Effectiveness of chitosan/propolis extract emulsion coating on refrigerated storage quality of crayfish meat (Astacus leptodactylus)

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ABSTRACT
In this study, propolis extract emulsions were used in chitosan coatings to keep the storage quality of crayfish meat during shelf-life (16 days). Crayfish meats were coated with a solution of chitosan containing propolis extract emulsions (0.3 and 0.6% v/v) and then stored at 4°C. Coatings protective effectiveness was determined by chemical analyses (pH, Thiobarbituric Acid, Peroxide value, Total volatile basic nitrogen, and K values), microbiological (Total aerobic mesophilic bacteria, Psychrotrophic bacteria H2S-producing bacteria, Yeasts- Moulds), and sensorial attributes (Odor, Taste, Firmness and Overall acceptance). Results demonstrate that chitosan coatings containing propolis extract are effective in controlling the growth of bacteria and chemical indices. These results can be beneficial for seafood processing sectors, as well as for food technologists.

Keywords: Chitosan-based coating; propolis extract emulsion; shelf life; crayfish (Astacus leptodactylus)

1. Introduction

Astacus leptodactylus are commonly found in lakes, ponds, and rivers in Turkey and low-fat, low-calorie, and rich in protein. However, it is susceptible to spoilage just like fish and other seafood and should be consumed fresh immediately after harvest, or measures should be taken to preserve its original freshness as much as possible. Therefore, adequate packaging technologies need to be developed for the preservation of fish or other foods (Bahadır Koca & Argun Uzunmehmetoğlu, 2018; Ojagh et al., 2010).

Synthetic preservatives are reported to delay the lipid peroxidation process and increase the shelf life of foods. But, synthetic additives can be affected by consumer health, so consumers tend to avoid foods prepared with chemical preservatives (Yazgan et al., 2020). Propolis is a natural product collected by honeybees from plants especially flowers and buds. Within, it is included in the GRAS list and is used as a bioactive food. Even though the composition of propolis changes depending on its source, it generally consists of 50% resin, 30% wax, 10% essential and aromatic oils, 5% pollen, 5% other organic compounds and mineral substances supplement. The main active ingredients of propolis are benzoic acid, cinnamic acid, phenols, ketophenols, hydroquinone, coumarins, and naphthahene (Galeotti et al., 2018).

Some researchers have reported that propolis can be used as a resource natural antioxidant and antimicrobial in meat and fish products (Gutierrez-Cortes & Suarez Mahecha, 2014; Hasck et al., 2015; Uçak, 2018). Moreover, many studies have reported that chitosan films or coatings enriched with plant extracts or essential oils improve the quality and shelf life of foods (Cai et al., 2018; Fadidloğlu & Emir Çoban, 2018; Fan et al., 2009; Shahbazi & Shavisi, 2018). However, according to our literature seek, there is not found any information on the application of propolis extract/chitosan coating to crayfish meat. Therefore, this study was planned to evaluate the effect of chitosan/propolis extract emulsion coating on the refrigerated storage quality and shelf life of crayfish (Astacus leptodactylus) meat.

2. Materials and methods

2.1. Materials
Chitosan (medium molecular weight: 190–310 kDa; deacetylation degree: 75–85%, viscosity of 200–800 cPs) and Tween-
80 were purchased from Sigma-Aldrich. Propolis liquid extract (≥98%, food-grade, FDA approved) was obtained from a commercial company (Talya herbal product Co., Turkey). All other reagents were procured in analytical grade.

2.2. Preparation of chitosan-based coating-forming emulsions

The propolis emulsion was prepared using the method of Wu et al. (2018). Propolis extract (0.3 and 0.6% v/v) and 2 g of surfactant Tween 80 were mixed and added 50 mL of deionized water, and then stirred for 1 h at 25°C. Afterward, again deionized water was added 50 mL. Propolis emulsions were acquired by stirring 6 h at 25°C.

