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Effects of Acetic Acid and Different Salt Concentrations on the Shelf Life of Caviar from Rainbow Trout (*Oncorhynchus mykiss* W. 1792)

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Abstract: In this study, the intention is the investigation of the chemical, microbiological and sensorial changes occurring during cold storage (4±1°C) of the caviar obtained from rainbow trout (*Oncorhynchus mykiss*, W. 1792) that is wet salted and added acetic acid in different ratios. If data obtained as results of this study, an effect on microbiological and chemical parameters of samples were observed depending on increasing acetic acid and salt ratios. In addition, it was determined any effect of acetic acid on the organoleptic of samples. In this study especially when the sensorial data were evaluated the shelf-life of the groups were determined as 14 days in 5% salt containing A and B groups as 21 days in 10% salt containing C and D groups.

Key words: Caviar, rainbow trout, shelf-life, salt, acetic acid, chemical, microbiological, sensorial

INTRODUCTION

As a result of scientific studies which have been conducted in various areas, positive effect of fish in diet on human health has become well-known. Increasing fish consumption in diet is very important with respect to both of economy and health. Nations which are aware of how balanced diet is important have been seeking new products which can satisfy consumers sensationally and may be prepared easily to enrich their protein resources in food industry and these nations have been making investments for this purpose (Varlik *et al*., 2004; Gogus and Kolsarici, 1993). The seafood, particularly fish eggs (caviar) which has high nutritional value are in a position to meet these needs of the people. However, it is among the most expensive food due to limited production opportunities as it can be obtained difficultly (Timur, 1982; McCune and Ingalls, 1988).

It is known that the caviar has been produced since 13th century and it was recorded that Russia has been producing caviar since 16th century. Towards the end of the 19th century, the caviar processing techniques developed in parallel to the development of fishing industry (Varlik *et al*., 2004).

Caviar produced in different technologies and especially are sold at high prices in the world markets. Quality of the caviar depends on the fish species that the eggs were used and in the technological processes that applied to the eggs. In the country, consumption of processed fish eggs is quite limited. The conventional preparation of caviar could not to able to obtain the desirable taste of consumers and by the way, it takes the category very expensive products (Karakas, 2008). The caviar production is mainly based on the salting process of fish eggs. In addition to the salting process, the freezing, smoking, canning and sausage production technologies are also used in the caviar production.

In this study, it was aimed to determine the chemical and sensory changes of eggs obtained from rainbow trout (*Oncorhynchus mykiss*, W. 1792) wet salted and treated with acetic acid and storaged at 4±1°C as 4 groups.

MATERIALS AND METHODS

Raw material: In this study, the eggs of broodstock fish of *Oncorhynchus mykiss* (Rainbow trout) species included in the Salmonidae family were investigated. The fish were sourced from Keban of DSI Number IX Regional Directorate and the study was conducted in two replicates.

Processing of eggs, added salt, acetic acid and storage: In this study, flow diagram of the production of obtained caviar samples is shown in Fig. 1.

Chemical and microbiogical analysis: To determine the shelf life of samples prepared in the study, sensorial, chemical and microbiological analyses were carried out in

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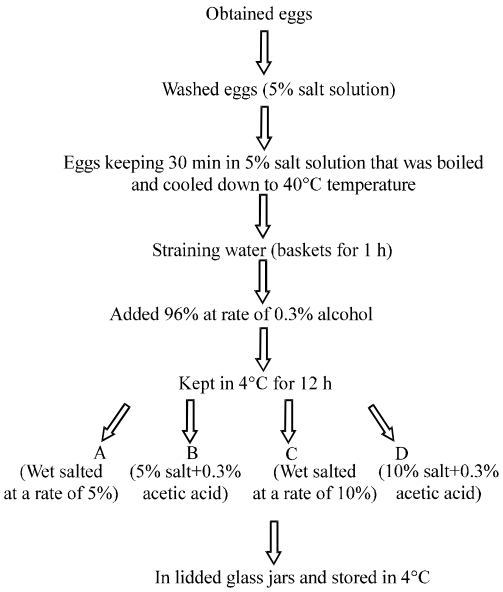

