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Research Article

Effect of electrochemically activated anolyte on the shelf-life of cold stored rainbow trout

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Abstract

The sensory, physicochemical and microbiological properties of electrochemically activated anolyte and flake ice chilled rainbow trout *(Oncorhynchus mykiss)* packed in low-density polyethylene/polyamide bags were studied during storage for 20 days at 0-4°C. Significant reduction of the sensory assessed scores was found. The fish treated with electrochemically activated anolyte was assessed as satisfactory fresh to 15 days of storage. On this day, their sensory scores of the state of the eyes were higher ($p < 0.05$) with 27.78%, skin appearance with 22.22% and taste of grilled trout with 18.75% respectively in comparison with flack ice chilled fish. After 20 days of storage the electrochemically activated anolyte treated rainbow trout characterised by 4.38% lower pH, 20.00% lower free fatty acids, 9.22% lower peroxide value, 17.65% lower TBARS, 5.85% lower free α-Amino nitrogen, 19.66% lower total volatile based-nitrogen, 4.28% lower total viable counts on the skin and 2.90 % in the muscle tissue, and 2.82% lower psychrophilic count on the skin and 2.86% in the muscle tissue. The conclusion was made that the chilling of the rainbow trout with electrochemically activated by maintaining satisfactory life-shelf anolyte could contribute to prolong the organoleptic andphysicochemical properties to the 15th day at 0-4°C.

Keywords: fresh fish, sensory characteristics, physicochemical properties, microbiological state, ECA chilling, cold storage

Abbreviations:

ANOVA - one-way analysis of variance
ECA - electrochemically activated anolyte
FFA - free fatty acids
POV - peroxide value
SD - standard deviation
TBARS - 2-thiobarbituric acid reactive substances

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Introduction

Freshwater fish is very perishable product, it is highly susceptible to both microbiological and chemical deterioration, due to its high water activity, neutral pH, presence of endogenous enzymes promoting proteolysis of muscle proteins and connective tissue, as well as a lipid hydrolysis and oxidation, and microbial spoilage. That is way, the development of effective preservation technologies for the shelf-life extending, as saving the nutritional value and sensory properties of aquatic organisms become increasingly necessary. Rapid chilling, ice storage and salting or with combination of natural preservatives (antimicrobials and antioxidants) have been used as traditional methods to extend the shelf-life of fish. The disadvantages of fish chilling with flake ice as a method of preservation are few (Magnussen et al. 2008). In particular, in today's market the consumers meanly connected those disadvantages with the quick loss of freshness (Verbeke et al. 2007) and not so good safety (Unnevehr 2000) of fish. Therefore, more often poses the question; can we preserve the benefits of chilling with ice and at the same time to prolong the shelf-life of fresh unpackaged fish?

Electrochemical active anolyte (ECA) has been reported to have strong bactericidal effects on most pathogenic bacteria that are important to food safety (Huang et al. 2008; Cloete et al. 2009). The antimicrobial effects of ECA are used in many spheres nowadays (Huang et al. 2008). They are used for disinfection of the technological equipment in factories and the working outfit of the workers in meat and dairy industry. It provides high quality disinfection of slaughterhouse premises, tools and accessories, staff hands, shoes and clothing, and (most importantly) the product safety. The anolyte is an ideal means of care for the animals, since it is non-toxic, does not damage the alive cells, and has the ability to instantly destroy all known pathogens in the complete exclusion of the emergence of resistant varieties. Anolyte water is also used in agriculture to extend the durability of vegetables (Worknen et al. 2003), to fight viral and fungal diseases of plants, seeds and seedlings and to stimulate plant growth (Thorn et al. 2012). The electrochemically activated anolyte is an alternative to chlorine for decentralized disinfection (Ghebremichael et al. 2011). There are indications for an unit which can be used to conduct the electrolysis of a solution of common salt in water, and to produce ECA water. A diaphragm (membrane) separates both chambers. In Anode-chamber so called Anolyte was being produced, that is used as disinfectant.

In this respect, based on knowledge about the benefits of ECA question arises could we use proven its antibacterial properties to extend the shelf-life of the fresh trout?

In the literature we cannot found data for applying the anolyte as tool for hygienic treatment of fresh fish or extending the shelf-life of rainbow trout. The aim of the present study was, to determine the possibilities for extending the shelf-life and freshness of rainbow trout by using ECA treatment during fish storage at 0-4°C.

