

Antioxidant properties of sodium acetate, sodium citrate and sodium lactate on lipid oxidation in rainbow trout (*Onchorhynchus mykiss*) sticks during refrigerated storage (4°C)

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Abstract

This study was carried out to investigate the rate of lipid oxidation in fresh rainbow trout (*Onchorhynchus mykiss*) and in the sticks treated by (2.5% w/v) sodium acetate (NaA), sodium citrate (NaC) and sodium lactate (NaL). The pH value, free fatty acid (FFA), thiobarbituric acid (TBA) and sensory evaluation (odor, flavor and color) were determined on 0, 3, 6, 9 and 12 days of storage. The results showed that TBA and FFA in control were significantly higher than those in the other groups ($P<0.05$). Sticks immersed in NaA indicated a significant difference in formation of free fatty acids in comparison with other sodium salt-treated samples on 9 and 12 days after storage. Sticks dipped in NaL had a maximum level of pH at the end of the storage, whereas samples treated by NaA achieved significantly the lowest value of pH, 9 days after storage. Organoleptic assessments of the samples expressed more acceptability of sticks immersed in sodium salt solutions than the control after 3 days of storage. These indicated that sodium salts, particularly sodium acetate, have antioxidant properties.

Keywords: Rainbow trout, Antioxidant, Sodium salt, Lipid oxidation, Refrigerated storage

Introduction

Fish products like fish sticks elaborated from fatty fish have lately attracted strong interest on fish processing industry and consumers due to their nutritional value, mainly associated to its high content of essential fatty acids, n-3 fatty acids (Puwastien *et al.*, 1999). These polyunsaturated fatty acids (PUFA) were reported to have beneficial effects on human health (Steffens, 1997), including lower blood triglycerides, reduce abnormal heart rhythms, reduce blood pressure and improve blood clotting regulation (Nettton, 1995). They are also considered to be susceptible to peroxide-tion damage (Richards & Hultin, 2002). Fatty fish such as rainbow trout (*Onchorhynchus mykiss*) are particularly sensitive to oxidative changes during storage which cause quality deterioration, lipid hydrolysis and formation of unpleasant odors (Gram *et al.*, 1990; Venugopal & Shahidi, 1996). To minimize the undesirable effects of lipid prooxidation, various strategies are being applied, including the use of refrigeration and addition of antioxidants to fish flesh (Pirini *et al.*, 2000; Scaife *et al.*, 2000). Antioxidative effects of sodium organic salts derived from citric, lactic and acetic acids have already been studied on color and lipid oxidation in n-3 oil fortified ground beef patties (Lee *et al.*, 2005), quality changes of packaged shrimp and catfish fillets (Zhuang *et al.*, 1996), and microbial damage of *Salmon* slices during tray-packaged storage at 1°C (Sallam, 2007). The main evaluation criteria of fresh quality of fish, sensory evaluation, were not assessed in these studies. The aim of the present study is the commercialization of refrigerated sticks of

rainbow trout and as well as the assessment of antioxidative effects of sodium salts on the stability of lipids in rainbow trout sticks during refrigerated storage.

Materials and methods

Rainbow trout was purchased from a local aquaculture farm located near Mahiran Village (Golestan province) with an average length and weight of 30 ± 8 cm and 400 ± 50 g, respectively. Specimens were washed several times in tap water and transported to the laboratory within 2 hours after catch, in boxes containing ice. Fish sticks were prepared using common household practices. First head, skin, tail, fins, and backbone were removed, then gutted and the resulting fillets were cut into 4 sticks (30 ± 5 g each). Sticks were randomly divided into 3 batches (3 kg each). The first and second one were dipped for 10 min in pre-chilled (4°C) aqueous solutions (2.5% w/v) of NaA, NaC and NaL (E Merck, Darmstadt, Germany), respectively, and the last one was immersed in pre-chilled distilled water as a control sample. The ratio of dipping solution to fish was 2.5 to 1. After draining for 5 min on a plastic sieve, the treatments were packaged separately and stored under refrigeration ($4 \pm 1^\circ\text{C}$). When required, the covering polyvinylidene film and Styrofoam trays were replaced. At each sampling occasion (0, 3, 6, 9 and 12 days) samples of each treatment were removed for subsequent chemical and sensory analysis.