Fig. 1: Flow diagram of caviar production

parallel once every 7 days of storage. The pH values, the moisture, ash, fat, protein, salt and Total Volatile Basic Nitrogen (TVB-N, mg/100 g) quantities, quantities, the thiobarbituric acid content, the total number of aerobic microorganisms (at 5 and 30°C incubation temperatures), yeast-fungi, coliform and Staphylococcus-Micrococcus numbers of the samples and sensorial analysis were conducted before processing, during processing and at certain days of storage (days 0, 7, 14, 21) of the roes.

The pH values of the samples were determined with a pH meter (EDT. GP 353) (AOAC, 1990). The drying cabinet procedure was used to determine the moisture content of the samples, the combustion method was used to determine the amount of ash in the samples samples (Gogus and Kolsarici, 1993) and the Soxhelet method was used to determine the amount of fat in the samples (AOAC, 1990). The raw protein contents of the products were determined by applying the Micro-Kjeldahl method (AOAC, 1990).

Mohr's method was applied to determine the amount of salt in the samples and the method specified by Varlik *et al.* (1993) was applied to determine the TVB-N quantity in the samples. The malonaldehyde amount in a 1000 g sample was calculated by reading the absorbance of red colours of the formed malonaldehits upon fat oxidation in samples that occurred with thiobarbituric acid in a glacial acetic acid environment at 538 nm (Tarladgis *et al.*, 1960).

About 5 g of sample was weighed in a special bag of an homogeniser (Stomacher 400), 45 mL of sterile 0.1% peptoned water was added to and the sample was homogenised. Dilutions of the samples (10^{-6}) were prepared using standard methods (Varlik *et al.*, 1993; Harrigan, 1998).

The samples were cultivated in laboratory under aseptic conditions and plaques containing 30-300 colonies were evaluated. Plate count agar media was used in counting the number of total aerobic microorganisms. The plaques were incubated at 30±1°C for 72 h and 5±1°C for 7 days before evaluation (Harrigan, 1998). Potato dextrose agar medium was used to count the number of yeast and fungi colonies after the cultivated plaques were incubated at 22±1°C for 5 days (APHA, 1976). Colonies of coliform group bacteria were counted after growth under incubation in violet red bile agar plates at 30±1°C for 24 h. Mannitol salt agar medium was used to count the number of Staphylococcus-Micrococcus growth colonies after the plaques were incubated at 37±1°C for 36-48 h (British Standards Institution, 1968).

Sensorial analysis: The caviar samples were analyzed at certain days of storage in terms of sensorial aspects. For this purpose in determining the quality attributes they were rated between 1 and 5 points. The products have been characterized by 10 panelists in terms of appearance, color, odor, texture and flavor. In the scoring, the assessment was conducted by using the following scheme (Kurtcan and Gonul, 1987) 1 = Very poor, 2 = Poor, 3 = Normal, 4 = Good, 5 = Very good.

Stastical analysis: The chemical and sensorial values obtained during the production and storage of the experimental prepared caviars were statistically analyzed. For statistical analysis, SPSS® 16.0 statistical computer program package was used. Independent t-test was performed to determination the differences between two groups and Kruskal-Wallis test was performed to determination differences amongst more than two groups.

RESULTS AND DISCUSSION

The chemical, microbiological and sensorial changes that occurred during storage at 4±1°C of rainbow trout caviar obtained by processing at different rates are shown in Table 1 (Fig. 2-4).

Chemical changes during storage: In this study, the chemical analysis of the raw roe shows the following characteristics: pH 7.83, moisture 61 (%), ash 2.15 (%), fat 11.65 (%), protein 24.77 (%), TVB-N (mg/100 g) 6.90, TBA 0.02 (mg malonaldehyde/1000 g).