Chitosan/propolis extract emulsion coating was prepared according to the method of Sun et al. (2019) a minor change. 2% (w/v) chitosan and 1% glycerol were dissolved in prepared propolis emulsions (100 mL) at 25°C and stirred for 2 h to obtain a homogeneous coating solution. The chitosan coatings were coded as Ch, Ch-0.3% PE and Ch-0.6% PE.

2.3. Preparation sample treatments

A total of 5 kg of live Crayfish of the same size (8–9 cm) obtained from Kebean Dam Lake in Elazığ were transferred to the laboratory under aseptic and cold conditions in 1 h. They were washed with tap water and boiled for 10 minutes at 100°C. After boiling, crayfishes were cooled and separated from shells. The deshelled crayfish were randomly divided into four groups: Cont (uncoated), Ch, Ch-0.3% PE and Ch-0.6% PE. The crayfish were individually immersed in the coating solutions for 2 min (crayfish/coating solution ratio: 1/3). Then, the coated crayfish were dried for 1 h in a cold air cabinet set at 10°C to form the edible coating and were stored at 4°C. All samples were separately packed in a sterile polyethylene bag and stored for 16 days (Figure 1). The present study was conducted as two parallels.

2.4. Chemical analysis

Ten grams of crayfish meat were homogenized in 100 mL of sterile distilled water. The pH was measured in triplicate at room temperature, according to the method reported by Gokalp et al. (2001). The total volatile base nitrogen (TVB-N) value was determined by using an automatic Kjeldahl apparatus according to the micro-titration methodology reported by Gharibzahedi and Mohammadnabi (2017). The TVB-N value was stated as mg/100 g fish muscle thiobarbituric acid (TBA) value was measured using the method reported by Kilic and Richards (2003). Spectrophotometric measurements were made based on the principle of malondialdehyde in samples to react with TBA reagent. The TBA values were described as mg MDA/kg seafood flesh. The PV value was assigned using the method of Shantha and Decker (1994). 2 g sample was mixed in 30 ml chloroform-glacial acetic acid solution (chloroform/glacial acetic acid, 3/2). Then 1 ml of saturated potassium iodide (KI) solution was added and mixed again. Subsequently, it was kept in the dark for 5 minutes, and 75 ml of pure water and 1 ml of starch solution were added and titrated with 0.1 M sodium thiosulphate (Na2S2O3) solution. The K-value was determined by an HPLC using 150 × 4.60 mm column (a Merck-Hitachi Model D-6500). The extraction procedure was based on that by Fan et al. (2008).

2.5. Microbiological analysis

Ten grams of crayfish meat were taken, transferred aseptically to a sterile stomacher bag containing 90 ml of buffered

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Figure 1. Flow diagram of coating processing with chitosan/propolis extract emulsions of Crayfish meat.

Figura 1. Diagrama de flujo del proceso de recubrimiento de la carne de cangrejo de rio con emulsiones de extracto de quitosano/propóleo.
peptone water, and mixed for 1 min using a Stomacher blender (Lab Stomacher Blender 400-8A 7021 Seward Medical, UK). Then, decimal dilutions (1:10, diluent: 0.1% peptone water) were prepared and inoculated on agar plates to determine:

a. the total mesophilic aerobic bacteria (TMAB) and psychrotrophic bacteria (PB) counts on pour plates of plate count agar incubated for 2 days at 30°C and 10 days at 5°C, respectively;
b. H2S-producing bacteria count assigned using iron agar plates incubated for 2 days at 30°C;
c. yeasts and molds determined using potato dextrose agar (PDA) and incubated for 5 days at 22°C. All counts were expressed as log CFU/g (Halkman, 2005).

2.6. Sensory evaluation

The sensorial attributes of crayfish meat were determined according to the method reported by Cai et al. (2015). Seven experienced panelists measured important quality parameters, such as odor, taste, firmness, and overall acceptability using a ten-grade hedonic score system about crayfish meat: 10.0–9.0 (excellent), 8.9–7.0 (good), 6.9–4.9 (fair), and 3.0–1.0 (rejectable).