The thiobarbituric acid index (TBA-i) was determined according to Natseba *et al.*

(2005) and expressed as mg malonaldehyde per kg fish flesh. 200mg of fish sample was homogenized in a 25ml balloon with 1-bothanol. 5ml of the homogenate was mixed well with 5 ml of TBA agent in a capped test tube. The mixture was heated in water bath (~95°C) for 2 hours and then cooled at room temperature. The color development was measured at 530nm as following where "As" is the absorbance of the sample against a blank sample of distilled water (Ab).

(mg malonaldehyde per kg fish flesh) TBA = $50(As - Ab) / 200$

Free fatty acid (FFA) content was measured as described by Egan *et al* (1997). 150g of comminuted fish sticks were mixed thoroughly with 250ml of chloroform and for 10 min and filtered with Wattman filtration paper. 10ml of the filtered liquid was used for oil sample weight after drying at 105°C. Free fatty acid content was expressed as oleic acid percent by the titration of filtered liquid (2ml) containing 3 droplets of phenolphthalein with 0.1 N NaOH as follows:

(% oleic acid) FFA = $(\text{ml of } 0.1 \text{ N NaOH used in the titration} \times 28.2 \times 100) / (\text{oil sample weight} \times 1000)$.

The pH value was recorded using a pH meter (713 pH meter, Metrohm Herisau Switzerland) and based on the method of Lanier (1992). Fish sample (5g) was homogenized thoroughly with 45ml of distilled water and the homogenate used for pH determination.

Sensory assessments included the evaluation of three parameters (flavor, color and odor) were conducted by five trained

panelists. According to the method of Karmer *et al.* (1966), 5 categories of acceptability were ranked: excellent quality (5), good quality (4), acceptable (3), fair quality (2) and reject (1). Samples of each treatment were taken at regular intervals and the scores given to the judgments were analyzed.

All data were presented as means $\pm$ standard error. The study was conducted as a 4 $\times$ 5 factorial experiment (factor A; NaA, NaC, NaL and control), five levels of storage time (factor B; t₁, t₂, t₃, t₄ and t₅) in three replications. ANOVA was used to search for significant differences among levels of each factor and also for interactions between two treated factors (storage $\times$ time). Data from pH, chemical and sensory measurements were subjected to ANOVA followed by least significant difference test (LSD) using Statistical Analysis System (SAS Institute, Inc., 1995). Also, comparisons between means for levels of each factor within other factor were performed.

Results

The initial pH in rainbow trout sticks ranged from 5.44 to 5.60 (Table 1). A significant increase ($P < 0.05$) was found in all samples except those treated with NaA up to the sixth day of storage (Fig. 1). On day 9, sticks dipped in NaA solution (2.5%) had significantly lower pH value than the control and other treated samples ($P < 0.05$).

The level of thiobarbituric reactive substances in the refrigerated sticks was found to increase significantly ($P < 0.05$)

with storage period (Table 2). By the sixth day, TBARS were above 0.8 for the control, whereas those sticks immersed in NaA aqueous solution did not reached the same level by the 12 days of storage. As shown in Fig. 2, it is deduced that the use of

sodium acetate solution (2.5% w/v) slowly increased the formation of thiobarbituric acid reactive substances in refrigerated rainbow trout sticks, particularly at the end of storage time when compared with NaL-treated samples.

Table 1: Changes in pH value during refrigerated storage under sodium acetate (NaA), sodium citrate (NaC), sodium lactate (NaL) and control (distilled water)

Factors	Storage Time (days)				
	t ₁ =0	t ₂ =3	t ₃ =6	t ₄ =9	t ₅ =12
NaA	5.60±0.006 ^a _D	5.62±0.040 ^{ab} _{CD}	5.71±0.040 ^c _C	5.91±0.018 ^b _B	6.21±0.034 ^b _A
NaC	5.60±0.023 ^a _E	5.70±0.043 ^a _D	5.90±0.010 ^a _C	6.05±0.030 ^a _B	6.25±0.021 ^b _A
NaL	5.45±0.020 ^a _D	5.50±0.060 ^b _D	5.84±0.060 ^{ab} _C	6.03±0.030 ^a _B	6.40±0.012 ^a _A
Control	5.44±0.040 ^a _E	5.60±0.040 ^{ab} _D	5.75±0.006 ^{bc} _C	6.02±0.051 ^a _B	6.19±0.000 ^b _A

Different script letters characterize significant differences in each column (a-c) and in each raw (A-E) for P<0.05.

