

Maintaining the quality of rainbow trout (*Oncorhynchus mykiss*) fillets by treatment of red onion peel extract during refrigerated storage

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Summary. Due to excessive onion wastes are produced in the food industry, possible ways of their utilization are need to be searched. Considering the consumers concerns about the synthetic ingredients, onion peels can be assessed as a natural source in foods. In this study, onion peel extract (OPE) was evaluated as natural antioxidant and antimicrobial agent in prolonging the shelf life of rainbow trout fillets. The fish fillets were treated with 5 and 10% OPE. One batch was left as control without extract. Peroxide value (PV), thiobarbituric acid (TBARS), para-anisidine value (p-Av), total mesophilic aerobic and total psychrophilic aerobic bacteria, Enterobacteriaceae count, and sensory quality of fillets were evaluated during 14 days of storage. OPE was able to delay lipid oxidation compared to control, since the scores of PV, TBARS and p-Av of the fillets treated with OPE showed lower levels. With the increase of OPE concentration, successful results were observed in the growth of bacteria and the lowest bacterial numbers were obtained from 10% OPE treated group. Shelf-life of fish was found as 6, 12 and 14 days for the control, 5% OPE and 10% OPE treated groups, respectively. The results revealed that OPE contributed to maintain the oxidative, microbiological and sensory quality of rainbow trout fillets during storage. Additionally, finding of this research could help to evaluation of food wastes and the industrial usage of these products as a natural preservative.

Keywords: Onion peel extract, food by-products, rainbow trout, microbiological quality, sensory quality

Introduction

Rainbow trout (*Oncorhynchus mykiss*) is a cold-water fish species and is one of the major aquaculture fish worldwide. Because of its desirable characteristics the demand for rainbow trout has increased significantly over the past decade. Nevertheless, it is very vulnerable to deterioration resulting in off-flavor and off-odour. The high water activity, neutral pH, large quantities of free amino acids, high amount of polyunsaturated fatty acids, and presence of autolytic enzymes (1) can cause the spoilage in trout fillet. Freshness is the most important and fundamental single criterion for judging the fish quality (2). The demand of the consumers is toward the

fresh refrigerated foods with prolonged shelf life, thus remarkable studies have been directed toward extending the shelf life of these foods (3,4). The typical storage and transfer method of the rainbow trout is using ice. However it spoils very quickly, thus extension of its shelf life is very important (5). However, cold storage and freezing cannot prevent the spoilage of fish alone.

Nowadays, innumerable synthetic and naturally sourced antioxidants are used to overcome the quality problems of foods. Since the synthetic additives are usually refused by consumers, demand for the natural sources food supplements has increased (6). In recent years, there has been an increasing interest on the evaluation of food by-products from food wastes and the

industrial usage of these products as antioxidants and antimicrobials in prolonging the shelf-life of food.

Annual production of onion (*Allium cepa* L.) is around 66 million tonnes and it is a versatile vegetable which can be consumed both fresh and processed form. It is a good source of dietary phytochemicals including phenolic compounds and flavonoids (7). Onion peels include high levels of flavonoids, but the peels are usually discarded before the processing of onions (8). It was reported that the main by-products of the onion bulbs after industrial peeling are dried skin, the outer two fleshy leaves and the top and bottom bulbs, which are not edible and are removed before processing (9,10). Besides all this, onion peel contains over 20 times more flavonoids (especially quercetin) than the onion flesh (11). Various studies conducted on the effects of onion skin and onion peel such as antioxidant, antimicrobial, antimutagenic, cytotoxic and anti-inflammatory effects (12-19), however as per our knowledge there is no study on the protection of fish quality with the addition of onion peel extract. Hence, the present study was intended to determine the effects of red onion peel extract, which has strong antioxidant and antimicrobial activity, on the quality of rainbow trout fillets during refrigerated storage.

Material and Methods

Extraction procedure

Approximately 1000 g of red onion peels (OPs) were collected from a local market in March 2018, Niğde, Turkey. OPs were washed twice in tap water and dried at 40 °C for 48 h. Dried OPs were ground into powder with a blender. The method of Tabaraki et al. (20) was used for the ultrasound-assisted extraction. OPs powder and solvent (ethanol 70%) were stirred (1:10, g:ml) in a flask and subjected to sonication for 60 min at ambient temperature in ultrasonic bath. Afterwards, the extracts were filtered through whatman no.1 filter paper and concentrated by using rotary evaporator (IKA, HB-10 digital, Germany) at 45 °C under vacuum.

