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Investigating the effect of decontaminants on microbiological and chemical properties of rainbow trouts

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Abstract: This study was designed to determine the effect of decontamination on the shelf life of whole rainbow trouts. For this purpose 0.5% cetylpyridinium chloride (CPC), 10% trisodium phosphate (TSP), 2.5% acetic acid (AA), 2.5% lactic acid (LA), 1200 ppm acidified sodium chloride (ASC) and control (tap water) were used as decontaminants. After the decontamination process, the samples were stored in cold storage and subjected to microbiological and chemical analyzes on days 0, 3, 6, 9, 12 and 15. Mesophilic bacteria, psychrophilic bacteria, lactic acid bacteria, *Pseudomonas* spp., *Enterobacteriaceae* and coliform bacteria were enumerated for the evaluation of microbiological quality, whereas pH, total volatile alkaline nitrogen (TVB-N), thiobarbituric acid (TBA) were determined for the evaluation of the chemical quality of fish samples. The study was repeated 3 times and 6 fish were used in each group corresponding to 108 fish in total. Microbiological samples were evaluated with a modification in USDA/FSIS chicken carcass method. The data of microbiological analysis showed that decontamination provided a significant improvement on the microbiological quality and the decontaminants used in this study extended the microbiological shelf life of rainbow trout. However, acidic decontaminants and TSP caused some changes in the physical properties of rainbow trouts. On the other hand, the use of CPC extended the shelf life of rainbow trouts without adversely affecting the texture. The microbiological sampling protocol used in this study was proved to be easier to apply and gave coherent results.

Keywords: Fish, organic acid, trisodium phosphate, cetylpyridinium chloride, shelf life

1. Introduction

Marine products, especially fish, are an essential part of a balanced and healthy diet. Because of its perishable nature, delivering fish and its products to consumers in high quality and reducing losses have been issues of the seafood sector. Even though cold storage slows down microbiological and enzymatic activities, fish meat deteriorates rapidly after catching [1]. Rainbow trout are widely traded and consumed in Turkey, thus has an essential place in the seafood sector [2–4]. Therefore, the extension of its shelf life and delaying the quality degradation during cold storage would be an excellent contribution to the field.

Various substances (acidic, alkaline, and neutral) are used to protect fish meat from rapid spoilage and extend the shelf life. TSP is among these substances that have an alkaline nature. Because of their ability to effectively dissolve proteins, alkaline compounds like TSP are the essential component of cleaning products. Their applicability depends on the pH and the buffering capacity of the solution. TSP solution is known for its high pH, ability to bind to the cell wall, ionic effect and bactericidal effect by thinning the lipid layer of the microorganism cells [5–7].

Organic acids are among the most commonly used compounds for the decontamination of cattle, pigs, lambs, and poultry carcasses. Organic acids act as preservatives by releasing proteins from carboxylic groups and lowering the pH of the medium. Organic acids are inexpensive and can be found naturally in many foods. They are safe, and there is no limitation on their daily intake. Another advantage of organic acids is that they cause an unnoticeable sensory change in the product when used [5,8]. As an organic acid,

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LA is known to have a bactericidal effect by disabling the proton pump in the bacterial cell membrane. It is also useful as a preservative by the ability to reduce the water activity of the food. Antimicrobial effects of lactic acid were investigated by many researchers, and it is reported to be effective against many pathogens and effective in degrading microorganisms [5–10].

Another organic acid that can be used for decontamination is AA. Acetic acid is one of the organic acids that can be used to remove encrusted dirt layers on material surface. Acidic cleaning compounds are effective substances in removing mineral deposits, and AA is among the agents that can be used for disinfection of organically produced foods. Both AA and LA have well-characterized biocidal properties and are generally recognized as safe (GRAS) [5,10,11].

ASC is one of the substances used in the decontamination of poultry carcasses. It can be applied by immersion methods or directly added to process water used in various carcass processing steps such as plucking, scalding, washing, and cooling [11,12].

CPC is a substance used frequently in poultry, fish, meat, fast foods, vegetables, fruits, and fruit juice. With a stable neutral pH, it is also a nonvolatile and water-soluble substance. It is effective against many pathogens such as *Salmonella, Listeria, E. coli* [4, 7, 8].