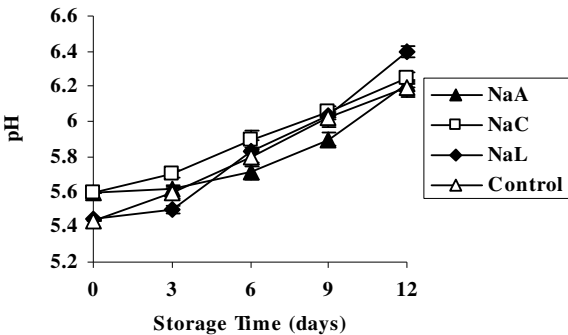
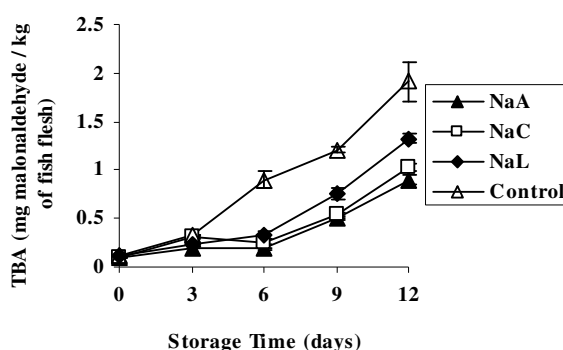


Figure 1: Comparison of pH value in rainbow trout sticks treated with NaA (sodium acetate), NaC (sodium citrate), NaL (sodium lactate) and under control. Bars denote standard error of the mean (n=3).

Table 2: Changes in TBARS value during refrigerated storage under sodium acetate (NaA), sodium citrate (NaC), sodium lactate (NaL) and control (distilled water)

Factors	Storage Time (days)				
	t ₁ =0	t ₂ =3	t ₃ =6	t ₄ =9	t ₅ =12
NaA	0.09±0.005 ^a _C	0.18±0.030 ^a _C	0.20±0.015 ^c _C	0.47±0.050 ^c _B	0.81±0.110 ^c _A
NaC	0.09±0.015 ^a _D	0.31±0.050 ^a _C	0.25±0.030 ^{bc} _C	0.54±0.040 ^{cb} _B	1.02±0.051 ^{bc} _A
NaL	0.11±0.011 ^a _C	0.23±0.035 ^a _C	0.33±0.060 ^b _C	0.75±0.050 ^b _B	1.32±0.152 ^b _A
Control	0.11±0.007 ^a _D	0.32±0.080 ^a _C	0.86±0.031 ^a _C	1.21±0.202 ^a _B	1.91±0.007 ^a _A

Different script letters characterize significant differences in each column (a-c) and in each row (A-D) for P<0.05.

**Figure 2: Comparative evolution of thiobarbituric acid (TBA-i) content in rainbow trout sticks treated with NaA (sodium acetate), NaC (sodium citrate), NaL (sodium lactate) and under control. Bars denote standard error of the mean (n=3).**

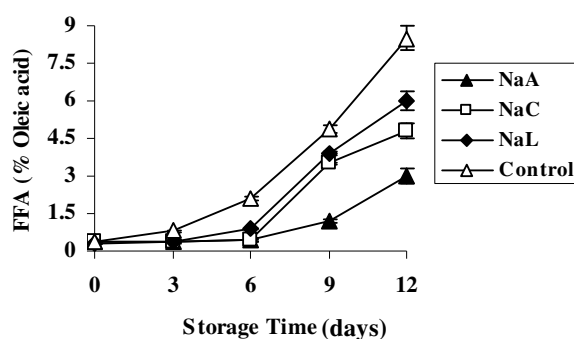
Lipid hydrolysis in rainbow trout sticks occurred during refrigerated storage (Table 3). In both treated sticks with sodium salts and the control, free fatty acids increased over storage time. There were also significant differences (P<0.05) among sodium salt treated samples and the control after 3 days of storage. By the end of storage time (day 12), however, NaA-treated sticks achieved significant (P<0.05) lower FFA value of 3% in comparison with the control and NaL- or NaC-treated sticks which attained a higher levels of 8.48%, 6.01% and 4.9%, respectively (Table 3 & Fig. 3).