Sample preparation

Rainbow trout (*Oncorhynchus mykiss*) fillets were commercially purchased from a local fish market in Ak-

saray, Turkey. Fish were harvested and transported to the laboratory in ice boxes in the same day. Totally 144 fillets (15 kg) were used for the study. The average weight and length of fillets were 109.54 ± 6.70 g and 20.98 ± 1.67 cm, respectively. After washing the fillets with tap water, the fillets were divided into three lots (48 fillets for each lot). Two lots were dipped in the onion peel extract (OPE) in concentrations of 5% and 10% (w/v). The other lot was used as control (C) without extract. All samples were placed in strapor plates and covered with stretch film. All samples were stored at 4 ± 1 °C during 14 days and were subjected to quality analysis during the storage period.

Lipid oxidation analyses

Peroxide value (PV) was determined according to method of AOAC (21). Approximately 2 g sample was stirred with 30 ml of solution including 3 chloroform:2 glacial acetic acid (v/v). After then 1 ml of saturated potassium iodide (KI) solution was added. The mixture was stored in a dark place for 5 min. Later on, 75 ml of distilled water was added and the mixture was titrated with sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) (0.1M) with the addition of starch solution as an indicator. The results were calculated as; $\text{PV meq/kg} = K \times (V - V_0) \times 12.69 \times 78.8 / w$

K is consumed (mol/l) $\text{Na}_2\text{S}_2\text{O}_3$ concentration, V is the volume of $\text{Na}_2\text{S}_2\text{O}_3$ consumed during titration (ml), V_0 is volume of the blank consumed during titration (ml), w is the weight of the sample (g).

Thiobarbituric acid (TBARS) was determined using the method of AOCS (22). Thiobarbituric acid content determinations were conducted depending on the principle of colorization of malondialdehyde present in the lipids with TBARS reagent. After addition of the same amount of TBARS reagent in the samples solved in n-butanol, the mixture was put in the water bath at 95 °C for 120 min. Results were calculated as; $\text{TBARS (mg MDA/kg)} = 50 \times (\text{The absorbance of lipid} - \text{The absorbance of blank}) / \text{sample weight (mg)}$

Analysis of para-anisidine (p-Av) was performed by IUPAC (23). 0.5 g sample was dissolved in 25 ml of n-hexane. The absorbances were detected at 350 nm (X_1). 1 ml of Para-anisidine reagent was added into the 5 ml of solution and kept in a dark place for 10 min, before the absorbance measuring (X_2) at the 350 nm was done. p-Av values were detected for this equation: $\text{p-Av} = 25 (1.2 \times [X_2 - X_1]) / \text{sample weight (g)}$

pH measurement

For the determination of pH value, a pH electrode was dipped into trout homogenates prepared with distilled water (1:1). All measurements were conducted at room temperature (24 ± 1 °C) using pH-meter (Thermo Scientific Orion 2-star, Germany).

Microbiological analyses

10 g of fish samples were mixed with 90 ml pre-chilled sterile ringer solution. Further decimal serial dilutions were used from this homogenate. In order to determine total psychrophilic bacteria and total viable counts Plate Count Agar (PCA) was used the plates were incubated at 8 °C for 7 days and 37 °C for 24–48 h, respectively. Yeast and mould were enumerated by plating on Potato Dextrose Agar (PDA, with pH 3.5) and incubated at 25 °C for 36–48 h. For the Enterobacteriaceae enumeration, pour plating method was used in Violet Red Bile Agar (VRBA). The plates incubated at 37 °C for 36–48 h.

Sensory analysis

Sensory evaluation was performed according to method of Maqsood and Benjakul (48) with slight modifications by training the panellists in two sessions using a 9-point scale. Ten experienced panellists aged between 25 and 35 years who were familiar with fish consumption participated in sensory test. Separated sensory test boxes were used for the evaluation of samples under daylight at 24 °C. In order to test the samples, panellists were asked to open the plates covered with stretch film. The samples were assessed in terms of odour, texture, color, appearance and general acceptance on a nine-point hedonic scale. A score of 9–7 indicated “very good”, a score of 6.9–4.0 “good”, a score of 3.9–1.0 denoted as spoiled (24).

Statistical analysis

All measurements were performed in triplicate and analysis was carried out using the SAS software (Statistical Analysis System, Cary, NC, USA). Variance analysis (ANOVA) was used to evaluate the data and 5% significance level of Duncan's test was based to compare the differences between means of parameters.