Since the abovementioned decontaminants have promising applications to food, this study aims to investigate the effects of decontamination by 0.5% CPC, 10% TSP, 2.5% AA, 2.5% LA, 1200 ppm ASC and water on the shelf life of whole rainbow trouts (ungutted trout). In the study, a more straightforward approach was used for sampling, which we believe provided more objective results. There are various sampling methods for microbiological analysis of fisheries, including surface extrusion (cm^2), sampling from the skin surface or muscles in different weights (10–25 g) and swabbing the skin surface with a sponge [5,6,12]. The use of these methods may not provide realistic results in whole fish whose digestive system has not been removed. Because the gill, tail, and head of the fish, which are the regions where the deterioration begins, are not taken into account with these sampling methods. In addition, the entire surface cannot be sampled in conventional methods. Thus, the results of other potentially contaminated surfaces are masked. Obtaining more realistic results in fisheries, including fish fillet samples, is only possible by sampling the whole surface area. Moreover, conventional methods are time-consuming and therefore not practical. To obtain more realistic results and simplify the sampling procedure in this study, the whole carcass rinse method used for sampling from chicken carcasses was applied to rainbow trouts.

2. Materials and methods

2.1. Materials

Fresh rainbow trouts (*Oncorhynchus mykiss*) (average weight 250 ± 25 g/per fish and a total of 108 fish) were obtained from an aquaculture farm located at Keban Dam Lake at Elazığ (Turkey). Fish samples were obtained from farm as whole fish and transferred in ice buckets within one hour to the laboratory. In this study, ungutted fish (with an intact digestive system) were used for decontamination applications. The decontaminants used in the experimental groups were; CPC (Sigma, Taufkirchen, Germany), TSP (Merck, Darmstadt, Germany), AA (Carlo Erba Reactifs, Val de Reuil, France), LA (Carlo Erba Reactifs) and ASC (Merck).

2.2. Methods

Decontaminations were performed with 0.5% CPC, 10% TSP, 2.5% AA, 2.5% LA, 1200 ppm ASC and tap water resulting in formation of 6 experimental groups [7,13]. Dilutions of decontaminants were conducted with tap water. A total of 6 fish were used per experimental group. Triplicate trials were conducted by sampling 108 fish in total. Tap water (chlorine free) was used for the control group to mimic the actual process conditions. The treatments were conducted by adding 2 L of decontamination liquid into a sterile steel container. The fish were decontaminated in this container for 2 min at 20 °C. Each fish was decontaminated individually. Then, each fish was placed on polyethylene plastic plates and covered with stretch film for storage at 4 ± 1 °C. Samples of each experimental group were examined for changes in chemical and microbiological properties at days 0, 3, 6, 9, 12, and 15 of storage.

2.3. Microbiological analysis

As mentioned above, the microbiological sampling method used for the chicken carcasses was modified to use for fish sampling in this study. In the technique used for chicken carcasses (whole carcass rinse procedure), 400 mL of sterile peptone water per carcass is added and mechanically shaken for 2 min. The sampling is made from this rinse [14]. For this study, the method was modified and 100 mL of sterile peptone water/per fish for rinsing was used. The whole fish was immersed in this water and shaken vigorously for 2 min. The rinse was decimally diluted up to $1/10^8$, and microbiological analyses were conducted.

The decimally diluted samples were plated onto MRS agar (LabM) for enumeration of lactic acid bacteria (30 °C for 72 h), Rose Bengal Chloramphenicol agar (LAB036, LabM, Lancashire, UK) for enumeration of yeast (25 °C for 5 days), *Pseudomonas* Agar Base (Lab108, LabM , Lancashire, UK) with cetrimide, fucidin, and cephaloridine (CFC, X108, LabM, Lancashire, UK) for enumeration of *Pseudomonas* spp. (37 °C for 48 h) [15]. To enumerate

aerobic mesophilic bacteria and aerobic psychrophilic bacteria, samples were pour plated on Plate Count agar (PCA, LAB010, LabM, Lancashire, UK) and incubated at 35 °C for 48–72 h, and at 7 °C for 7 days, respectively. Coliforms and *Enterobacteriaceae* were enumerated by using the double-layered pour plate method on VRB agar and VRB-G agar respectively (Lab031, LabM, Lancashire, UK) after incubation at 35 °C for 24 h [15].

2.4. Chemical analysis

For chemical analysis, samples containing both muscle and skin were cut from the dorsal part, just behind the head of the fish. The pH values of fish meat samples were measured according to AOAC (1990) [16]. Thiobarbituric acid reactive substances (TBA) were determined by a selective third-order derivative spectrophotometric method [17]. TBA content was expressed as mg of malondialdehyde (MDA)/kg for fish samples. Determination of total volatile basic nitrogen (TVB-N) was based on the method of Varlik et al. (1993) [18].

2.5. Statistical analysis

All microbiological and analytical determinations were made on days 0, 3, 6, 9, 12, and 15 of cold storage. The study was repeated three times and duplicate results were obtained from each determination. The data were subjected to analysis of variance (ANOVA) according to the treatment × storage time model. The numbers of bacteria were converted to $\log_{10}$ cfu/g. The means were separated according to general linear models (GLM) using Fisher's least significant difference (LSD) test, and the statistical significance level was accepted as 5% (SAS Institute, Inc., Cary, NC, USA).