Taste rating was conducted on treated rainbow trout sticks throughout 12 day-storage at 4°C (Table 4). As refrigerated storage time lengthened, flavor scores in treated rainbow trout sticks and in the control were significantly decreased (P<0.05). No significant differences (P>0.05) have been observed throughout the first 3 days of storage among different samples. Stored control sticks in refrigeration were considered organo-leptically unacceptable by 6th day of storage (Fig. 4).

Table 3: Changes in FFA content during refrigerated storage under sodium acetate (NaA), sodium citrate (NaC), sodium lactate (NaL) and control (distilled water)

Factors	Storage Time (days)				
	t ₁ =0	t ₂ =3	t ₃ =6	t ₄ =9	t ₅ =12
NaA	0.37±0.027 ^a _C	0.34±0.070 ^b _C	0.43±0.082 ^c _C	1.22±0.300 ^c _B	3.00±0.190 ^c _A
NaC	0.36±0.030 ^a _C	0.37±0.034 ^b _C	0.44±0.031 ^c _C	3.51±0.300 ^b _B	4.90±0.331 ^b _A
NaL	0.33±0.056 ^a _C	0.41±0.016 ^b _C	0.86±0.060 ^b _C	3.90±0.370 ^{ab} _B	6.01±0.200 ^b _A
Control	0.34±0.042 ^a _D	0.80±0.064 ^a _D	2.12±0.145 ^a _C	4.85±0.510 ^a _B	8.48±0.100 ^a _A

Different script letters characterize significant differences in each column (a-c) and in each row (A-D) for P<0.05.

**Figure 3: Comparative evolution of free fatty acid content in rainbow trout sticks treated with NaA (sodium acetate), NaC (sodium citrate), NaL (sodium lactate) and under control. Bars denote standard error of the mean (n=3).****Table 4: Changes in taste score during refrigerated storage under sodium acetate (NaA), sodium citrate (NaC), sodium lactate (NaL) and control (distilled water)**

Factors	Storage Time (days)				
	t ₁ =0	t ₂ =3	t ₃ =6	t ₄ =9	t ₅ =12
NaA	5.00±0.000 ^a _A	4.80±0.200 ^a _A	3.80±0.374 ^a _B	3.20±0.200 ^a _{BC}	2.60±0.245 ^a _C
NaC	5.00±0.000 ^a _A	4.60±0.245 ^a _{AB}	4.00±0.320 ^a _B	2.80±0.245 ^a _C	2.40±0.245 ^a _C
NaL	5.00±0.000 ^a _A	4.40±0.245 ^a _A	3.00±0.320 ^a _B	2.20±0.200 ^b _C	1.40±0.245 ^b _D
Control	5.00±0.000 ^a _A	2.60±0.245 ^b _B	2.00±0.320 ^b _C	1.20±0.200 ^c _D	1.00±0.000 ^b _D

Different script letters characterize significant differences in each column (a-c) and in each row (A-D) for P<0.05.

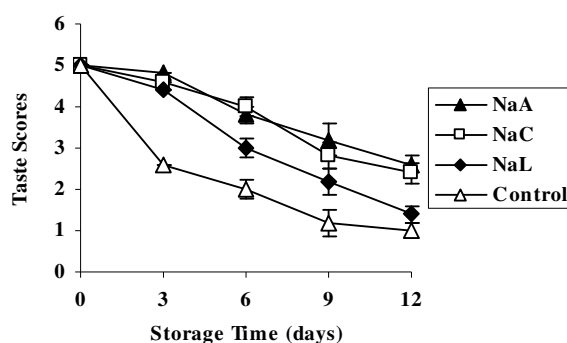


Figure 4: Comparison of taste scores given to rainbow trout sticks treated with NaA (sodium acetate), NaC (sodium citrate), NaL (sodium lactate) and under control. Bars denote standard error of the mean (n=3).