Results and Discussion

Lipid oxidation analyses

Peroxides, the primary product of lipid oxidation, are unstable compounds, forming aldehydes, ketones, and alcohols that are volatile products causing off-flavor in products (25). Changes in the peroxide value (PV) of rainbow trout treated with OPE are shown in the Fig. 1. Initially, PV of the rainbow trout fillets was determined as 2.98 meq/kg and significant differences ($p < 0.05$) were observed between the control and OPE treated groups. A good quality of fish lipid should comprise less than 5 meq/kg peroxide (25). PV of the control group increased to 5.67 meq/kg on the 6th day of the storage and reached at 10.96 meq/kg at the end of the storage period. This increase in PV is attributed to the formation of hydroperoxides, i.e. primary oxidation products. The group treated with 10% OPE showed the lowest PV during the storage period and reached at 4.67 meq/kg at the end of the storage, while this value was 7.33 meq/kg in the group treated with 5% OPE. Ozogul et al. (26) reported the PV of the rainbow trout fillets as 4.07 meq/kg at the beginning of the study and this value was 18.23 meq/kg at the end of the storage (24 days). Similar hydroperoxide contents were determined in rainbow trout fillets by other researchers (27,28). The evaluation of peroxide value reveals that the treatment of OPE has a great effect in retarding primary lipid oxidation in rainbow trout fillets compared to control and OPE treated groups gave significantly ($p < 0.05$) lower PV than the control during the storage period.

In order to assess the degree of the secondary oxidation products, such as aldehydes or carbonyles, thiobarbituric acid is a widely used method (29–31). The initial TBARS value was 0.90 mg MA/kg and increased to maximum level of 4.66 mg MA/kg for the control and 4.41 mg MA/kg for the 5% OPE treated group at the end of the storage (Fig. 2.). TBARS values increased in all groups during storage period and, significant differences ($p < 0.05$) were observed for TBARS between the control and the OPE treatment groups. It was reported that TBARS values of 1–2 mg MA/kg of fish flesh are usually regarded as the limit for normal odour or flavour (32). When the trout fillets were declined by the sensory panel, TBARS value was

1.22 mg MA/kg for the control at 6 day. Among the all groups, 10% OPE treated group showed the lowest TBARS values during the storage period. Other studies have also reported similar initial TBARS values for the rainbow trout fillets (26-28). According to their observation, lipid oxidation level in the rainbow trout fillets decreased with the addition of natural extracts.

The para-anisidine values (p-Av) of rainbow trout fillets treated with different concentrations of OPE during the storage are presented in Fig. 3. At the beginning of the storage, p-Av of rainbow trout fillets was 1.95 which was lower than the values reported by the other studies (33-35). The p-Av in all samples increased during the storage period and control group was significantly ($p < 0.05$) higher than the groups treated with different concentrations of OPE. It was reported that para-anisidine value essentially reflects how the lipid has been handled and stored versus peroxide value, which measures current oxidation (34). At the end of the storage, p-Av levels were determined as 20.54, 13.46 and 10.74 in control, 5% OPE and 10% OPE treated groups, respectively. Application of OPE to rainbow trout fillets was effective on retarding the formation of p-Av and this value was remained lower in these groups than the acceptable limit of 20 which reported by Gokoglu et al. (33).

Microbiological analyses

Changes in the total viable counts (TVC) in the rainbow trout fillets during storage at $4 \pm 1^\circ\text{C}$ are represented in Fig. 4(a). Initial microbial count of fillets was determined as 1.48 log CFU/g which was lower than the value reported by the other studies (26,36,37) (3-4 log CFU/g). Total viable count in the control group increased more quickly than those of the groups treated with onion peel extract (OPE). The highest bacteria numbers were observed in the control group and reached at 6.42 log CFU/g at the end of the storage period, while the lowest bacteria counts were obtained from 10% OPE treated followed by 5% OPE treated groups (5.42 and 5.90 log CFU/g, respectively). Oz (38) reported the initial TVC of rainbow trout fillets as 2.80 log CFU/g. Considering the proposed limits (7 log CFU/g) for fresh fish (39) the results of the present study indicate that OPE treated fillets were in good quality. Sensory analyses correlated with microbiologi-

cal analyses, since the application of OPE extended the shelf life of rainbow trout fillets around 8 days.

