, Su, & Lee, 2010). The Sensory evaluation of samples was done after 3 days of storage.

2.9 | Statistical analysis

Analysis of all data was performed by One-way analysis of variance (ANOVA) and Duncan's New Multiple Range Test in SAS version 9.1. The statistical significance of differences between mean values was proved at $p < .05$.

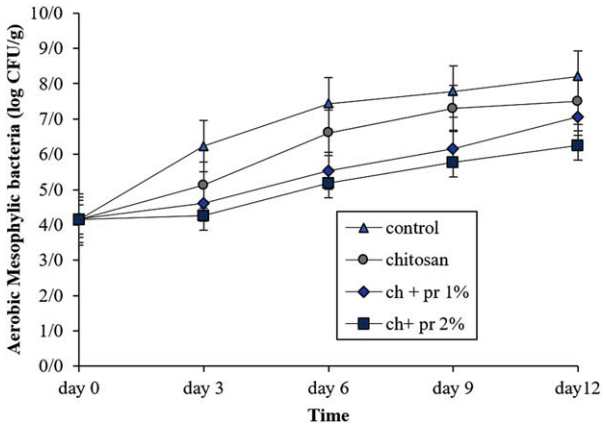

FIGURE 1 Total aerobic mesophilic bacteria count in chicken fillet coated by chitosan and ethanolic extract of propolis during 12 days storage at 4 °C

3 | RESULTS AND DISCUSSION

3.1 | Microbiological properties

The number of bacteria including aerobic mesophilic, psychotrophic, lactic acid, coliform and *S. aureus* bacteria in four different treatment groups was counted and the results are shown in Figures 1–4 and Table 1, respectively. The number of aerobic mesophilic bacteria in the control group increased with time and its log reached to 8.1 in day 12. In the chitosan group, the count was 7.4 log at day 12 in which a significant decrease ($p < .05$) shows in comparison with the control group. Samples treated with 1% propolis and chitosan had a decrease in number of aerobic mesophilic bacteria (7 log in the last day). By increasing the extract of propolis to 2%, the antimicrobial effects got stronger as the number of bacteria reached only 6.2 in day 12. The log of number of bacteria in last two groups was significantly lower than control and chitosan groups.

Yingyuad et al. (2006) showed that chitosan coating in a PVDC/nylon pouch decreased the microbial count of grilled pork during refrigerated storage. Petrou et al. (2012) also indicated that 1.55% chitosan

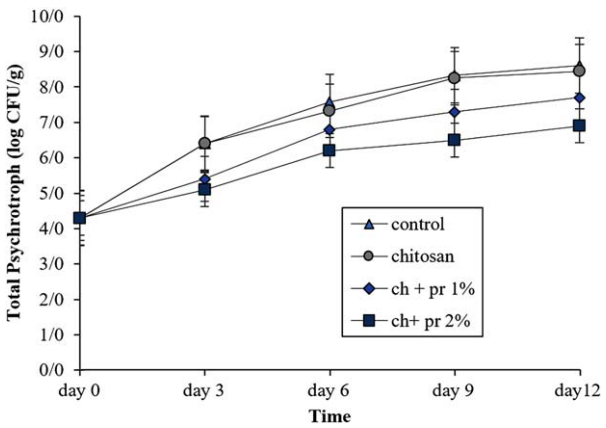

FIGURE 2 Psychotrophic bacteria count in chicken fillet coated by chitosan and ethanolic extract of propolis during 12 days storage at 4 °C

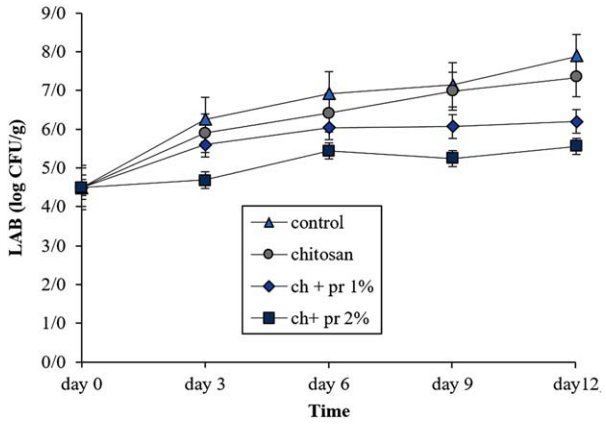

FIGURE 3 Lactic acid bacteria count in chicken fillet coated by chitosan and ethanolic extract of propolis during 12 days storage at 4 °C

coating is effective in inhibiting bacterial growth on chicken breast meat. Some authors suggested that 1% chitosan can reduce the bacterial number by an average of 2 log CFU/g in beef (Darmadji & Izumimoto, 1994).