Table 1: Chemical analyses values of processed roes

	Percentage				
Results	Moisture	Ash	Fat	Protein	Salt
A	53.41	4.48	10.04	26.43	1.89
B	54.50	4.51	10.44	26.36	1.45
C	52.86	5.23	9.75	27.23	2.45
D	55.16	5.12	9.78	25.32	2.13

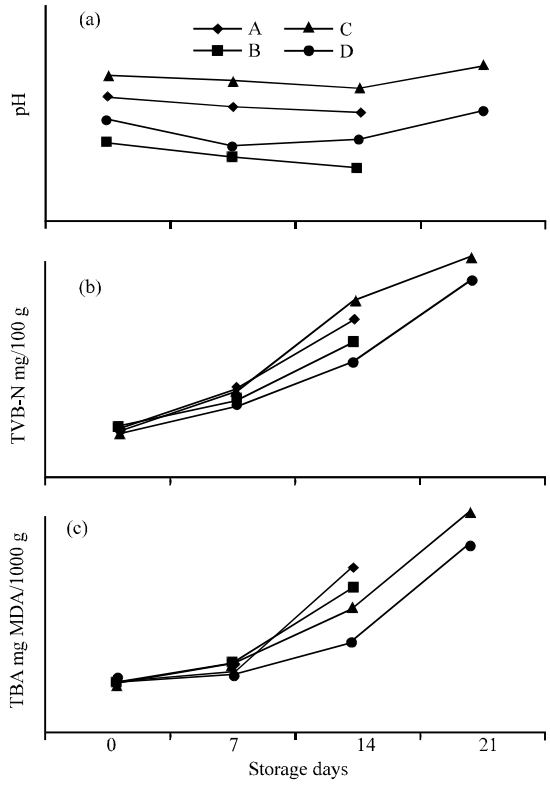

Fig. 2: Chemical changes of the different caviars types during storage at 4±1°C; a) pH (A: 6.07, 6.02, 6.00; B: 5.86, 5.80, 5.74; C: 6.16, 6.14, 6.11, 6.21; D: 5.96, 5.85, 5.87, 6.00); b) TVB-N (A: 7.12, 13.32, 23.43; B: 7.00, 11.34, 20.12; C: 7.10, 13.00, 26.23, 32.43; D: 6.78, 10.90, 16.80, 28.76); c) TBA (A: 1.01, 1.20, 3.30; B: 0.98, 1.34, 2.92; C: 1.00, 1.43, 2.48, 4.43; D: 1.06, 1.20, 1.80, 3.76)

In the study, the 7.83 pH value identified in raw roes at the end of salting was reduced to 6.07 in the A group, to 5.86 in the B group, to 6.16 in the C group and to 5.96 in the D group. At the end of storage, these pH values of the samples increased. In another study, the pH value was determined to 7.80 in the raw roes obtained from rainbow trout and the pH values of the samples were observed to increase at the end of the storage period (Inanli *et al*., 2010). Bledsoe *et al.* (2003) found pH values of 5.8 for red caviar and 5.45 for black caviar. The difference between those findings and researchers can be attributed to differences in the fish species from which the caviar were obtained and the different treatments applied. In this study, the chemical analysis of the raw roe showed the following: pH 7.83, moisture 61 (%), ash 2.15 (%), fat 11.65 (%), protein 24.77 (%) TVB-N 6.90, TBA (mg malonaldehyde/1000 g) 0.02.