The effects of OPE on the total psychrophilic bacteria counts were shown in the Fig. 4(b). Ibrahim Sallam (3) indicated that in fresh fish at chilled temperatures, gram-negative psychrotrophic bacteria group are responsible from aerobic spoilage. At the beginning of the study, the total psychrophilic bacteria counts of rainbow trout fillets were 2.47 log CFU/g and increased with the storage time. OPE was significantly ($p < 0.05$) affected the total psychrophilic bacteria counts in the rainbow trout fillets, since the bacteria counts remained lower than 7 log CFU/g in group treated with 10% OPE. When the control group was rejected by sensory evaluation on the 6th day, total psychrophilic bacteria counts were 5.98 log CFU/g and reached 7.02 log CFU/g on the 10th day of the storage. Volpe et al. (40) monitored the bacterial changes in the rainbow trout fillets and reported that the initial total psychrophilic bacteria counts increased from a initial value of 4.02 log CFU/g to final level of 8.88 log CFU/g. In the present study, bacteria counts were found 7.88, 7.54 and 6.70 log CFU/g in control, 5% OPE and 10% OPE treated groups, respectively at the end of the storage. Similar results were observed by Ozogul et al. (26) with the initial bacteria level in rainbow trout fillets during ice storage (2.40 log CFU/g). Berizi et al. (27) observed lower total psychrophilic bacteria counts in rainbow trout fillets coated with chitosan combined with pomegranate peel extract during frozen storage.

Total Enterobacteriaceae counts of rainbow trout fillets are shown in Fig. 4(c). Enterobacteriaceae is a hygiene indicator in fish, and also it may be in the microflora of trout (36,41). The initial Enterobacteriaceae counts were determined as 1.60 log CFU/g and increased with the storage time for all groups. OPE treated groups showed significantly ($p < 0.05$) lower bacteria counts than the control group and the lowest bacteria counts were obtained 10% OPE treated group. Enterobacteriaceae counts were 3.64 log CFU/g when the control group was rejected by sensory panel. At the end, Enterobacteriaceae counts were 5.46, 5.10 and 4.91 log CFU/g in control, 5% OPE and 10% OPE treated groups, respectively. Yazgan et al. (42) reported that the initial Enterobacteriaceae counts were 2.15

log CFU/g for sea bream and sea bass, and showed increase with storage time. Addition of garlic to the fish diet reduced the Enterobacteriaceae counts in the rainbow trout during storage (38). It was reported that Enterobacteriaceae are a significant part of the spoilage microflora of trout fillets (36,43,44).

pH assesment

The effects of OPE on pH changes of rainbow trout fillets during the cold storage is depicted in Fig. 5. The initial pH value of the rainbow trout fillets was 6.61 and increased in all groups with some small fluctuations during the storage period. In all groups, 10% OPE treated group showed significantly ($p < 0.05$) lower pH values untill at the end of the storage period. Other findings reported higher initial pH value in rainbow trout fillets varied between 6.64 and 6.89 with fluctuations during the storage (26,38,45). Yerlikaya et al. (34) reported that during the post-mortem period pH value tends to increase because of the degradation of nitrogenous compounds. In the present study, the pH value of the rainbow trout fillets showed increase and reached at 6.94, 6.84 and 6.76 in control, 5% OPE and 10% OPE treated groups at the end of the storage.

Sensory analyses

Since the freshness is an important parameter in evaluation of fish quality, sensory properties of fish are very important for the consumers. The results of sensory analyses (odor, texture, color, apperance, general acceptance) of rainbow trout fillets treated with different concentrations of OPE are given in Table 1. The results showed that the best sustainability was observed in group treated with 10% OPE, followed by 5% OPE. The lowest sensory scores were determined in the control group with the shelf-life of 6 days (odor: 3.10, texture: 3.00, color: 3.70, apperance: 3.60 and general acceptance: 3.80). The increase in OPE concentration improved the appearance of rainbow trout fillets with extended shelf life. The shelf-life of rainbow trout fillets was extended 6 and 8 days in 5% OPE and 10% OPE treated groups, respectively. This is in aggrement with previous studies on the shelf-life of rainbow trout fillets treated with different natural plants, herb and oils (27,28). In the present study, general acceptance of fillets treated with 5% and 10% OPE were 3.00 (12th

day, declined) and 5.80 (14th day). Krizek et al. (46) reported the shelf life of rainbow trout fillets as 11-16 days at 3°C which is higher than the shelf-life of the control group in our study. According to Liu et al. (47), specific color, firmness, elasticity of muscles and odor of fresh rainbow trout are degraded during storage time. The sensory results of present study showed that the shelf life of rainbow trout fillets were determined as 6, 12 and 14 days for the control, 5% and 10% OPE treated groups, respectively.

Conclusions

OPs have high levels of flavonoids, however they are usually discarded before onions are processed for the consumption. The food industry produces a large amount of onion waste, therefore possible ways of their utilization is need to be searched. In the present study, influence of OPE has been investigated as natural antioxidant and antimicrobial agent in maintaining the oxidative, microbial and sensory quality of rainbow trout fillets. The use of OPE enhanced the quality of rainbow trout fillets with 6 and 8 days extended shelf-life for 5% and 10% OPE treated groups, respectively. Finding of this research could help to evaluation of food wastes and the industrial usage of these products as a natural preservative.

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