Materials and Methods

Fish. The rainbow trout *(Oncorhynchus mykiss, Walbaum)* was purchased by the fish farm 'White River' village Levski, municipality of Karlovo, in Plovdiv district (Bulgaria). The fish was on the age of 13 months and with almost identical mass of 320±20g. Eighty one fish were caught alive put into plastic barrels and covered over with a flake ice. The fish was culled by a rapid decrease in temperature due to backfilling it with a flake ice, causing cold related stress (hypothermia).For approximately one hour the fish was transported to the laboratory of the Department of Meat and Fish Technology of the University of Food Technologies, Plovdiv and fish was eviscerated as soon as possible and divided into three groups. The first group consists 9 fish. This sample was used as initial control group. The fish from this group were analysed 2h *post mortem.* The results obtained were used as initial values (0 day of storage). Against them they were evaluated the depth of the current changes in the experimental and control samples during the refrigeration storage. The remaining two groups (control and experimental samples) consist 36 pieces of fish each. The fish from experimental sample was immersed in plastic

barrels in cold (4°C) ECA for half an hour. In the same time the fish from control sample was left in plastic barrels covered with a flake ice. The fish from control and experimental samples were packed in air in low-density polyethylene/ polyamide bags with thickness 75μm. In every one pack were put three pieces of fish. After that both samples were labelled and stored 20 days at 0-4°C. A sampling was carried out at 5, 10, 15 and 20 days of storage. Both the control and experimental sample for each day of study (5, 10, 15, 20 day of storage time) were opened in three packages with 3 fish. The first package was used to conduct sensory analysis, the second one for carrying out the chemical analyses and the third one for microbiological experiments. For preparation the average laboratory samples was used as the light and dark musculature as well the skin of the fish.

Electrochemical active anolyte (ECA). For purposes of this experiment electrochemical synthesis anolyte type ANK (Bakhir 2005) was used. For activation of low mineralized water to 2g.L^{-1} was used portable laboratory stand Module 1 (Association Activated water, Sofia, Bulgaria). In the stand was mounted instantaneous flow electrochemical activator (reactor) type 11 MB (Bahir et al. 1997).
The eleventh litters of ECA were made. The parameters of the used ECA were measured before the solutions were used (Table 1).

Table 1. Properties of obtained electrochemical active anolyte

Indicators	Average value of the incoming water	Average value of the obtained anolyte
pH value	7.98	2.71
Total dissolved solids (TDS), mg.L^{-1}	371	239
Oxidation reduction potential (ORP), mV	283	1421
Conductivity, S.m^{-1}	669	400

Sensory evaluations. The sensory analysis of the samples was made using the scale from 'sensory assessment score sheets for fish and shellfish (Archer 2010) was used. The scheme undergo on some changes and modifications for the specific needs in our case. The sensory quality of rainbow trout was evaluated by a panel of five trained members. Panellists were scored for sensory characteristics using ten grade scale for samples of fresh fish referring to the appearance of skin and condition of the eyes, and nine-point hedonic scale grade scales for taste assessment of grilled fish (1, dislike extremely to 9, like extremely). The scores were statistically processed and shown as final grade.

Preparation of samples for physicochemical analysis. Average laboratory samples were prepared by homogenization of the fish meat. From every one average laboratory sample, the quantity required has calibrated, as described in the appropriate method for research.

pH value. pH value of the samples was determined from a meat-water mixture (Korkeala et al. 1986). 10g of the homogenized sample was put into 90ml of distilled water and the pH value was measured with pH meter Microsyst MS 2004 (Microsyst, Plovdiv, Bulgaria), equipped with temperature and combined pH electrode type Sensorex Combination Recorder S 450 CD (Sensorex pH Electrode Station, Garden Grove, USA).

Free fatty acids. The acid value was measured using free fatty acids as an indication of hydrolytic rancidity of fish oil (Gheisari 2011). FFA in the oil extracted from a fish sample was determined by titration with a solution of potassium hydroxide (KOH) with 0.1N concentration and with phenolphthalein as a colour indicator. The acid value (or free fatty acid content) was determined by AOAC method 940.28 (Latimer 2012). The acid value and the percentage fatty acid were calculated from the expression bellow:

$$Acid\ Value = (56.11\ molarities\ of\ NaOH \times Titter\ value):\ Weight\ of\ fish\ oil \quad (1)$$

$$FFA = 0.503 \times Acid\ Value,\ \%\ oleic\ acid \quad (2)$$

Peroxide value. POV was determined spectrophotometrically based on the oxidation of Fe^{2+} to Fe^{3+} in the presence of hydroperoxides and

the formation of a coloured complex between obtained Fe^{3+} and SCN as described by (Hornero-Méndez et al. 2001). The absorbance at 470nm was measured against the water blank (correction of the spectrum baseline at 670nm was performed). The absorbance was measured by twin ray spectrophotometer UV-VIS Camspec, model M550 (Camspec Ltd, Sawston, Cambridge, United Kingdom).