3. Results

3.1. Aerobic mesophilic bacteria count

Results show that there were increases in the number of total aerobic mesophilic bacteria during storage (Tables 1a–1c). However, the increase was found to be higher in the control group than in other groups ($p < 0.05$). Even on the first day of application, the decontaminants reduced the microbial load significantly ($p < 0.05$). Moreover, the increases determined in treatment groups during the storage period were less than the increase in the control group.

3.2. Psychrophilic bacteria count

Results of our study showed that, decontaminants significantly reduced the psychrophilic microorganism load on the first day of application and the growth was retarded during the storage period. Increases in the psychrophilic bacteria count of treatment groups were less than the control group during the storage period ($p < 0.05$).

3.3. Lactic acid bacteria count

Lactic acid bacteria load increased during the storage period in all experimental groups and the increases were statistically significant ($p < 0.05$). However, the increases of lactic acid bacteria in the groups treated with 0.5% CPC and 1200 ppm ASC were significantly less than the increases in the control group and other treatment groups. The lactic acid bacteria increases in the other experiment groups treated with 10% TSP, 2.5% LA and 2.5% AA were found to be similar to the control group (Table 1a).

3.4. *Pseudomonas* spp. count

In this study, the differences in the *Pseudomonas* spp. load of the samples were found to be statistically insignificant on the first day of storage between all experimental groups ($p > 0.05$). However, it was determined that there were increases of *Pseudomonas* spp. counts in all groups during storage but, the increase was significantly higher in the control group than in other groups ($p < 0.05$) (Table 1b). The results show that decontamination was most effective on *Pseudomonas* spp. count with the application of 2.5% AA.

3.5. Coliform bacteria and *Enterobacteriaceae* count

The growth of coliform bacteria was lower in all treatment groups than in the control group during the storage period ($p < 0.05$) (Table 1b). Results indicate that all the decontaminants were effective on coliform bacteria. But the most effective decontaminants were ASC and TSP. Moreover, decontaminants effectively reduced the coliform count and reduced the growth rate during storage (Table 1c). Results of *Enterobacteriaceae* count were similar to coliform bacteria results. ASC and TSP was the most effective decontaminants in suppressing the *Enterobacteriaceae* growth during storage (Table 1b).

3.6. Yeast count

The number of yeast cells following the treatments decreased significantly in all treatment groups ($p < 0.05$), except for the 2.5% acetic acid-treated group ($p > 0.05$) (Table 1c). The fact that acetic acid was not effective in reducing yeast count might be related to concentration and application method. The most effective treatments in reducing yeast count on rainbow trouts were TSP and ASC applications.

3.7. pH

Results showed that decontaminants applied in the study did not cause different effects on pH. Only significant changes were observed in the pH values of samples from TSP treated group ($p < 0.05$).

3.8. TVB-N value

TVB-N value increased significantly in all experimental groups with increasing storage time but none of the TVB-N values exceeded the limit of consumable quality at the end of the storage ($p < 0.05$). Among the

Table 1a. The effects of different decontaminants on the microbiological properties of rainbow trout (log_{10} cfu/mL) (n: 3, N: 2).