As a high nutritional value food, the chemical composition of caviar varies according to fish species and processing techniques. In this study, the amount of moisture in raw roes was determined to be 61.30% on average. The amount of moisture contained in caviar varies according to the fish species from which it is obtained and according to processing techniques. As the caviar is gathered at the end of the applied salting process, the amount of moisture is reduced by the water that is expelled when salt enters the roes. In the study, the moisture content of raw roes was reduced at the end of the salting operation. In a different study, the amount of moisture in raw roes was determined to be 61.16% on average (Inanli *et al*., 2010). Ozpolat and Patir (2010) determined the moisture value in raw roes obtained from trout to be 63.90% on average; 49.17% in caviar at the end of salting and 50.40% at the end of storage. Sengor *et al.* (2000) detected the percentage of moisture to be 52.43% in raw roes obtained from flathead mullet, 35.34% in salted caviar and 23.58% in waxed caviar. Bledsoe *et al.* (2003) determined the amount of moisture in raw roes obtained from salmon to be 50% and reported that the moisture rate of the caviar changed according to the type of salmon. Namely, the amount of moisture retained by different types of caviar have been reported to be 50-60% in *Oncorhynchus gorbuscha*, 55-56% in *Oncorhynchus keta*, 56-58% in *Oncorhynchus nerka* and 51-70% in *Oncorhynchus ishawischa*. Wirth *et al.* (2000) reported that the moisture content in caviar obtained from sturgeon varies between 38 and 53%. Inal has declared the moisture in good-quality caviar to be 45%. The variation in the findings can be attributed to the diversity of the fish species from which caviar was obtained and to the different salt rates and processing techniques applied. When these findings are examined, it can be concluded that the applied salt rate and waiting time during caviar processing affect the moisture content of the products.

In the study, the average amount of ash was detected to be 2.21% in raw roe, 6.38% at the end of salting in the group in which 4% salt was applied and 8.84% in the group in which 8% salt was applied. In another study, the average amount of ash was detected to be 2.21% in raw roe, 6.38% at the end of salting in the group in