TBARS. TBARS was determined using the method of (Botsoglou et al. 1994), by recommendations of (Jebelli Javan et al. 2012). Calibration curves were constructing by plotting values of peak height at 521.5nm were measured using twin ray spectrophotometer UV-VIS Camspec, model M550 (Camspec Ltd, Sawston, Cambridge, United Kingdom), as they are printed on the instrumental chart in arbitrary units, versus known concentration of MDA in the final reaction mixtures. MDA in samples was calculated using formula:

$$MDA = 16 \times C \times V: W, \mu g.kg^{-1} \quad (3)$$

where C is the MDA concentration ($ng.cm^{-3}$) in the sample extracts according to the calibration curve, V is a dilution factor of sample extract (cm^3) if any, W is the weight (kg) of the sample.

Total volatile base nitrogen. The total volatile basic nitrogen was determined according to the method described by (Goulas and Kontominas 2005).

Free α-amino nitrogen. Firstly an extraction of soluble proteins was made with help of buffer pH 7.3 homogenized with 2.5g of meat and left for one night in refrigerator further developed by (Abernathy et al. 2009). The absorbance on 570nm was measured after 20min with twin ray spectrophotometer UV-VIS Camspec, model M550 (Camspec Ltd, Sawston, Cambridge, United Kingdom).

Microbiological analysis. Decimal dilutions were performed (10^{-8}) and 1mL was removed from each dilution and cultured by the pour plate technique on plate count agar (PCA, Merck Bulgaria Ltd, Sofia, Bulgaria) for mesophilic (TVC) and psychrophilic counts (PTC) in triplicate. The total mesophilic aerobic bacteria count was incubated 72h at 28ºC (Gelabert et al. 2003). Psychrophilic count was determined in a similar method to that for TVC, except that plates were incubated at 7°C for 10 days (Cousin et al. 1992).

Statistical analysis. Statistical analyses were run in triplicate and results were reported as mean values ± standard deviation (SD). Data were subjected to analysis of variance (one-way ANOVA Excel 5.0). A p-value less than 0.05 ($p \leq 0.05$) was considered statistically significant.

Results and Discussion

Sensory evaluations

Panel evaluated sensory scores of the samples are shown in Fig. 1, 2 and 3.

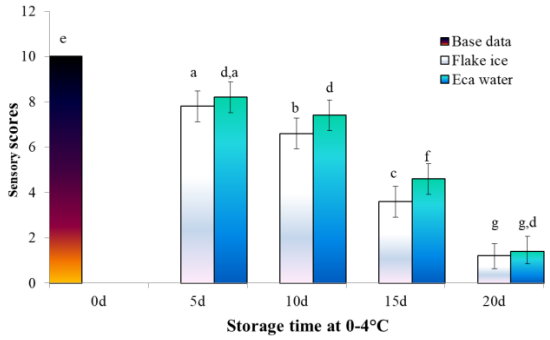

Figure 1. Sensory scores for state of the eyes of chilled rainbow trout (*Oncorhynchus mykiss*) preliminary treated with ECA.

Data were expressed as mean ± SD (n = 3). a, b, c, d, e, f, g - different letters indicated that values of the means are significantly different ($p < 0.05$).

The results showed a significant reduction in sensory scores after 20 days storage at both samples. After 20 days of storage, in two samples a significant reduction of the sensory assessed scores ($p < 0.05$) were found (Fig. 1, 2 and 3). Those results are a proof that the fish deterioration was flowed. After 15 days of storage, the development of deterioration in the control samples fish was

obvious. The panellists were reporting for adopting a putrescence smell, rancid to butter taste, and softer texture of fish fillets of control samples on 15th day of storage.