	Days	Control	0.5% CPC	1200 ppm ASC	10% TSP	2.5% LA	2.5% AA
Aerobic mesophilic bacteria	0	3.3 ± 0.1^{aY}	1.59 ± 0.9^{aX}	2.4 ± 0.1^{aXY}	2.5 ± 0.1^{aXY}	2.3 ± 0.1^{aXY}	2.8 ± 0.2^{aY}
	3	5.29 ± 0.2^{bZ}	2.25 ± 0.7^{aX}	2.9 ± 0.1^{abXY}	3.66 ± 0.3^{bY}	3.44 ± 0.6^{bY}	3.29 ± 0.5^{aY}
	6	6.43 ± 0.2^{cZ}	3.47 ± 0.7^{bX}	3.7 ± 0.7^{bXY}	4.3 ± 1.1^{bXY}	4.4 ± 0.8^{cXY}	4.6 ± 0.4^{bY}
	9	7.6 ± 0.1^{dY}	6.1 ± 0.1^{cX}	5.63 ± 0.2^{cX}	5.51 ± 0.5^{cX}	5.63 ± 0.5^{dX}	5.3 ± 0.4^{bX}
	12	8.68 ± 0.1^{eY}	7.54 ± 0.3^{dX}	6.59 ± 0.1^{cX}	7.41 ± 0.3^{dX}	7.52 ± 0.3^{eX}	6.5 ± 0.4^{cX}
	15	9.42 ± 0.2^{eZ}	8.6 ± 0.2^{eYZ}	7.9 ± 0.2^{dXY}	8.4 ± 0.3^{eXY}	8.3 ± 0.2^{eXY}	7.6 ± 0.3^{dX}
Psychrophilic bacteria	0	3.06 ± 0.5^{aY}	1.09 ± 0.1^{aX}	1.61 ± 0.4^{aX}	1.16 ± 0.2^{aX}	1.18 ± 0.2^{aX}	1.23 ± 0.3^{aX}
	3	4.0 ± 0.6^{abY}	1.96 ± 1.0^{aX}	2.6 ± 0.2^{abX}	2.3 ± 0.9^{aX}	2.6 ± 0.8^{bX}	2.1 ± 0.8^{aX}
	6	4.9 ± 0.7^{bY}	3.47 ± 1.4^{bX}	3.5 ± 0.9^{bcX}	3.85 ± 0.5^{bX}	3.35 ± 0.6^{bX}	3.43 ± 0.9^{bX}
	9	5.77 ± 0.6^{bY}	4.6 ± 1.6^{cXY}	4.32 ± 1.0^{cX}	5.3 ± 0.2^{cXY}	4.7 ± 0.6^{cXY}	4.1 ± 1.2^{bX}
	12	7.0 ± 0.2^{cY}	5.75 ± 1.4^{cX}	5.35 ± 1.1^{dX}	6.5 ± 0.2^{dXY}	5.78 ± 0.7^{cdX}	5.43 ± 1.0^{cX}
	15	8.23 ± 0.4^{dZ}	7.0 ± 1.1^{dXY}	6.1 ± 1.2^{dX}	7.48 ± 0.2^{dYZ}	6.94 ± 0.5^{dXY}	6.5 ± 0.7^{cXY}
Lactic acid bacteria	0	2.6 ± 0.3^{aY}	< 1.00	1.4 ± 0.3^{aX}	1.7 ± 0.3^{aXY}	1.4 ± 0.4^{aX}	1.57 ± 0.5^{aX}
	3	3.2 ± 0.8^{abZ}	1.53 ± 0.2^{aX}	2.06 ± 0.5^{abXY}	2.6 ± 0.5^{aYZ}	2.4 ± 0.9^{abYZ}	2.6 ± 0.2^{aYZ}
	6	4.02 ± 0.9^{bY}	2.8 ± 0.2^{bX}	2.75 ± 0.5^{bX}	3.9 ± 0.6^{bY}	3.0 ± 1.0^{bXY}	3.9 ± 0.7^{bY}
	9	5.38 ± 0.6^{cY}	3.98 ± 0.4^{cX}	4.4 ± 0.2^{cXY}	5.07 ± 0.3^{cY}	4.8 ± 0.4^{cXY}	5.16 ± 0.3^{cY}
	12	7.14 ± 0.1^{dY}	5.38 ± 0.2^{dX}	5.4 ± 0.1^{cdX}	6.3 ± 0.3^{dXY}	6.4 ± 0.2^{dXY}	6.1 ± 0.1^{cXY}
	15	7.96 ± 0.1^{dY}	6.37 ± 0.2^{dX}	6.45 ± 0.1^{dX}	7.3 ± 0.1^{dXY}	7.3 ± 0.1^{dXY}	7.4 ± 0.2^{dXY}

a, b, c, d: Means in the same column with different superscripts are statistically different ($p < 0.05$).
X, Y, Z,W: Means in the same line with different superscripts are statistically different ($p<0.05$).
CPC: cetylpyridinium chloride, TSP: trisodium phosphate, AA: acetic acid, LA: lactic acid, ASC: acidified sodium chloride.

experimental groups, the increases in TVB-N were less in the groups treated with 0.5% CPC and 1200 ppm ASC than in the other groups. The increases in other groups were found to be relatively similar to the control group (Table 2).

3.9. TBA value

TBA values continuously increased in all groups ($p < 0.05$), but none of them exceeded the consumable quality limits.

4. Discussion

There are not many recent studies in the literature regarding decontamination treatments on fish and fisheries [19–23]. The studies seem to have focused on mostly chicken meat and carcasses in terms of chemical decontamination [24–29].

For fish, recommended maximum limit for aerobic bacteria count is 7 log_{10} cfu/g [30]. This level was exceeded in the control group on the 9th day of storage. The counts exceeded 7 log_{10} cfu/g in treatment groups of CPC, TSP and LA on the 12th day and in the groups of which ASC and AA were applied, on the 15th day of storage. Although ASC and AA applications effectively suppressed aerobic mesophilic bacteria, the differences in the counts were low among the treatment groups. Monirul et al. (2019) also found AA treatment to be effective in reducing the total plate count of silver carp fish [31]. But the authors reported the combined use of AA and ascorbic acid was more effective. In the present study, we investigated the effects of the decontaminants separately, but in future studies, combinations of the decontaminants might be investigated to determine the most effective combination. Marshall and Kim (1996), also reported success in reducing aerobic bacteria on catfish by decontamination with LA and AA [22]. The effectiveness of ASC in reducing aerobic bacteria was also demonstrated on broiler carcasses [25]. Similar to our results, some researchers determined that decontaminants effectively reduced the aerobic bacteria count in related studies [23, 24, 26]. Palmer et al. (2010) investigated the effects of CPC treatment of fish on *Listeria* spp. and total plate counts. They reported 2.4–2.9 log reductions in total plate counts similar to our results [20]. Jasass (2008) compared the effectiveness of TSP, LA and AA in reducing aerobic plate count of chicken carcasses after immersing in the decontaminants and subsequently in tap water [27]. Although the reductions were similar, they reported that LA was more effective than TSP and

