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EFFECT OF SODIUM ACETATE, CHILLI AND TURMERIC ON THE SHELF LIFE OF REFRIGERATED TILAPIA FILLET

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ABSTRACT

The aim of this study was to determine the effects of turmeric powder, chilli powder and sodium acetate on the shelf life of refrigerated tilapia fillets at 5ºC. Six treatments were applied to the tilapia fillets which were salt (S), salt and turmeric (ST), salt and chilli (SC), salt, turmeric and sodium acetate (STNaA) and salt, chilli and sodium acetate (SCNaA) and control. The samples were analyzed in 15 days period for microbiological, chemical and physical analyses. It was found that there were significant differences ($p<0.05$) in all microbiological analysis of fish treated with STNaA and SCNaA compared to other treatments. Microbiologically, the shelf life of fillets treated with STNaA and SCNaA was 12 days, fillets treated with ST, SC and S were 6 days, and only 3 days for control fillet. In terms of TVB-N and TMA values, ST fillets were still acceptable up to 12 to 15 days of refrigerated storage. As for TBA values, they were in acceptable range for all treatments throughout 15 days storage. The texture quality in decreasing order was given by fillets with SCNaA, SC, STNaA, ST, S and control treatment. In conclusion, the best treatment to extend the shelf life of refrigerated Tilapia fillet was given by STNaA, followed by SCNaA and ST.

Key words: Shelf life, tilapia, turmeric, chilli, acetic acid

INTRODUCTION

Food sources such as fish are rich in proteins, micronutrients, minerals and polyunsaturated fatty acids. Fish provides 16.6 percent of the world's population of animal proteins intake (FAO, 2014). Fish is categorized in highly perishable commodity (Chakravarty *et al*., 2015) which easily spoiled through autolysis, lipolysis and microbial action (Jeyasanta & Patterson, 2014). Excessive production of fish require an appropriate preservation method to prevent wastage and ensure sustainable protein source in the future. There is increasing demand for high quality ready-to-cook fish products with natural preservatives to yield a quality and safe products.

The preservative effect of organic salts with low molecular weight including acetic acid, lactic and citric have been used in controlling microbial growth, acceptance in sensory attributes and prolong the shelf life of food products including meat (Sallam & Samejima, 2004) and fish (Boskou & Debevere, 2000). Effects of organic salt on the shelf life of refrigerated sliced salmon has been reported by Sallam (2006). They found that dipping in 2.5% (w/v) of sodium acetate, sodium lactate and sodium citrate solution for 10 minutes can extend the shelf life of sliced salmon 4-7 days longer than that of control. Ghaly *et al*. (2010) reported that 1000 ppm benzoic acid are set as the limit for marinated or similarly cold-processed packaged fish and meat.

Antimicrobial agents practically used in the aquaculture industry which added in the feed as an additives in preventing fish disease (Chenia, 2015). Yano *et al*. (2006) found that turmeric inhibited the growth of microbial especially *Vibrio para-haemolyticus* in fish at chiller storage, while Khalafalla *et al*. (2015) reported that thyme extract

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can maintain the quality parameters and extend the shelf life of refrigerated Nile tilapia fillets for 9 days longer than control one. In this study, the effects of acetic acid, salt, chilli powder and turmeric powder on the microbiological, chemical and physical quality of refrigerated tilapia fillets was investigated.

MATERIALS AND METHODS

Raw material

Uniform size of freshwater tilapias were obtained from a local farm and immediately transported to the laboratory in ice. The scales were removed, filleted (110 g average weight/fillet), rinsed using water and allowed to drain for 5 minutes at the room temperature. Turmeric powder and chilli powder were prepared in the laboratory. The microbiological substrates used were purchased from Merck Chemicals (Germany). Analytical grade of other chemicals were used in this study.

Preparation and treatments of fish samples

For each replicate, tilapia fillets were divided into six treatment which were control (CO), salt (S), salt and chilli powdered (SC), salt and turmeric powdered (ST), chilli powdered, salt and sodium acetate (dipped) (SCNaA), and contain turmeric powdered, salt and sodium acetate (dipped) (STNaA). For each replicate of treatment, 18 packs of marinated tilapia fish were prepared. Each pack contained a piece of tilapia fillet weighed 110 g. At each time interval, 3 packs of tilapia fillets were sampled for microbiological, chemical and physical analysis.