2.7. Statistical evaluation

The IBM SPSS®26 (SPSS Inc., Chicago, IL, USA) statistical package was used for data processing. The statistical significance of the differences between the groups and storage days of the chemical and microbiological data determined as a result of the analyses carried out in the study was determined using variance analysis (ANOVA) and Duncan’s multiple range tests. Differences were regarded as statistically significant at p < 0.05. All experiments were replicated two times for all groups.

3. Results and discussion

3.1. Chemical quality

The pH of the crayfish meat used in this study was 5.72. In all groups, the pH value reduced at first but then increased throughout storage (Figure 2a). The reason reduced in the pH value of samples can be caused by the formation of lactic acid from glycogen in fish muscles (Fan et al., 2008; Manju et al., 2007). In contrast, the rising pH value was reported to adhere to the release of protein metabolites, like ammonia and trimethylamine by the activity of deteriorating bacteria. While for the Ch coatings containing propolis liquid extract samples, the pH value observed was low compared to control samples during the storage period (p < .05). Furthermore, lower pH changes were determined in the Ch+0.3% PE and Ch+0.6% PE samples. It can be deduced that the lower pH values of the Ch+0.3% PE and Ch+0.6% PE samples combined with chilling could increase microbial prevention and cause prolongation of the quality of crayfish meat (Abd El-halim El-Sherif & Abd El-Ghafour, 2015).

Alterations in the TVB-N of the samples with storage are demonstrated in Figure 2b). Coated groups demonstrated a tendency of decreasing TVB-N value throughout the first 4 days of storage. Subsequently, a rise was determined until the end of storage. These results were also similar to the findings of other researchers. The rise in TVB-N value was particularly clear in Control and Ch crayfish samples, on the other hand, crayfish samples with Ch+PE coatings supplemented with PE significantly decreased the value of TVB-N during storage. Gimenez et al. (2002) reported a value of 25 mg/100 g fish meat as the beginning of spoilage. In this study, TVB-N values remained below this limit of admissibility until at the end of storage in Ch+PE coated groups, meanwhile, the limit of admissibility was exceeded on day 8 and day 12 for control and group coated with Ch, respectively. These conclusions are in parallel with the findings of Martínez-Alvarez et al. (2008), Tsironi et al. (2009), and Nekuie Fard et al. (2015). Ojagh et al. (2010) declared that chitosan coatings combined with cinnamon oil effectively suppress the rise in TVB-N value and protect refrigerated rainbow trout fillets.

The changes in the PV of all the groups during chilled storage are presented in Figure 2(d). At the start of the storage period, the PVs were determined as 0.96 meqO2/ kg, 0.90 meqO2/kg, and 0.92 meqO2/kg for the crayfish coated with Ch, Ch+0.3% PE and Ch+0.6% PE, respectively. The PV increased during storage time, as awaited, the control group had the highest PV values and PV might be significantly affected (p < .05) by PE concentration in the coating solutions; however, which were lower than that of Ch+0.3% PE. Similar results were also revealed for the yellowfin tuna meat (Thunnus albacares) coated with chitosan/lemon peel extract (Sabu et al., 2020) who mentioned that supplemented coating with lemon peel extract was efficient in restraining the formation of lipid oxidation products. A study conducted by Viji et al. (2015) determined similar findings in Indian mackerel coated with a combination of citrus peel and mint leaf extracts.

The variations in the TBA value of crayfish coated along storage time are indicated in Figure 2(c). The initial TBA values were in the range of 0.52±0.12 to 0.44 ± 0.07 mg MDA/kg (p < .05); however, the TBA values increased as gradually until the end of storage time in all groups. This increase was slower observed in crayfish samples coated with Ch containing PE when compared with other samples (p < .05). Similar results were reported by Thaker et al. (2017), Fadiloglu and Emir Çoban (2018), and Sun et al. (2019), in meat and fish coated with coatings containing essential oil. After day 12, the control group and there were no significant differences among the Ch group (p > .05). According to these results, propolis extract might be used to delay lipid oxidation in the active coatings for shellfish and fish products. Yu et al. (2017) reported that the chitosan coating combined with essential oils (clove, cinnamon, and lemongrass) has strong antioxidant activities and preservative effects for antioxidant enzyme activities.