Table 1b. The effects of different decontaminants on the microbiological properties of rainbow trout (log_{10} cfu/mL) (n: 3, N: 2).

	Days	Control	0.5% CPC	1200 ppm ASC	10% TSP	2.5% LA	2.5% AA
Pseudomonas spp.	0	1.05 ± 0.1^{a}	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00
	3	1.58 ± 0.3^{a}	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00
	6	2.67 ± 0.1^{bZ}	1.84 ± 0.1^{aY}	2.1 ± 0.1^{aYZ}	1.3 ± 0.2^{aXY}	1.3 ± 0.1^{aXY}	1.21 ± 0.1^{aX}
	9	4.17 ± 0.1^{cZ}	2.54 ± 0.2^{bY}	2.7 ± 0.1^{abY}	2.38 ± 0.2^{bY}	2.25 ± 0.1^{bY}	1.59 ± 0.1^{aX}
	12	6.3 ± 0.3^{dW}	4.51 ± 0.4^{cZ}	3.1 ± 0.1^{bXY}	3.62 ± 0.2^{cY}	4.23 ± 0.2^{cZ}	2.62 ± 0.2^{bX}
	15	7.2 ± 0.2^{eW}	5.95 ± 0.3^{dZ}	4.51 ± 0.2^{cY}	4.53 ± 0.3^{dY}	5.55 ± 0.2^{dZ}	3.73 ± 0.1^{cX}
Enterobacteriaceae	0	1.78 ± 0.2^{a}	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00
	3	1.86 ± 0.1^{aY}	1.1 ± 0.1^{aX}	1.3 ± 0.2^{aX}	1.04 ± 0.1^{aX}	1.38 ± 0.1^{aX}	1.25 ± 0.1^{aX}
	6	2.66 ± 0.1^{bY}	1.5 ± 0.2^{aX}	2.35 ± 0.1^{bY}	1.8 ± 0.1^{bXY}	2.48 ± 0.2^{bY}	2.58 ± 0.1^{bY}
	9	4.68 ± 0.3^{cY}	3.09 ± 0.1^{bY}	3.27 ± 0.1^{cX}	3.28 ± 0.2^{cX}	3.61 ± 0.2^{cX}	3.41 ± 0.1^{cX}
	12	6.47 ± 0.4^{dZ}	4.48 ± 0.1^{cY}	3.74 ± 0.1^{cX}	3.8 ± 0.2^{cXY}	4.59 ± 0.1^{dY}	4.61 ± 0.3^{dY}
	15	7.03 ± 0.2^{eZ}	5.75 ± 0.1^{dY}	4.71 ± 0.1^{dX}	4.54 ± 0.1^{dX}	5.4 ± 0.2^{eY}	5.61 ± 0.2^{eY}

a, b, c, d, e: Means in the same column with different superscripts are statistically different ($p < 0.05$).
X, Y, Z: Means in the same line with different superscripts are statistically different ($p < 0.05$).
CPC: cetylpyridinium chloride, TSP: trisodium phosphate, AA: acetic acid, LA: lactic acid, ASC: acidified sodium chloride.

Table 1c. The effects of different decontaminants on the microbiological properties of rainbow trout (log_{10} cfu/mL) (n: 3, N: 2).