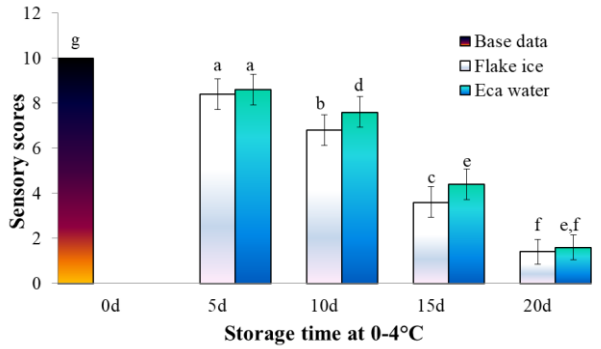

Figure 2. Sensory scores for skin appearance of chilled rainbow trout (*Oncorhynchus mykiss*) preliminary treated with ECA.

Data were expressed as mean ± SD (n = 3). a, b, c, d, e, f, g - different letters indicated that values of the means are significantly different ($p < 0.05$).

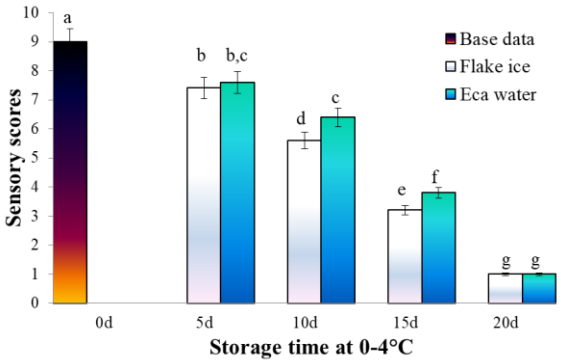

Figure 3. Sensory scores for taste of grilled rainbow trout (*Oncorhynchus mykiss*) fillet preliminary treated with ECA.

Data were expressed as mean ± SD (n = 3). a, b, c, d, e, f, g - different letters indicated that values of the means are significantly different ($p < 0.05$).

The results of sensory and microbiological analysis are in good agreement (Fig. 4 a, b, c, d). The results obtained demonstrated that the treatment of rainbow trout chilled in flaky ice does not lead to the adverse effects and to unacceptable sensory properties of the fish. The panellists were reporting for adopting a putrescence smell, rancid to butter taste, and softer texture of fish fillets of control samples on 15th day of storage. The results of sensory and microbiological analysis are in good agreement (Fig. 4 a, b, c, d). The results obtained demonstrated that the treatment of rainbow trout chilled in flaky ice does not lead to the adverse effects and to unacceptable sensory properties of the fish. Our results are similar to those reported by Rezai et al. (2007) about chilled wild trout whose shelf-life was indicated on 9-11 days and by Arashisar et al. (2004) about vacuum packaged rainbow trout immersion treated with sodium acetate solution (2%), which shelf-life was 15 days at 2±1°C. Contrary to us Chytiri et al. (2004) determined a shelf-life of 15-16 days when the whole cultivated rainbow trout, and 10-12 days when the fillets, were stored in ice. After the 5th, 10th and 15th day of storage sensory scores of samples treated with ECA were significant ($p < 0.05$) higher in comparison with control samples. According to the results of sensory analysis trout treated with ECA were assessed as satisfactory fresh to 15 days of refrigeration storage. On this day, their sensory scores were higher ($p < 0.05$) with respectively 27.78% (Fig. 1), 22.22% (Fig. 2) and 18.75% (Fig. 3) in comparison with control samples.

Judging by the results of the sensory analysis we can conclude that treated with ECA rainbow trout kept organoleptic qualities to 15 day of refrigeration, while control samples up to 10 day. Moreover, after 15 days of storage at 0-4°C, treatment of rainbow trout using ECA contributes to better preservation of the skin's appearance and taste of grilled fish fillet.

Physicochemical properties

The pH values of the both samples were significantly increased ($p < 0.05$) during the storage at 0-4°C was determined (Table 2). Up to 10 days of storage pH value of the two samples did not differ statistically significant ($p > 0.05$). Upon further storage pH values of the experimental trout samples rises less was found (Table 2). After 15 days of storage pH of experimental samples is lower by 1.01% and after 20 days – 4.38%. Higher pH values recorded for flake ice chilled trout after 15 day of storage may be attributed to the rapid

Table 2. Changes of pH, FFA, POV, TBARS, FAN and TVBN of chilled rainbow trout (*Oncorhynchus mykiss*) preliminary treated with ECA during 20 days of storage at 0-4°C

Data were expressed as mean ± SD (n = 7). [a, b, c, d, e, f, g, h] - different letters indicated that values of the means are significantly different ($p < 0.05$).