Results indicate a decreasing trend in scores given to odor in all samples during storage (Fig. 5). Sticks immersed in NaC, NaL and distilled water (as the control) solutions, were rated below 3 after 6 and 9 days at 4°C except for those treated with NaA which were rated above 3 even by the 12th day (Table 5).

Changes in the color of refrigerated rainbow trout sticks were also examined

(Table 6). Storage time had a significant effect on the color scores for each of the control and treated samples. The surface of treated sticks, especially those treated with NaA, was not severely discolored and remained acceptable after 12 days storage. Analysis of color scores showed panel preference for sticks dipped in 2.5% NaA and NaC aqueous solutions over refrigerated control and samples treated with NaL.

Table 5: Changes in odor score during refrigerated storage under sodium acetate (NaA), sodium citrate (NaC), sodium lactate (NaL) and control (distilled water)

Factors	Storage Time (days)				
	t ₁ =0	t ₂ =3	t ₃ =6	t ₄ =9	t ₅ =12
NaA	5.00±0.000 ^a _A	4.60±0.245 ^a _A	4.40±0.245 ^a _A	3.60±0.245 ^a _B	3.40±0.245 ^a _B
NaC	5.00±0.000 ^a _A	4.40±0.245 ^{ab} _A	4.20±0.200 ^a _A	3.00±0.320 ^{ab} _B	2.20±0.374 ^b _C
NaL	5.00±0.000 ^a _A	4.20±0.374 ^{ab} _A	2.80±0.374 ^b _B	2.40±0.000 ^b _{BC}	1.60±0.245 ^{bc} _C
Control	5.00±0.000 ^a _A	3.60±0.245 ^b _B	2.40±0.400 ^b _C	1.600±0.245 ^c _D	1.00±0.000 ^c _D

Different script letters characterize significant differences in each column (a-c) and in each row (A-D) for P<0.05.

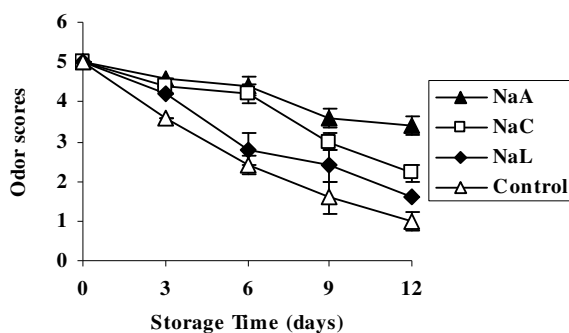


Figure 5: Comparison of odor scores given to rainbow trout sticks treated with NaA (sodium acetate), NaC (sodium citrate), NaL (sodium lactate) and under control. Bars denote standard error of the mean (n=3).

Table 6: Changes in color score during refrigerated storage under sodium acetate (NaA), sodium citrate (NaC), sodium lactate (NaL) and control (distilled water)

Factors	Storage Time (days)				
	t ₁ =0	t ₂ =3	t ₃ =6	t ₄ =9	t ₅ =12
NaA	5.00±0.000 ^a _A	4.80±0.200 ^a _A	3.80±0.200 ^a _B	3.40±0.245 ^a _{BC}	3.00±0.000 ^a _C
NaC	5.00±0.000 ^a _A	4.60±0.245 ^a _A	3.60±0.245 ^a _B	2.40±0.245 ^b _C	2.60±0.245 ^a _C
NaL	5.00±0.000 ^a _A	4.40±0.245 ^a _B	3.20±0.200 ^{ab} _C	2.00±0.245 ^c _D	1.80±0.200 ^b _D
Control	5.00±0.000 ^a _A	3.40±0.245 ^b _B	2.60±0.245 ^b _C	1.60±0.245 ^c _D	1.00±0.000 ^c _E

Different script letters characterize significant differences in each column (a-c) and in each row (A-E) for P<0.05.