	Days	Control	0.5% CPC	1200 ppm ASC	10% TSP	2.5% LA	2.5% AA
Coliform	0	1.63 ± 0.1^{a}	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00
	3	2.08 ± 0.2^{aY}	< 1.00	1.44 ± 0.1^{aX}	< 1.00	1.00 ± 0.1^{aX}	< 1.00
	6	2.69 ± 0.1^{bZ}	1.4 ± 0.2^{aXY}	1.5 ± 0.1^{aXY}	1.9 ± 0.4^{aY}	1.06 ± 0.1^{aX}	1.4 ± 0.1^{aXY}
	9	4.4 ± 0.3^{cW}	2.49 ± 0.1^{bY}	3.24 ± 0.2^{bZ}	2.58 ± 0.1^{bY}	1.75 ± 0.1^{bX}	1.9 ± 0.1^{aXY}
	12	5.74 ± 0.1^{dZ}	4.18 ± 0.1^{cY}	3.6 ± 0.2^{bXY}	3.29 ± 0.2^{cX}	3.31 ± 0.3^{cX}	3.5 ± 0.4^{bXY}
	15	6.54 ± 0.1^{eZ}	5.46 ± 0.1^{dY}	4.29 ± 0.1^{cX}	4.38 ± 0.1^{dX}	4.51 ± 0.2^{dX}	4.52 ± 0.2^{cX}
Yeast	0	1.73 ± 0.4^{a}	< 1.00	< 1.00	< 1.00	< 1.00	1.44 ± 0.2^{a}
	3	2.6 ± 0.4^{bZ}	1.05 ± 0.8^{aX}	1.2 ± 0.1^{aX}	1.02 ± 0.1^{aX}	1.6 ± 0.1^{aXY}	1.9 ± 0.1^{abY}
	6	2.8 ± 0.1^{bcZ}	1.67 ± 0.1^{bX}	1.58 ± 0.3^{aX}	2.17 ± 0.2^{bY}	2.19 ± 0.1^{bY}	2.1 ± 0.1^{bXY}
	9	3.36 ± 0.1^{c}	3.1 ± 0.1^{c}	3.15 ± 0.1^{b}	3.51 ± 0.1^{c}	3.34 ± 0.1^{c}	3.38 ± 0.1^{c}
	12	4.4 ± 0.2^{dYZ}	4.1 ± 0.1^{dXY}	3.76 ± 0.2^{cX}	3.85 ± 0.1^{cX}	4.5 ± 0.2^{dYZ}	4.96 ± 0.3^{dZ}
	15	5.7 ± 0.1^{eZW}	5.0 ± 0.1^{eXY}	4.63 ± 0.2^{dX}	4.7 ± 0.1^{dX}	5.5 ± 0.1^{eYZ}	6.1 ± 0.3^{eW}

a, b, c, d, e: Means in the same column with different superscripts are statistically different ($p < 0.05$).
X, Y, Z, W: Means in the same line with different superscripts are statistically different ($p < 0.05$).
CPC: cetylpyridinium chloride, TSP: trisodium phosphate, AA: acetic acid, LA: lactic acid, ASC: acidified sodium chloride.

AA at reducing the number of aerobic bacteria. Our results also showed that LA application, although statistically similar, caused more bacterial reduction than TSP and AA application (Day 0, Tables 1a–1c). On the other hand, Bal'a et al. (1998) reported that LA was the least effective at reducing the aerobic plate count of catfish among other acids such as malic acid, tartaric acid, acetic acid, citric acid, lactic acid and hydrochloric acid [21]. However, the concentration used in their study (%2) was lower than ours.

Psychrophilic bacteria are one of the critical microorganism groups responsible for the degradation of fisheries [32]. Rainbow trout is expected to have a higher number of psychrophilic or psychrotrophic bacteria, which may adversely affect shelf life, due to the fact that it is surrounded by cold water in its natural environment.

Table 2. The effects of different decontaminants on the chemical properties of rainbow trout (n: 3, N: 2).