The antibacterial property of propolis is indicated in many studies (Gonsales, Orsi, Fernandes Júnior, Rodrigues, & Funari, 2006; Kujumgiev et al., 1993; Popova, Silici, Kaftanoglu, & Bankova, 2005; Sforcin, Fernandes, Lopes, Bankova, & Funari, 2000). It is in accordance with our results which showed that adding propolis extract to chitosan coating inhibit bacterial growth in our samples. If the extract of propolis used alone (liquid form), such as spraying it on fillets, after a while due to its high volatility the concentration of the product is reduced which resulted in less antimicrobial effects (Bodini, Sobral, Favaro-Trindade, & Carvalho, 2013; Duman & Özpolat, 2015). But when the propolis and chitosan used together, it helps to maintain the characteristics of the extract for a long time.

According to our study, adding ethanolic extract of propolis reduced the number of psychrotrophic bacteria so that the log of bacteria in day 6 did not reach 7, and this reduction was significant in

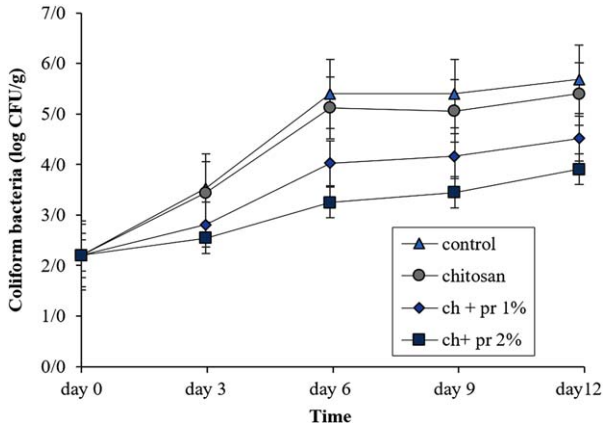

FIGURE 4 Coliform bacteria count in chicken fillet coated by chitosan and ethanolic extract of propolis during 12 days storage at 4 °C

TABLE 1 The *S. aureus* count (mean ± SD) in chicken fillet coated by chitosan and ethanolic extract of propolis during 12 days storage at 4 °C

Treatment	Storage days				
	0	3	6	9	12
Control	0.00[a]*	0.00[a]	1.61 ± 0.02[a]	1.53 ± 0.01[a]	3.44 ± 0.06[a]
Chitosan	0.00[a]	0.00[a]	1.92 ± 0.04[a]	2.54 ± 0.04[b]	3.93 ± 0.01[a]
Chitosan + 1% Propolis	0.00[a]	0.00[a]	0.00[b]	0.00[c]	2.62 ± 0.01[b]
Chitosan + 2% Propolis	0.00[a]	0.00[a]	0.00[b]	0.00[c]	1.30 ± 0.02[c]

*Values followed by the same letters are not significantly different at the 0.05 level.

respect to control group up to day 9 ($p < .05$), but after that, the difference was not significant. Ethanolic extract of 1% and 2% propolis significantly decreased the number of psychrotrophic bacteria so that the log of bacterial numbers was 7 in day 12. The results showed that chitosan and ethanolic extract of propolis significantly reduced the growth rate of psychrotrophic bacteria. These results were in accordance with those obtained by Jeon, Kamil, and Shahidi, (2002) which the log of psychrotrophic bacterial numbers in fish coated by chitosan in day 12 was less than 6, while this value was obtained in day 6 for uncoated samples. Other investigators also indicate that chitosan has antimicrobial effects to some extent on some psychrotrophic bacteria such as *Pseudomonas* (López-Caballero, Gómez-Guillén, Pérez-Mateos, & Montero, 2005; Tsai, Su, Chen, & Pan, 2002).