The initial K-value for fresh crayfish were determined as 11.35%. In all the groups, the K-value rose during storage at 4°C (Figure 2e). During storage, the K-values of the control and Ch groups were found to be higher than the groups containing propolis (p < .05). Many researchers have reported that the K-value is a good freshness index for determining quality during storage (Fan et al., 2009). Besides, the K-value varies according to the processing, the season in which the fish was caught, and the fish species. Dong et al. (2019) reported that Allium sativum EO can prevent the degradation of ATP and keep the quality of Pseudosciaena crocea. Cinnamon bark EO might prevent the degradation of ATP and retain the freshness of grass
Carp fillet (Huang et al., 2017). Chitosan at 2% was determined to be effective in lowering the K-value (Hassoun et al., 2020). According to our results, it might be supposed that the Ch+PE coatings prohibited the degradation of ATP and keep on quality of crayfish during refrigerated storage. Similar results were reported by Liu et al. (2019) for crayfish coated by a gelatin-containing red pitaya peel methanol extract.

3.2. Microbiological quality

The microbial number of all groups gradually increased with storage time. In accordance with the International Standards (ICMSF [International Commission on Microbiological Specifications for Foods], 1986), the maximum admissible limit of TMAB in raw fish is 7 log CFU/g. In the present study, the initial TMAB counts of crayfish meat were determined to

**Figure 2.** Changes in chemical quality a) pH, b) TVB-N, c) TBA, d) PV and e) K-value crayfish coated with Ch coatings throughout storage at 4°C. (Cont: without coated; Ch: coated with Chitosan; Ch+0.3% PE: coated with Chitosan+0.3% Propolis extract; Ch+0.6% PE: coated with Chitosan+0.6% Propolis extract).

**Figura 2.** Cambios en la calidad química a) pH, b) TVB-N, c) TBA, d) PV y e) valor K del cangrejo de río recubierto con recubrimientos de Ch durante el almacenamiento a 4°C. (Cont: sin recubrimiento; Ch: recubierto con chitosán; Ch+0.3% PE: recubierto con chitosán+0.3% de extracto de propóleo; Ch+0.6% PE: recubierto con chitosán+0.6% de extracto de propóleo).
be $3.22 \log \text{CFU/g}$. The TMAB counts of samples were significantly different between uncoated group and chitosan/propolis emulsions coated groups ($p < .05$). On day 4, the TMAB in all groups was below $5 \log \text{CFU/g}$; however, on day 12 the control group reached a count of $8.40 \log \text{CFU/g}$. This count was greater than the maximal admissible limit of ICMSF. Ucak et al. (2020) reported lower TMAB numbers in gelatin-coated trout fillets containing propolis. The lower TVC of samples may be attributed to the antimicrobial activity of propolis. These results can be explained by the phenolic compounds contained in propolis and polyphenolic fractions such as flavonoids (Pobiega et al., 2019; Spinelli et al., 2015).

The main microorganisms that cause spoilage of fish and fish products stored in cold conditions are gram-negative psychrophilic bacteria. As displayed in Figure 3(b), the rise in the psychrotrophic bacteria (PB) count in the control group was greater than in the coated groups. Initially, the psychrotrophic bacteria count was $2.88 \log \text{CFU/g}$, which increased to $8.00 \log \text{CFU/g}$ at day 20 of the storage for control groups.
While the psychrotrophic bacteria counts for groups coated with 0.3 and 0.6% PE containing were 6.12 and 5.40 log CFU/g, at the end of storage. Similar results were found in studies for chitosan/cinnamon oil and fish gelatine/GL-βCD-curcumin for fresh fish protection (Jouki et al., 2014; Sun et al., 2019). Ucak et al. (2020) reported that during the storage period, PB of fillets coated with gelatin films containing propolis were lower than those of the gelatin film coated samples and control samples.