	Days	Control	0.5% CPC	1200 ppm ASC	10% TSP	2.5% LA	2.5% AA
pH	0	6.4 ± 0.1	6.4 ± 0.1	6.4 ± 0.1	6.5 ± 0.1	6.5 ± 0.1^{b}	6.4 ± 0.1
	3	6.4 ± 0.1	6.4 ± 0.1	6.4 ± 0.1	6.4 ± 0.1	6.4 ± 0.1^{ab}	6.4 ± 0.2
	6	6.4 ± 0.1	6.4 ± 0.1	6.4 ± 0.2	6.5 ± 0.1	6.4 ± 0.1^{ab}	6.38 ± 0.1
	9	6.36 ± 0.1^{X}	6.38 ± 0.1^{X}	6.3 ± 0.1^{X}	6.53 ± 0.1^{Y}	6.3 ± 0.2^{aX}	6.36 ± 0.1^{X}
	12	6.3 ± 0.2^{X}	6.3 ± 0.1^{X}	6.3 ± 0.1^{X}	6.5 ± 0.1^{Y}	6.3 ± 0.1^{aX}	6.3 ± 0.1^{X}
	15	6.34 ± 0.1^{X}	6.35 ± 0.1^{X}	6.35 ± 0.1^{X}	6.54 ± 0.1^{Y}	6.29 ± 0.1^{aX}	6.3 ± 0.1^{X}
TVB-N (mg/100g)	0	4.18 ± 0.3^{a}	3.51 ± 0.2^{a}	3.98 ± 0.1^{a}	3.84 ± 0.2^{a}	3.77 ± 0.2^{a}	3.16 ± 0.3^{a}
	3	4.99 ± 0.4^{a}	4.22 ± 0.2^{a}	4.55 ± 0.2^{a}	4.89 ± 0.5^{b}	5.51 ± 0.1^{b}	4.94 ± 0.2^{b}
	6	6.6 ± 1.8^{bYZ}	4.94 ± 0.1^{aX}	7.2 ± 0.4^{bYZ}	8.22 ± 0.8^{cZ}	5.9 ± 0.3^{bXY}	7.91 ± 0.3^{cZ}
	9	9.12 ± 1.1^{cX}	8.33 ± 0.6^{bX}	8.89 ± 0.1^{cX}	11.5 ± 0.4^{dY}	8.95 ± 0.5^{cX}	11.2 ± 0.6^{dY}
	12	14.3 ± 0.8^{dY}	13.2 ± 0.6^{cXY}	12 ± 0.69^{dX}	16.1 ± 1.3^{eZ}	13.5 ± 0.9^{dXY}	14.6 ± 0.6^{eYZ}
	15	18.7 ± 0.8^{eY}	16.3 ± 0.4^{dX}	16.4 ± 0.4^{eX}	22.1 ± 0.4^{fZ}	16.6 ± 0.4^{eX}	17.4 ± 0.9^{fXY}
TBA (mg MA/kg)	0	0.22 ± 0.1^{a}	0.16 ± 0.1^{a}	0.22 ± 0.1^{a}	0.23 ± 0.1^{a}	0.24 ± 0.1^{a}	0.23 ± 0.1^{a}
	3	0.41 ± 0.1^{a}	0.4 ± 0.1^{ab}	0.4 ± 0.1^{ab}	0.61 ± 0.1^{a}	0.4 ± 0.1^{ab}	0.57 ± 0.2^{a}
	6	1.33 ± 0.4^{b}	0.5 ± 0.2^{ab}	1.19 ± 0.1^{bc}	0.96 ± 0.1^{a}	1.14 ± 0.1^{b}	0.9 ± 0.4^{ab}
	9	1.6 ± 0.3^{bXY}	1.13 ± 0.4^{bX}	2.0 ± 0.1^{cYZ}	2.1 ± 0.2^{bYZ}	2.55 ± 0.3^{cZ}	1.5 ± 0.2^{bXY}
	12	3.6 ± 0.1^{cYZ}	1.37 ± 0.6^{bX}	4.2 ± 0.7^{dZW}	3.5 ± 0.4^{cYZ}	4.88 ± 0.4^{dW}	2.79 ± 0.2^{cY}
	15	5.05 ± 0.1^{dY}	2.39 ± 0.8^{cX}	5.5 ± 0.8^{eYZ}	5.4 ± 0.3^{dYZ}	6.27 ± 0.1^{eZ}	5.6 ± 0.6^{dYZ}

a, b, c, d, e: Means in the same column with different superscripts are statistically different (P<0.05).
X, Y, Z, W: Means in the same line with different superscripts are statistically different (p < 0.05).
CPC: cetylpyridinium chloride, TSP: trisodium phosphate, AA: acetic acid, LA: lactic acid, ASC: acidified sodium chloride, TVB-N: total volatile basic nitrogen, TBA: thiobarbituric acid.

Our results showed that decontamination adversely affected the increase of psychrophilic bacteria during storage. In another study conducted on chicken breasts, it was determined that TSP and sodium chloride were effective in reducing psychrophilic bacteria [6]. Nykänen et al. (1998) reported that LA treatment reduced the psychrophilic bacteria count and adversely affected their growth during storage of rainbow trouts [23]. Similarly, in the study conducted by Hecer and Ulusoy (2011) it was also determined that AA, LA and sodium lactate applications effectively inhibited psychrophilic microorganisms in deboned poultry meat samples [28]. The authors stated that lactic acid was the most effective decontaminant. But the psychrophilic bacteria counts of the samples from different treatments groups were hardly different at the end of the storage. In our study, the psychrophilic bacteria counts of the samples from different treatment groups were also statistically similar. Therefore it can be concluded that the effectiveness of the treatments in reducing and suppressing the psychrophilic bacteria was similar (Table 1a).

Applications of CPC and ASC were more effective in reducing lactic acid bacteria. Similar results were obtained in related studies [26,29]. The effects of various decontamination methods on lactic acid bacteria may vary depending on the product, application method and concentration. Since lactic acid bacteria are not very competitive in cold-stored fish and produce fewer unwanted metabolites than gram-negative bacteria, they are believed to have a minor role in spoilage of fisheries. Although some researchers found a correlation between lactic acid bacteria and spoilage of fisheries, the adverse effects caused by lactic acid bacteria are found to be strain-dependent. On the other hand, lactic acid bacteria can play a significant role in the spoilage of the fresh fish especially if the a_w is reduced for preservation, i.e. by salt addition [33]. Therefore, suppressing the lactic acid bacteria can still confer advantages in extending the shelf life and delaying the unwanted changes in fisheries.