It is obvious in Figure 3 that no significant antimicrobial effect was observed on lactic acid bacteria by chitosan coating. The log of number of lactic acid bacteria reached 7.7 in the last day in control group and 7.3 in chitosan group. In the ethanolic extract of 1% propolis group, the number of lactic acid bacteria was recorded as 6.2 in day 12 which is remarkably less than control group. Besides, 2% propolis was better in respect to 1% propolis, where the log of lactic acid bacteria in day 12 was 5.5.

López-Caballero et al. (2005) indicated that chitosan has no obvious effect on inhibiting the growth of lactic acid bacteria. In another study, Lee, Park, Jung, and Shin (2002), demonstrated that chitosan coating could even stimulate the growth of some of these bacteria in lower concentrations (0.1–0.5%).

In the control group, the log of number of bacteria varied from 2.2 to 5.7. In chitosan treatment group, the number of coliforms was recorded to be 5.4 in day 12, and there was not any significant difference between control and chitosan groups from day 6 up to day 12 (Figure 4). Adding 1% and 2% propolis resulted in reduction of bacterial count and they reached to 4.6 and 3.7 in day 12, respectively. In this study, the number of coliforms in treated samples by propolis was significantly less than those in control and the 2% propolis was more efficient in comparison with others.

Some authors suggested that chitosan could prevent the growth of some coliform bacteria while it is not very effective against some others (Kanatt et al., 2008). López-Caballero et al. (2005) reported that

chitosan coating was effective in reducing number of gram-negative bacteria. Various studies proved that propolis affects gram-positive bacteria more than gram-negative ones (Silva, Rodrigues, Feás, & Estevinho, 2012; Siripatrawan, Vitchayakitti, & Sanguandeekul, 2012). The reason is due to the outer membrane of gram-negative bacteria that limits the penetration and diffusion of hydrophobic compounds in lipopolysaccharide cover of bacteria (Brumfitt, Hamilton-Miller, & Franklin, 1989).

According to Table 1, in the control samples, the log of *Staphylococcus aureus* numbers raised from 0 to 3.4 in 12 days. In the chitosan-treated fillets, the number of *S. aureus* in day 12 was recorded as 3.9, and there was no significant difference in these groups. Conversely, in samples treated with 1% and 2% of propolis extract, the log of bacterial count was 0 until day 9, but it was increased to 2.6 and 1.3 in the last day, respectively. These findings show the inhibitory properties of propolis against *S. aureus*. Besides, the number of *S. aureus* in 1% propolis group was more than that in 2% propolis group. It means that the higher concentration of propolis, the less bacterial growth, which is due to high antimicrobial impacts of propolis against such microorganism. Siripatrawan et al. (2012) showed propolis has a higher impact on gram-positive bacteria in comparison to gram-negative bacteria, which is similar to the findings obtained in the present study. Other studies, in contrast to ours, showed the effect of chitosan in preventing the growth of *S. aureus* in some meat products (Kanatt et al., 2008; Tajik, Moradi, Rohani, Erfani, & Jalali, 2008).

3.2 | Chemical evaluation

3.2.1 | TBARS evaluation

According to our results, TBARS formation in the samples treated by propolis in both concentrations was significantly lower than control group ($p < .05$). There was no significant difference recorded between 1% and 2% propolis up to day 9, but in day 12 this difference was significant. The difference of TBARS between control samples and chitosan treatment sample was also not significant. Samples coated by chitosan and 2% propolis had less increase of TBARS in respect to other treatments up to day 12 (Figure 5). This reduction may be due to phenolic and flavonoid compounds of propolis and subsequently the effect of these compounds on free radicals and TBARS. Hence, the secondary compounds of lipid oxidation such as TBARS decreased. Some researchers indicated that chitosan coating has positive effects on reducing TBARS (Fan et al., 2009; Jeon et al., 2002; Sathivel, 2005; Sathivel, Liu, Huang, & Prinyawiwatkul, 2007). But López-Caballero et al. (2005) observed that chitosan coating has no significant influence on TBARS production in fish meat. Antioxidative feature of propolis was demonstrated in the study of Siripatrawan et al. (2012) which is in agreement with our research.