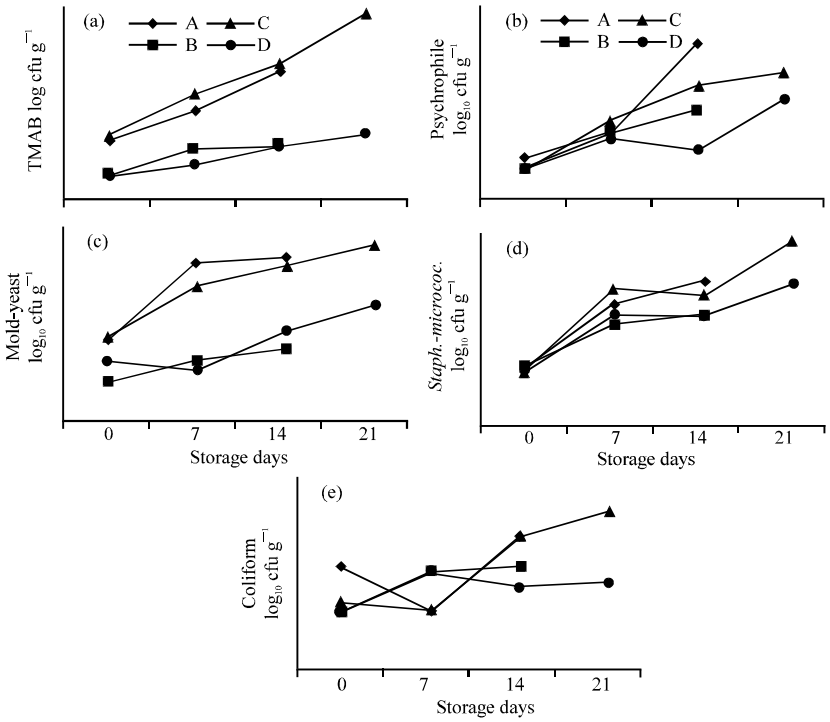

Fig. 3: Microbiological changes in the caviars types during storage at 4±1°C; a) TMAB (A: 2.27, 3.34, 4.93; B: 0.95, 1.94, 2.05; C: 2.51, 3.98, 5.06, 7.00; D: 0.95, 1.38, 2.02, 2.46); b) PAB (A: 1.33, 2.26, 5.10; B: 0.95, 2.16, 2.96; C: 0.98, 2.63, 3.79, 4.20; D: 0.98, 1.98, 1.65, 3.30); c) Mold-yeast (A: 1.90, 3.72, 3.85; B: 0.95, 1.41, 1.69; C: 2.02, 3.18, 3.65, 4.17; D: 1.41, 1.19, 2.11, 2.74); d) Stapylococcus-Micrococcus (A: 0.98, 2.14, 2.52; B: 0.98, 1.76, 1.95; C: 0.95, 2.40, 2.29, 3.23; D: 0.95, 1.95, 1.90, 2.48) and e) Coliform bacteria (A: 1.67, 0.95, 2.19; B: 0.95, 1.59, 1.68; C: 1.13, 0.95, 2.19, 2.59; D: 0.95, 1.58, 1.37, 1.43)

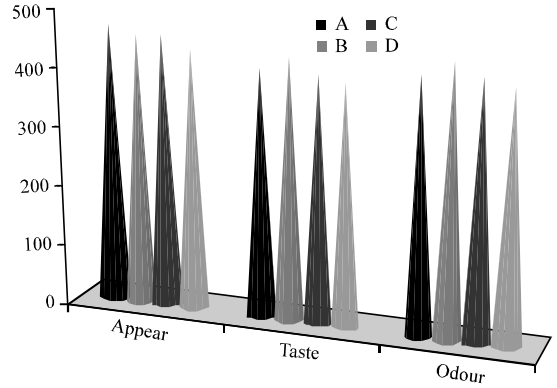

Fig. 4: The values of sensorial quality in the caviars of different types during storage at 4±1°C

which 4% salt was applied and 8.84% in the group in which 8% salt was applied; at the end of storage, these values were determined to be 6.28 and 8.13%, respectively (Inanli *et al*., 2010). The value obtained by Ozpolat and Patir (2010) in their research on the average amount of ash in the raw roes of rainbow trout (2.01%) is close to those found in the findings. In the same study, the determined value was (12.44%) in salted caviar which is higher than those determined from the findings due to the different salt rate. In a study conducted on the roes of flathead mullet, the ash rate was determined to be 5.08% in raw roes, 7.17% in processed roes and 10.14% in waxed roes (Sengor *et al*., 2000). Altug and Bayrak (2003) detected a 7% ash content in red caviar. It can be said that the diversity of these findings is derived from the different processing techniques applied and the different fish species studied.

The chemical composition of caviar varies depending on fish species and processing techniques. In this study, the amount of fat in the roes obtained from rainbow trout was determined to be 11.65%. In a study by Inanli *et al*. (2010), the fat amount in roes obtained from rainbow trout was determined to be 11.70% and did not change much during storage. In another study, the fat amount was identified to be 10.9-19.4% on average in caviar obtained from salmon fish (Wirth *et al*., 2000). Himelbloom and Crapo (1998) detected 11% fat in their caviar processed

with salmon (Ikura). Sengor *et al.* (2000) detected the fat content in roes obtained from flathead mullet to be 6.89% in raw caviar, 11.58% in processed caviar and 21.27% in waxed caviar. In another study related to caviar obtained from salmon, it was reported that the fat content varied between 8 and 25% according to fish species (Bledsoe *et al.*, 2003). The fat amounts found in the study were relatively similar to those in the literature and it can be concluded that the differences were due to the differences between fish species and the applied processing techniques.

In this study, the average protein content was determined to be 24.77% in raw roes. Inanli *et al.* (2010) report on average protein content of 24.87% in raw roes from rainbow trout. In a study on flathead mullet (*Mugil cephalus*), the protein amounts were determined to be 25.52% in roes, 35.38% in salted caviar and 40.83% in waxed caviar (Sengor *et al.*, 2000). Wirth *et al.* (2000) have noted that caviar obtained from sturgeon has a protein content between 26.2 and 31.1%. Bledsoe *et al.* (2003) have specified the protein amounts in caviar obtained from different trout species and prepared by different techniques as follows, 23% in *Oncorhynchus gorbuscha*, 27% in *Oncorhynchus keta* and 20% in *Oncorhynchus nerka* and 21% in *Oncorhynchus ishawyischa*. In this study, the researchers determined that the amounts of protein were in agreement with the majority of findings reported in the literature and the differences can be attributed to differences in species and processing techniques.