Examined parameter	Base data	Rainbow trout (*Oncorcynchus mykiss*) chilled with flake ice stored at 0 - 4°C Control sample				Rainbow trout (*Oncorhynchus mykiss*), chilled by immersion in ECA stored at 0 - 4°C			
	Days of storage								
	0d	5d	10d	15d	20d	5d	10d	15d	20d
pH value	6.65^{a} ± 0.02	6.74^{b} ± 0.03	6.83^{c} ± 0.03	6.99^{d} ± 0.02	7.63^{e} ± 0.05	6.71^{b} ± 0.02	6.81^{c} ± 0.02	6.92^{f} ± 0.01	7.31^{g} ± 0.07
Free fatty acids, % oleic acid	0.20^{a} ± 0.02	0.29^{b} ± 0.01	0.65^{c} ± 0.02	0.84^{d} ± 0.03	1.02^{e} ± 0.03	0.29^{b} ± 0.02	0.62^{f} ± 0.01	0.78^{g} ± 0.02	0.85^{h} ± 0.04
Peroxide value, $\mu eqO_2.kg^{-1}$	2.78^{a} ± 0.3	8.30^{b,c} ± 0.21	8.90^{b} ± 0.34	10.97^{d} ± 0.13	15.16^{e} ± 0.57	7.72^{f} ± 0.23	8.31^{b} ± 0.18	9.90^{g} ± 0.17	13.88^{h} ± 0.44
TBARS, mg MDA.kg^{-1}	0.12^{a} ± 0.03	0.41^{b} ± 0.02	0.50^{e} ± 0.04	0.59^{c} ± 0.02	0.80^{d} ± 0.02	0.39^{b} ± 0.02	0.43^{b,h} ± 0.04	0.53^{f} ± 0.03	0.68^{g} ± 0.04
Free α-Amino nitrogen, mg.100 g^{-1}	8.24^{a} ± 0.07	11.34^{b} ± 0.07	13.93^{c} ± 0.21	17.06^{d} ± 0.17	19.52^{e} ± 0.14	11.17^{b} ± 0.17	13.01^{f} ± 0.33	16.00^{g} ± 0.18	18.44^{h} ± 0.12
TVB-N, mg.100 g^{-1}	12.48^{a} ± 0.67	15.25^{b} ± 0.33	19.95^{c} ± 0.27	26.38^{d} ± 0.45	37.12^{e} ± 0.13	15.11^{b} ± 0.34	18.69^{f} ± 0.33	23.92^{g} ± 0.36	31.02^{h} ± 0.26