The varieties of H2S-producing bacteria (Figure 3c), yeast and mold bacteria (Figure 3d), and Pseudomonas spp. bacteria (Figure 3e) populations indicated a similar tendency with TAM and psychrophilic bacteria. On day 20, groups coated with Ch containing PE obtained lower counts.

3.3. Sensorial quality

The sensory evaluation results are shown in Figure 4. At the beginning of the storage, a glossy and smooth layer of Ch-based coatings was seen on the crayfish sample surfaces, resulting in increased scoring. Therefore, the sensory score of the control group was lower than the coated groups (p < .05). With the progress of storage, the sensory scores of all groups decreased. A faster decrease was detected in the control group (p < .05). Control and Ch were reported inadmissible by the panelists in terms of odor and taste on day 12 and day 16, respectively. Also, the Ch+0.6% PE group even on day 16 was considered to be consumable by panelists. These data suggest that 0.6% of PE concentration is more effective in Ch based coatings. Spinelli et al. (2015) reported that the sensory quality of fresh fish burgers containing microencapsulated propolis improved. Ucak et al. (2020) found similar results. They reported that combining PE with gelatin films had a positive effect on trout fillet.

4. Conclusion

The incorporation of propolis liquid extract into a crayfish chitosan coating increased the effectiveness of the coating. Ch+PE treatment increased the crayfish shelf life by ~7 days compared to the control group. Chemical, microbiological and sensorial analyses showed that the Ch+0.6%PE coating had an obvious effect on the quality of crayfish 4°C coating and their shelf lives. Therefore, PE combined with coatings might be useful as a safe natural protective for seafood.

Conflicts of interest

The authors declare no conflict of interest.
Uçak, I. (2018). Determination of the lipid oxidation level in fish oil enriched with propolis extract. GIDA, 43(3), 523–532. https://doi.org/10.15237/gida.GD18031

Uçak, I., Khalily, R., Carrillo, C., Tomasevic, I., & Barba, F. J. (2020). Determination of the lipid oxidation level in fish oil enriched with propolis extract. GIDA, 43(3), 523–532. https://doi.org/10.15237/gida.GD18031

Uçak, I., Khalily, R., Carrillo, C., Tomasevic, I., & Barba, F. J. (2020). Potential of propolis extract as a natural antioxidant and antimicrobial in gelatin films applied to rainbow trout (Oncorhynchus mykiss) fillets. Foods, 9(11), 1584. https://doi.org/10.3390/foods9111584

Viji, P., Binsi, P. K., Visnuvinayagam, S., Bindu, J., Ravishankar, C. N., & Gopal, T. K. S. (2015). Efficacy of mint (Mentha arvensis) leaf and citrus (Citrus aurantium) peel extracts as natural preservatives for shelf-life extension of chilli stored Indian mackerel. Journal of Food Science and Technology, 52(10), 6278–6289. https://doi.org/10.1007/s13197-015-1788-1

Wu, J., Sun, X., Guo, X., Ji, M., Wang, J., Cheng, C., ... Zhang, Q. (2018). Physicochemical, antioxidant, in vitro release, and heat sealing properties of fish gelatin films incorporated with β-cyclodextrin/curcumin complexes for apple juice. Preservation Food and Bioprocess Technology, 11(2), 447–461. doi.org/10.1007/s11947-017-2021-1

Yazgan, H., Burgut, A., Durmuş, M., & Koske, A. R. (2020). The impacts of water and ethanolic extracts of propolis on vacuum packaged sardine fillets inoculated with Morganella psychrotolerans during chilly storage. Journal of Food Safety, 40(2), e12767. https://doi.org/10.1111/jfs.12767

Yu, D., Xu, Y., Jiang, Q., & Xia, W. (2017). Effects of chitosan coating combined with essential oils on quality and antioxidant enzyme activities of grass carp (Ctenopharyngodon idellus) fillets stored at 4 °C. International Journal of Food Science and Technology, 52(2), 404–412. https://doi.org/10.1111/ijfs.13295