The salt used in preparing caviar is a factor that affects both the storage period and the flavour of the product. In this study, the amounts of salt in the processed caviar were determined to be 1.45 and 2.45%. The salt rate was observed to decrease with storage time. Excessive salt will disrupt the taste of a product as well as create digestion problems. Therefore, the salt level should be determined with precision.

During the storage of fish and seafood products, TVB-N values increase with deterioration. In this research, this value was determined to be 6.90% mg/100 g in raw roes, 7.12% mg/100 g in the 5% wet salted group and 7.04 mg/100 g in the 10% wet salted group on the 1st day of storage and as the days progressed, these values increased. The limit values related to the TVB-N quantities differ between fish and other seafood products. For example, Huss (1995) reports a TVB-N quantity in just caught fresh fish as being 5-20 mg/100 g and fresh acceptable limit values as being 30-40 mg/100 g. Varlik *et al.* (1993) considers the quality classification of TVB-N values up to 25 mg/100 g as very good, up to 30 mg/100 g as good, up to 35 mg/100 g as marketable and >35 mg/100 g as corrupted. Long reported the edibility limit values for freshwater fish as being 32-34 mg/100 g and Alperden *et al.* (1981) reported that these values were also valid for caviar. In this study, the TVB-N quantities at the upper limit values were determined at the end of storage of samples not included in the sensorial evaluation. TVB-N value in the storage of fish and seafood products, increases in parallel to the deteriorate. In this research, this value was determined as 6.90% mg/100 g in raw roes as 7.12% mg/100 g in the 5% wet salted group and as 7.04 mg/100 g in the 10% wet salted group at the 1st day of storage and as the days progressed it has increased. The limit values related to the TVB-N quantities differ in the fish and other seafood products. Namely; Huss (1995) reports TVB-N quantity contained in a just caught fresh fish as 5-20 mg/100 g and the fresh acceptable limit values as 30-40 mg/100 g. Varlik *et al.* (1993) considers the quality classification according to the TVB-N values up to 25 mg/100 g as very good, up to 30 mg/100g as good, up to 35 mg/100 g as marketable and >35 mg/100 g as corrupted. Long have reported the edibility limit values for freshwater fish as 32-34 mg/100 g and Alperden *et al.* (1981) have reported that these values were also valid for caviar. In this study, the TVB-N quantities at the upper limit values were determined at the end of storage on the samples not included in the sensorial evaluation.

Inanli *et al.* (2010) have reported TVB-N values of 6.95% mg/100 g in raw roes from rainbow trout, 7.06% mg/100 g in the 4% salted group and 7.04 mg/100 g in the 8% salted group. Safari and Yosefian (2007) determined average TVB-N values of 8.98 mg/100 g in raw roes obtained from *Acipenser persicus* fish, 11.34 mg/100 g in processed roes and 25.48 mg/100 g in products stored at -3°C. In a study on rainbow trout caviar (Ozpolat and Patir, 2010), the detected TVB-N values were 6.19 mg/100 g in raw roes, 6.64 mg/100 g in salted roes and 6.99 mg/100 g at the end of storage (84th day). In this research, the values identified in raw roes and in salted roes at the end of salting were consistent with those of Ozpolat and Patir (2010) and the inconsistency of the other values is due to the different processes applied and different species studied.