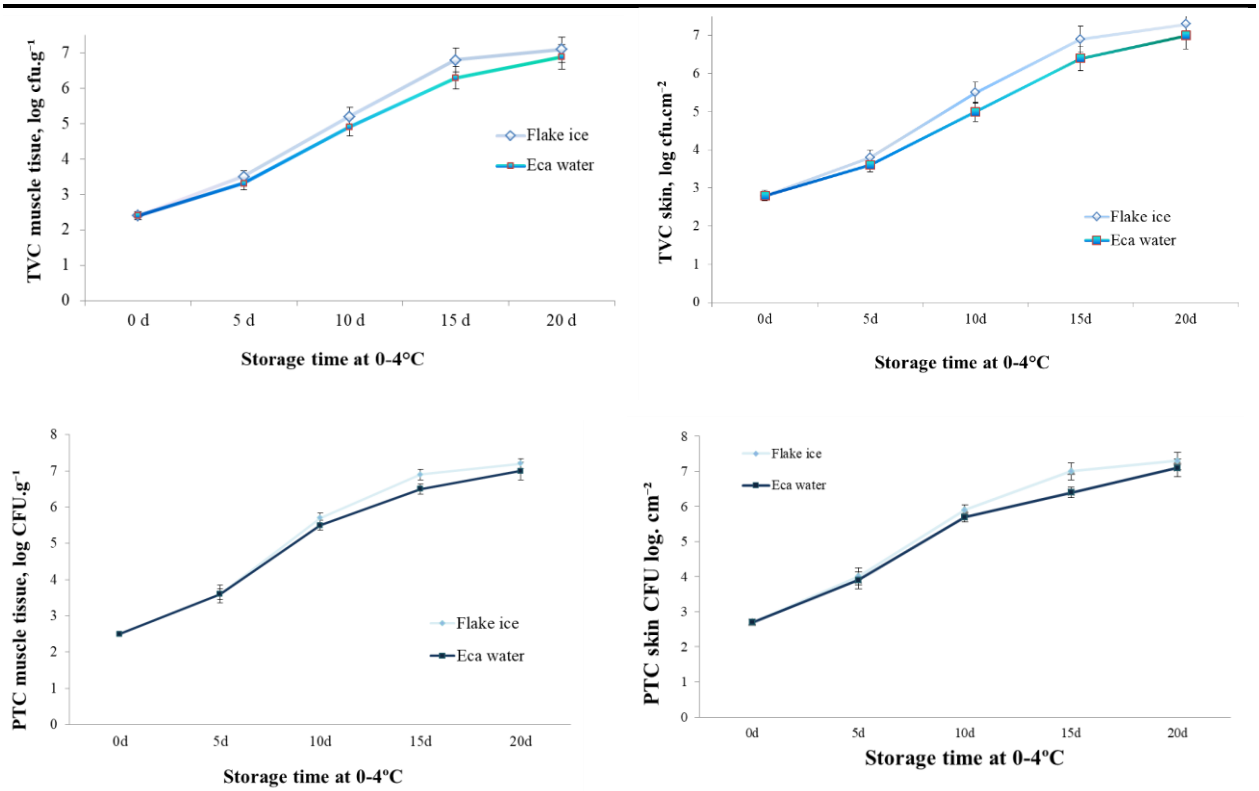

Figure 4 a, b, c, d. Microbiological changes of total viable counts and psychrophilic counts on skin and in muscle tissue of rainbow trout chilled with ECA treatment and classically flake ice chilled during storage at 0-4ºC.

spoilage of the product and the formation of alkaline compounds of autolysis and bacterial metabolites (Atrea et al. 2009).

FFA significantly increased ($p < 0.05$) from 0.20% oleic acid to 1.02% oleic acid at 20 day in flake ice chilled and 0.85% oleic acid in ECA treated samples respectively were determined (Table 2).

The found FFA increases probably is a result of triglyceride chemical or enzyme mediated hydrolysis (Barthet et al. 2008) of trout lipids. In our study lipid hydrolysis occurred to a great extent at the end of the storage period. It is possible to have a relationship between FFA release and loss of trout freshness (Özogul et al. 2005; Rodríguez et al. 2006). Significantly low ($p < 0.05$) values of FFA content in experimental samples during storage can be explain with strong bactericidal effects of ECA water solution and decrease of the microbial growth. FFA formation produced at the beginning of storage may be a result of endogenous enzymes lipases and phospholipases activity (Whittle et al. 1990). Later on (after the end of the lag phase), microbial activity should be important, so that FFA formation should mostly be produced as a result of bacterial enzyme activity.

Permanently increase the POV of both samples during their storage was found (Table 2). More accelerated increase in POV was observed in control samples. On the 15th day of storage POV of control sample was increased 3.95 times, and on the 20th 5.45 times respectively. By comparison POV experimental sample was increased 3.56 and 4.99 times on the 15th and 20th day of storage, respectively. These results indicate that the fish treated with ECA solution exhibits greater oxidative stability during refrigeration storage. POV in these samples were with 7.51%, 7.10%, 10.81% and 9.22% lower at the 5th, 10th, 15th and 20th day of storage as compared to the control samples. A smaller percentage difference in POV can be explaining as results of formation of primary inducted products of lipid oxidation (Szwarc-Rzepka et al. 2013). Perhaps that significant differences in POV between samples especially on 15th and 20th day of storage were due to ECA solution treatment reduces the possibility of active oxygen species to attack the unsaturated fatty acids from trout oil which are mainly responsible for the lipid oxidation initiation in fish (Dragoev et al. 2014).

In comparison to POV, TBARS increase up in fewer rates to 20 days of storage for both samples was found. The control samples showed significantly higher ($p < 0.05$) TBARS at 20th day of storage with 17.65% comparing with the ECA treated samples. One possible explanation of the inhibition of lipid oxidation is that due to result of immersion of rainbow trout in ECA water solution it acts as a barrier between the fish and the surrounding air, slowing down the diffusion of oxygen from the surroundings via the fish surface Thus, the reported results for the POV and TBARS (Table 2) are in good agreement the identified sensory changes in the taste of grilled fish because mg MDA.kg^{-1} is usually regarded as limit beyond which fish will normally develop an objectionable odour and taste (Peters et al. 1985).