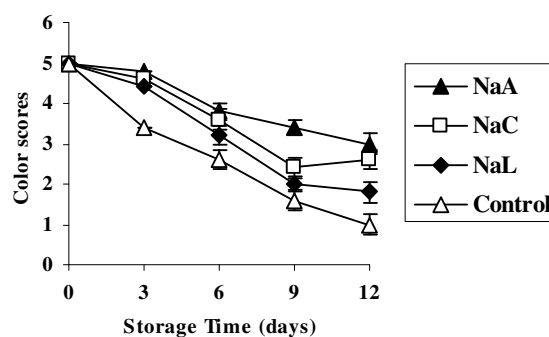


Figure 6: Comparison of color scores given to rainbow trout sticks treated with NaA (sodium acetate), NaC (sodium citrate), NaL (sodium lactate) and under control. Bars denote standard error of the mean (n=3).

Discussion

The effects of aqueous solutions of sodium salts (2.5% w/v) on the shelf-life of refrigerated rainbow trout sticks were studied on the basis of the generation of FFA, TBA, changes in pH and sensory values in this study. During storage the quality of fish degrades due to a complex process in which a number of physical, chemical and microbiological forms of deterioration are implicated (González *et al.*, 2005). Oxidative rancidity is an important organoleptic characteristic for rejection or approval of fish after prolonged storage (Amanatidou *et al.*, 2000).

As showed, by the end of storage, pH values of either sodium salt-treated sticks or the control significantly ($P<0.05$) increased as shown in Fig 1. Increases in pH may be attributed to the production of volatile base compounds by bacterial activity (Cann *et al.*, 1983). Similar observations were made by Al-Sheddy (1999) who reported that pH of camel meat was not significantly ($P<0.05$) affected under sodium acetate treatment (10% w/w) in comparison to sodium lactate (5% v/v of 60% solution) and trisodium citrate (1.5% w/w)-treated samples after 12 days storage at 4°C.

TBARs reactive materials that is generally produced in substantial amounts only from fatty acids containing three or more double bonds (Nawar, 1985), found in relatively high concentrations in Mediterranean fatty fish such as Sardines, Mackerel and Rainbow trout (Simeonidou *et al.*, 1998; Auborge, 2001; Chytiri *et al.*, 2004). In the current study, all sticks treated with sodium salt solutions of organic acids had significantly lower TBA

values after 3-days of storage than the control ($P<0.05$). In contrast, Sallam (2007) demonstrated that dipping sliced salmon in NaL did not produce any significant reduction in the TBA content ($P>0.05$) in the comparison with the control during 15 days storage at 1°C. However, results from previous study revealed a significant lower TBA value for catfish fillets treated with 2% sodium lactate after 2 days and through 8 days at 1°C as compared with the control (Williams *et al.*, 1995). The approximately more increases in TBA content for both of the control and treated sticks in this study indicated that in rainbow trout, which is a fatty fish, lipids are highly susceptible to the autoxidation during refrigerated storage as previous reports proved that (Simeonidou *et al.*, 1998). Although the fish may be acceptable for consumption with lower than 8 mg malonaldehyde/kg (Schormüller, 1969), it has been suggested that values more than maximum levels of TBARs (3-4mg malonaldehyde/kg) indicates lipid oxidation and loss of quality in fish flesh (Taskaya *et al.*, 2003). In the present study, TBA content for both the control and treated sticks of rainbow trout were much lower than proposed limit through the 12-days storage.

Examination of free fatty acids deemed to be important since it has been proved that accumulation of FFA is related to some extent to texture deterioration by interacting with proteins (Mackie, 1993; Sikorski & Kolakowska, 1994) and to lipid oxidation enhancement (Miyashita & Takagi, 1986;

Yoshida *et al.*, 1992). In this experiment, the free fatty acids content evolution was quite similar under both sodium salts treatments and the control up to the sixth day of storage, showing a slight increase with time. Afterwards, a lower FFA formation was observed for fish samples treated with NaA than in their counterparts immersed in NaL, NaC and distilled water as control.