Fat oxidation is one of the changes that cause the corruption of a product. The colour of oxidised products is yellowish brown and their taste is bitterish. One of the criteria that indicates fat oxidation is the thiobarbituric acid count. According to researchers, the TBA count must be <3 in a very good material and should not be >5 in a good material. The limit for consumption is between 7 and 8 (Varlik *et al.*, 1993; Sinnuber and Yu, 1958). In the raw roes used in this study, the TBA count was 0.02 mg

MDA/1000 g on average which increased during production and storage. According to the TBA values determined at the end of storage, the product exceeded the limit of a good material. In the present study, no significant differences were found among groups in terms of chemical values ($p>0.05$).

Microbiological changes during storage: In this study, the bacteria count determined by microbiological analysis of raw roe was as follows: total mesophile aerobic bacteria 4.74 $\log_{10}$ cfu g^{-1}, psycrophile 4.23 ($\log_{10}$ $cfug^{-1}$, yeast-mould 4.20 $\log_{10}$ $cfug^{-1}$, Staphylococcus-Micrococcus 1.00 $\log_{10}$ cfu g^{-1} and coliform 1.00 $\log_{10}$ cfu g^{-1}.

In general, the muscle of newly caught fish from healthy waters is sterile. Microorganisms generally exist in skin, gills and intestines. The number of microorganisms ranges between 10^2 and 10^6 cfu cm^{-2} in skin and 10^3 and 10^9 cfu g^{-1} in gills and intestines. Microorganisms may spread over muscles in gills and intestines depending on the processes applied after fish capture, ambient temperature and time. As a result, the quality of fish degrades depending on the microorganism species and the people who eat them may suffer from infection or toxicity. Therefore, information regarding microorganisms in fish muscle is very important from the point of view of health and storage (number and species of microorganisms) (Gram and Huss, 1996, 2000).

Psychrophiles are microorganisms responsible for the spoilage of fish. It has been evidenced that dominant microflora in decayed fillets are psychrophile microorganisms (Clingman and Hooper, 1986; Dalgaard *et al*., 1993).

In this study, the total number of aerobic microorganisms (Psychrophiles) obtained after incubation at 5°C of microbiologically cultivated samples of caviar was 4.23 $\log_{10}$ cfu g^{-1}; the, total number of aerobic microorganisms (Mesophiles) obtained after incubation at 30°C of microbiologically cultivated samples of caviar was 4.74 $\log_{10}$ cfu g^{-1} and the number of yeast-mold colonies was found to be 4.20 $\log_{10}$ cfu g^{-1}. In this study, the number of Staphylococcus-Micrococcus and coliform colonies was found to be 1.00 $\log_{10}$ cfu g^{-1}. These values decreased after salting and increased with the addition of acetic acid during the storage period.

The results of the present study show that there are significant differences among the groups studied (A-D) in terms of the numbers of total mesophilic aerobic bacteria and yeast-mold ($p<0.01$). In addition, the statistical differences were found to be significant between only the wet salted (A, C) and acetic acid added (B, D) groups ($p<0.01$).

Sensorial analysis during storage: In this study, the samples processed and stored at +4°C and were evaluated by 10 panelists in terms of sensorial aspects on the analysis days. At result of the evaluations, a significant difference between the groups was not found. It is ultimately the rate of acetic acid used is an important result because not affect the caviar organoleptic properties.

In the present study, it was found that there were no significant differences among groups in term of sensorial values ($p>0.05$).

CONCLUSION

Chemical, microbiological and sensory characteristics during the storage at 4±1°C of experimentally prepared four groups of caviar from rainbow trout were investigated. In this study, it was determined that acetic acid could be also used in the process of salting, it has got no affected negative effects on the organoleptic properties of caviar samples and but especially acetic acid affected on negative the samples microbiologically. Also increasing of salt rate affected positively the shelf life, values of D group containing 10% salt and 0.04% acetic acid and nearest to the values of consumability, respectively. In this study, it was concluded that using rates of acetic acid no effected on the sensory characteristics, antimicrobial effect of acid could taken advantage of caviar production.

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