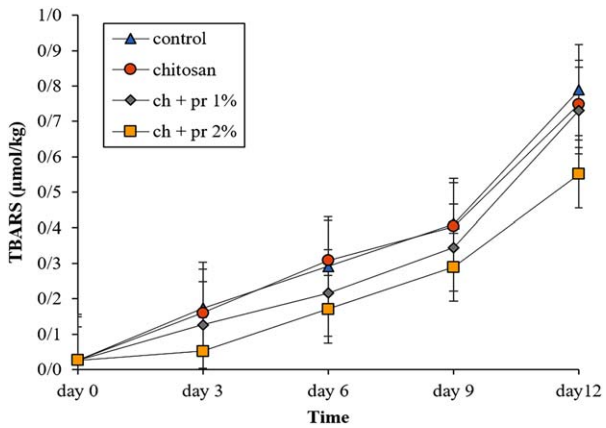

FIGURE 5 TBARS value in chicken fillet coated by chitosan and ethanolic extract of propolis during 12 days storage at 4 °C

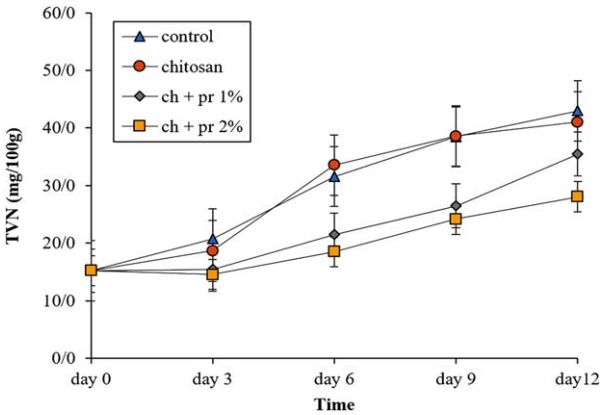

FIGURE 6 TVN value in in chicken fillet coated by chitosan and ethanolic extract of propolis during 12 days storage at 4 °C

3.2.2 | Total volatile nitrogen (TVN)

TVN results for fillet during 12 days has been shown in Figure 6. Based on Iran Veterinary Organization instructions, the acceptable value for TVN is 28 mg/100g. In control category, TVN increased by time and its value reached 31.6 in day 6 and 43 in the final day. In the chitosan-treated category, this number reached to 26.6 in day 6 which is still in acceptable, but in days 9 and 12 the value passed this criterion. In both groups with propolis extracts, TVN remained significantly lower throughout the study. TVN in samples treated with chitosan plus 1% propolis measured 26.5 mg/100 g in day 9 which is still satisfactory. In

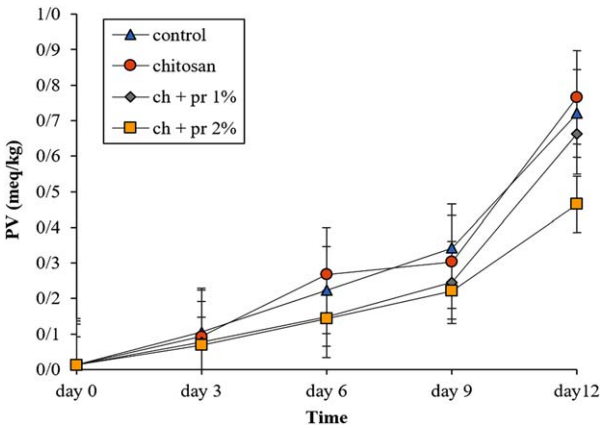

FIGURE 7 Peroxide value in chicken fillet coated by chitosan and ethanolic extract of propolis during 12 days storage at 4 °C