Pseudomonas spp., which is the primary cause of spoilage of milk especially stored in cold, meat, eggs and seafood, is natural members of fish microbiota [34]. In all treatment groups, decontamination was effective in reducing *Pseudomonas* spp. growth during storage, but AA was the most effective. This result is not surprising

since acetic acid is known to have an antimicrobial effect against *Pseudomonas* spp. strains and have been utilized as an antiseptic in medical treatments [35]. Moreover, acetic acid has various applications in fish meat, and can affect microbial growth considerably [34].

The most effective decontaminants on *Enterobacteriaceae* were ASC and TSP. Similar to our results, the effectiveness of TSP in reducing *Enterobacteriaceae* count was demonstrated in chicken carcasses [30]. Bosilevac et al. (2004) determined that ASC was effective against *Enterobacteriaceae* in ground beef products, but lower concentrations provided better organoleptic properties [36]. For coliform bacteria count similar results were obtained in the related studies [7,25,28]. Hecer and Ulusoy (2011), investigated the effect of LA, AA and sodium lactate on decontamination of chicken meat [28]. They reported that *Escherichia coli*, one of the most important members of the coliform bacteria, could be significantly affected by the treatments. A study investigating the single and combined effects of lactic acid, cetylpyridinium chloride, and trisodium phosphate on *E. coli* determined that viable *E. coli* counts decreased by treatments [7]. Kemp et al. (2000) reported that ASC especially at concentrations of 850 and 1200 ppm was very influential in reducing coliforms on treated broiler carcasses [25].

The most effective decontaminants on yeast count were TSP and ASC. Trisodium phosphate and sodium chloride are reported to be highly effective against yeast-mold in chicken breasts [26].

It has been reported that the pH value of fresh fish is between 6.0 and 6.5. The pH rises with spoilage during storage, and the pH around 6.8–7.0 is considered to be the maximum limit for consumption. But the pH value is not always a definitive criterion an should be supported by organoleptic, chemical and microbiological tests [37,38]. In this study, pH was affected in only TSP treated samples. Kim and Kim (2000) also stated that TSP application increased the pH value of chicken [39]. On the other hand, Sallam and Samejima (2004) reported that the pH remained constant during the storage period but differed from the control group [26]. The pH changes in our study appear to be relatively more stable (Table 2). Since the fish used in the study are sourced from fresh-water, they contain low amounts of TMAO and volatile bases and this could explain the pH stability.

The TVB-N value consists mainly of trimethylamine and ammonia with the effect of endogenous enzymes and bacteria found in fish. TVB-N value is reported to increase in fish and other seafood depending on the storage period. The species, sex, nutritional status and age of the fish, alongside with the hunting season and region are highly effective on the TVB-N value. Varlik et al. (1993) have defined the quality classification according to TVB-N values as; "very good" up to 25 mg/100 g, good up to 30 mg/100 g, "tradable" up to 35 mg/100 g, and "rotten" if more than 35 mg/100 g [18]. In the study, none of the TVB-N values exceeded the limit for consumable quality at the end of the storage.

TBA value is considered to be an important indicator of lipid oxidation. While the level of TBA increases due to the oxidation of the fats in the meat tissue, the TBA measurement gives information about the rancidity in the meat. As in the examples of this study, TBA is expected to increase with the storage time. The number of TBAs in a very good material should be less than 3, but not more than 5 mg and the limit for meat to be considered "consumable" is between 7 and 8 mg MDA/kg [18]. In some of the related studies [40,41], the TBA value was reported above the limit of consumable quality during cold storage of fish, while in some others [24,42] similar results to our results were reported. Increases in TBA values can vary depending on various factors, such as duration and temperature of the storage, packaging method, type of processing, and type of fish.

5. Conclusion

The results of the study showed that TSP, ASC, LA, AA and CPC can be used to extend the shelf life of fish and improve its microbiological quality. Even though they were effective in reducing the microbial load of rainbow trouts, acidic decontaminants (ASC, LA, AA) and TSP were found to cause whitening in the mucosa (retina), tail and fin tip of the fish. Due to the alkaline nature of the TSP excessive serous liquid was formed on the surface of the fish during the decontamination, but the liquid did not cause any problem during storage. On the other hand, since CPC is a decontaminant that has neutral pH, it did not cause any changes in the physical properties of fish samples. It was concluded that alkaline (TSP) and acidic (ASC, AA, LA) decontaminants may change customer preferences as they cause changes in the physical properties of the fish. Therefore, the most suitable decontaminant was CPC.

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