A significantly ($p < 0.05$) increases of FAN: 2.36 times for control sample and 2.24 times for ECA treatment respectively at the end of the storage was determined (Table 2). Likely FAN content, which reflect the degree of release of free amino acids from muscle proteins due to the action of two endogenous proteolytic enzymes, and those of microbial origin (Viji et al. 2015). At a later stage of storage these free amino acids undergo further degradation and release volatile bases and other low molecular weight nitrogen containing compounds. In our study after 20d of storage, both samples passed the upper limits of FAN content for fish 17-18mg.100g^{-1} (Viji et al. 2015).

An increase of TVB-N value was indicated for both samples throughout the 20 d storage 2.97 and 2.49 times for flake ice and respectively for ECA chilled rainbow trout samples (Table 1). TVB-N in all samples was below then limit of 35 mgN.100g^{-1} for raw fish (95/149/EC: Commission Decision) except the flake ice chilled rainbow trout on the 20 day of storage. As the TVB-N is a result of spoilage by either bacterial or enzyme degradation (Özogul and Özogul 2000) the higher TVB-N values determined after 15th days of storage (Table 2) is in good agreement with sensory scores of two trout samples.

Microbiological analysis

The data from microbiological analysis of flake ice and ECA treated chilled rainbow trout were presented in Fig. 4 a, b, c, d. Initial value (0 day) TVC of fish on the skin and in muscle were 2.8 log cfu.cm^{-2} and 2.4 log cfu.g^{-1}, which is proof of high quality of fish. Those results are in good agreement with the results of Chytiri et al. (2004) and Frangos et al. (2010). The microbiological limit recommend-ded by the Christian and Roberts (1986) for TVC at 30°C is 7 log cfu.g^{-1} or log cfu.cm^{-2} for fresh water and marine species. In our study this limit was passed by flake ice chilled rainbow trout on skin surface and in muscle tissue at 20th day of storage, while ECA treated samples passed the limit just on the skin surface at 20 day of storage.

TVC increased during the storage especially in control sample until 15 days of storage (Fig. 4 a, b). Significantly lower TVC content ($p < 0.05$) of ECA treated samples was detected from the 10th day till the end of storage. When the fish is unacceptable to the consumer judging by the sensory quality, its microbiological state deteriorated too, was found.

TVC content on skin surface and in muscle tissue of the both samples was 6.9 and 6.4 log cfu.cm^{-2}, 6.8 and 6.3 log cfu.g^{-1} respectively.

The psychrophilic counts (PTC) of chilled fish were higher than the TVC (Fig. 4 c, d), indicating that fish bacterial flora is composed mainly of psychrophilic bacteria (Duan et al. 2010). Continuous increase of PTC during storage in all samples was observed (Fig. 4 c, d). On 20th day of storage all samples were reached over 7 log cfu.cm^{-2} skin and 7 log cfu.g^{-1} muscle tissue (Fig. 4 a, b, c, d). Our results are in contract with those reported by Gimenez et al. (2002) estimated aerobic psychotropic count to 7 log cfu.g^{-1} for vacuum or modified atmosphere packaged rainbow trout fillet after 14-17 days.

Significant differences ($p < 0.05$) in favour of ECA treated samples were detected for PTC on skin and in muscles on 10, 15 and 20 days of storage.

Our results may be explained by the strong bactericidal effect of ECA on many pathogenic bacteria due to it pH in range of 4.0 – 9.0 (Huang et al. 2008) and mostly at ORP range -700 to +200 mV (Scott 2004).

The high ORP of ECA decreases the microbial growth in a result of oxidation of cells' sulfhydryl compounds (Park et al. 2004) and damage of the cellular membranes (Liao et al. 2007).

Conclusions

Based on the sensory evaluations, physicochemical properties, and microbiological data, the shelf-life of electrochemically activated anolyte chilled rainbow trout packed in low-density polyethylene/ polyamide bags after gutting and evisceration refrigerated at 0-4°C was approximately 15 d.

After that time of storage, the fish had deteriorated. The shelf-life of chilled in flack ice rainbow trout prolong from 10 to 15 days when the fish was initially treated by cold electrochemically activated anolyte.

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