Significant effect of storage time on acceptability scores verified in control and sodium salt-treated (2.5% w/v) rainbow trout sticks after 12-days storage at 4°C. All sensory scores (odor, flavor and color) for rainbow trout samples treated with sodium organic salt solutions and under control treatment showed a decreasing acceptability pattern. In the current study, sticks treated with NaC and NaA solutions had flavor scores that were significantly ($P<0.05$) distinguishable from the control and NaL-treated samples at the end of storage period. Results of color evaluation of the present study confirmed those of Al-Sheddy's reports (1999) about a significant ($P<0.05$) reduction in surface discoloration of camel meat dipped in sodium acetate (10% w/w) when compared to controls and other samples. Mendonca *et al.* (1989) demonstrated that the presence of sodium acetate with acetic acid improved color quality of pork chops than the use of acetic acid alone. Odor change was detected in sticks treated with NaA and the panelists characterized the new odor as mild acetic acid-like even at the first day of storage ($P<0.05$). By the end of storage, only samples treated with NaA solution

remained acceptable and continued to exhibit desirable odor as mild acetic acid-like odor in the present trial. A previous study demonstrated that camel meat treated with sodium acetate (10% w/w) alone or combined with Bifidobacteria (5%) had significantly the least strong off-odors than other samples after 9-days of storage at 4°C (Williams *et al.*, 1995). Based on scores given to sensory attributes, a shelf life of 9 (NaA), 6 (NaC) and 3 (NaL and Control) days were achieved in this experiment. Manju (2007) reported that vacuum-packaging in conjunction with 2% sodium acetate can be safely used to extend the shelf life of Pearlsplit samples up to 15 days at 2°C. Results from previous study proved that a combination of sodium acetate (dip treatment) and Bifidobacteria extended the shelf life of refrigerated catfish fillets.

This study demonstrates that NaA is the best chemical treatment to prolong the shelf-life of stored rainbow trout sticks in refrigeration at 4°C at least by 9 days compared to other treatments. To evaluate the effect of sodium acetate on the sensory quality of fish, further studies such as microbiological analysis is proposed, which has not been investigated in the present experiment.

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خواص آنتی اکسیدانتي استات سدیم، سیترات سدیم ولاکتات سدیم بر اکسیداسیون چربی استیک‌های قزل آلاي رنگین کمان (*Onchorhynchus mykiss*)

طی نگهداری در یخچال (۴ درجه سانتیگراد)

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چکیده

این مطالعه به منظور بررسی نرخ اکسیداسیون چربی در استیک‌های ماهی قزل‌آلای رنگین کمان (*Onchurhynchus mykiss*) و پس از غوطه‌وری در محلول‌های استات سدیم، سیترات سدیم و لاکتات سدیم (۲/۵ درصد) در مقایسه با تیمار شاهد (غوطه‌وری در آب مقطر) صورت گرفت. آزمایشات تعیین pH، FFA، TBA و ارزیابی حسی (بو، طعم و رنگ) در روزهای صفر، ۳، ۶، ۹ و ۱۲ پس از نگهداری انجام شد. نتایج حاکی از آن بود که میزان FFA و TBA نمونه‌های شاهد بطور معنی‌داری بالاتر از سایر تیمارها بود ($P < 0.05$). همچنین شاخص FFA در استیک‌های غوطه‌ور شده در محلول استات سدیم در مقایسه با سایر محلول‌های نمکی اختلاف قابل توجهی (روزهای ۹ و ۱۲) نشان داد ($P < 0.05$). در پایان دوره نگهداری حداکثر میزان pH در نمونه‌های غوطه‌ور در محلول لاکتات سدیم مشاهده شد ($P < 0.05$), در حالی‌که نمونه‌های تیمار استات سدیم در روز نهم ارزیابی بطور معنی‌داری دارای کمترین میزان pH بودند ($P < 0.05$). بررسی‌های ارگانولپیک نمونه‌ها بی‌انگر مقبولیت بیشتر استیک‌های غوطه‌ور در محلولهای نمک سدیم پس از ۳ روز نگهداری نسبت به شاهد بود ($P < 0.05$). با توجه به نتایج بدست آمده، استفاده از نمک‌های سدیم بخصوص استات سدیم در به تعویق انداختن اکسیداسیون چربی و افزایش دوره نگهداری استیک‌های ماهی قزل‌آلای رنگین کمان بعنوان نوعی آنتی اکسیدان توصیه می‌گردد.

کلمات کلیدی: قزل‌آلای رنگین کمان، نمک‌های سدیم، آنتی‌اکسیدانت، اکسیداسیون چربی، نگهداری در یخچال