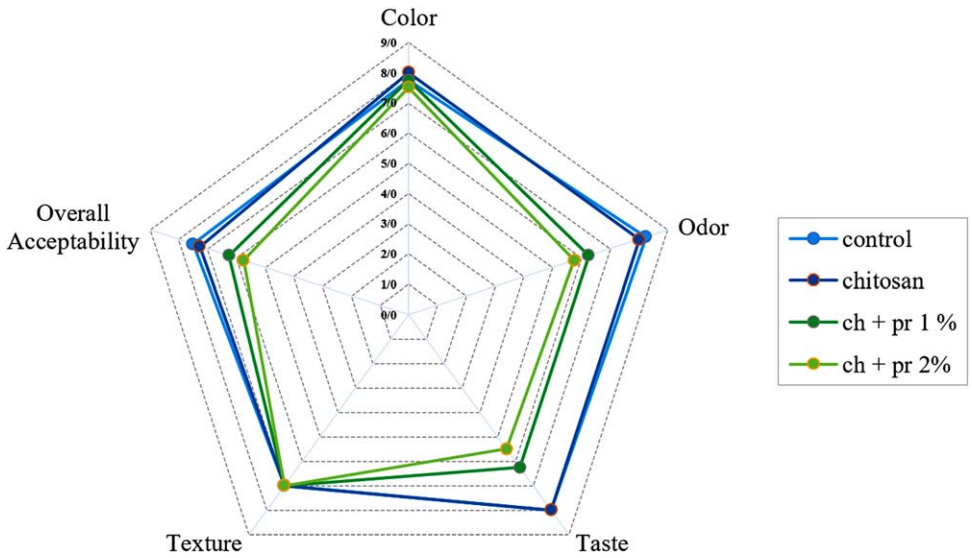

FIGURE 8 Sensory evaluation of samples coated by chitosan and ethanolic extract of propolis during 12 days storage at 4 °C

the 12th day, 1% propolis samples TVN was 35.5. In the chitosan plus 2% propolis treatment, TVN value reached only to 28.1 mg/100 g which is just above the criteria mentioned.

Studies such as Fan et al. (2009) and López-Caballero et al. (2005) indicated that chitosan alone and in combination with other materials could notably decrease TVN production. This can be due to reduction in bacterial numbers and oxidative ability of bacteria to separate TVN from amine compounds (Fan et al., 2009; López-Caballero et al., 2005). Propolis had a significant impact on TVN in our study, which was in accordance with findings obtained by previous researchers such as Ali, Kassem, and Atta-Alla (2010).

3.2.3 | Peroxide value

Oxidative spoilage in all samples was not significantly different in first 3 days of our investigation. In 6th and 9th days, this value in specimens containing propolis was less than control and chitosan groups (Figure 7). No remarkable variation observed in peroxide value between 1% and 2% propolis up to day 9, but in the last day, the difference was statistically significant.

The present study showed that propolis postponed primary oxidation of fillet, while this parameter in control group was sharply increased by time. High antioxidant power of ethanolic extract of propolis can be corresponded to certain compounds especially phenols and flavonoids in the extract (Sepici-Dincel, Açıkgöz, Çevik, Sengelen, & Yeşilada, 2007).

3.3 | Sensory evaluation

The samples were evaluated based on 9-point hedonic scale, and the score of 7 or more considered satisfactory. The summary of the sensory results (color, odor, taste, texture, and overall acceptance) are stated in Figure 8. The results showed that the control specimens and samples treated with chitosan had a high value of all sensory parameters and there was no significant difference between them. The changes of color and texture in samples treated with both concentrations of propolis samples was not significant in comparison with the control group, whereas the notable declines in odor, taste, and overall acceptance were observed ($p < .05$). The samples coated by chitosan and 2% propolis had obviously lower sensory values. Although samples treated by 1% propolis had lower values than control and chitosan groups, they were still in acceptable ranges. Chitosan alone and with low concentrations of propolis had no remarkable adverse impact on sensory features, but higher amount of propolis could decrease some of the sensory properties of fillets.

Kanatt et al. (2008) indicated that chitosan coating had no undesirable influence on meat products. Fan et al. (2009) also suggested that chitosan coating has positive effects and increases shelf life of fish meat.

4 | CONCLUSION

The present study shows that chitosan and propolis can improve the chemical and microbial properties of chicken fillet and increase its shelf life. The shelf life of uncoated samples was 3 days, while it was observed to be more than 10 days for samples treated by chitosan and propolis. In addition, combination of chitosan and propolis due to their synergetic effect can improve the chemical and microbial properties significantly. Despite some concerns about sensory values of samples treated with combination of chitosan and propolis extract, propolis and chitosan can be used for enhancing the shelf life of fillet.

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