Treatment with sodium acetate (prior to marination with salt and spices)

Treatment with sodium acetate was carried out using dipping method. Tilapia fillets were dipped into pre-chilled sodium acetate (2.5%, w/v) for 10 min. Dipping in sodium acetate was carried out prior to marination with salt and spices. The ratio of fish fillet to dipping solution was 1 to 2.5% (w/v). Fish was drained for 5 min after dipping at room temperature on sterile stainless steel wire mesh screen.

Treatment with spices

The marinating process was performed in spice solution in addition of equal amount of salt (NaCl). The ratio of salt and spices powdered was 1: 1. The amount of the spices and salt was used was 1% of tilapia fillets. For each sampling interval, two tilapia fillets were placed in the Styrofoam tray and covered with cling wrap film. The packaged tilapia fillets was subsequently labeled and stored at 5ºC. Packaged tilapia fillets were sampled for analysis at storage days 0, 3, 6, 9, 12, and 15. Tilapia fillets without any treatment were considered as a control samples.

Microbiological analysis

Plate Count Agar was used to determine aerobic plate count and psychotrophic, *Pseudomanas* agar to determine its count. All microbiological analyses were carried out according to Sallam (2006).

Texture Analysis

Texture Analyzer Machine (TA XT 2 Plus, USA) was used to analyze the firmness of the fillets. To check the firmness, a Spherical Probe which known as 5 mm Spherical Probe P/5S using 5 kg load cell was used. Four locations were measured for each tilapia fillets.

Measurement of TBA value

The 2-thiobarbituric acid (TBA) was determined according to the procedure by Schmedes and Holmer (1989). TBA value was calculated in mg malonaldehyde per kg of fish.

Total volatile base nitrogen (TVBN) and trimethylamine (TMA)

Determination of TVBN and TMA were determined using the modified method and the same experimental procedure of TVBN was used for the TMA measurement (Malle & Poumeyrol, 1989). The only difference is the addition of 20 mL of 35% (v/v) formaldehyde to the distillation tube to block the primary and secondary amines, whilst leaving only the tertiary amines to react. The amount of TVBN and TMA were calculated from the volume of 0.05 M H_2SO_4 acid used for titration and the results were expressed in mg nitrogen/100g of sample.

Statistical analysis

All experimental were carried out in triplicate. All data were stated as mean ± standard deviation. Data were subjected to analysis of two way variance (ANOVA) using statistical analysis system software of SPSS. Differences among the mean values of the different treatments were determined using Least Significant Difference (LSD), and the significant difference was defined at $p<0.05$.

RESULTS AND DISCUSSION

Aerobic plate counts

Figure 1a shows the effects of different treatments on aerobic plate counts (APC) of tilapia fillets during storage at 5ºC. As expected, APC

increased with storage time and there was a significant difference in APC count between all treatments at the same time interval. The variation in initial bacterial count could be due to differences in microbial quality of the whole tilapia purchased as well as antimicrobial effect of the spices and/or salt applied on tilapia fillets. By day- 3, APCs in tilapia fillets for all treatments were still below 6 $\log_{10}$ CFU/g, except for control sample which reached 7.35, which is above the maximum recommended limit of 7 $\log_{10}$ CFU/g for aerobic condition in raw fish (ICMSF, 1986), indicating a microbiological shelf life of about 6 days for the non-treated control samples. This finding is in agreement with Amanatidou *et al*. (2001) and Khalafalla et al. (2015) whom reported a commercial shelf life is usually limited to only 1 week when fish is stored between 2 and 8ºC without any treatment. Commonly, the number of total spoilage bacteria for fish products which is stored aerobically about 7–9 $\log_{10}$ CFU/g. Nevertheless, the acceptability index used much lower for total microbial counts in guidelines, specifications and standards setting (Ólafsdóttir *et al*., 1997).

By day-6 of storage, only APCs in tilapia fillets for STNaA and SCNaA were still below 7 $\log_{10}$ CFU/g. This shows that dipping of tilapia fillets treated with STNaA and SCNaA significantly delayed the microbial growth and extended the shelf life of the product up to 12 days, respectively and the tilapia fillets treated with sodium acetate revealed significantly lower APC (7.25 $\log_{10}$ CFU/g (STNaA) and 7.36 $\log_{10}$ CFU/g (SCNaA), respectively in comparison to that of control (11.54 ± 4.41 $\log_{10}$ CFU/g) (Figure 1a). This study found that the shelf life of tilapia treated with STNaA and SCNaA increased up to 12 days compared to other treatments that only lasted for 3-6 days.

***Pseudomonas* bacteria count**

Figure 1b shows a significant difference between all the treatments and time intervals (except for day-15). *Pseudomonas* counts were slightly lower than the APCs, indicating the importance of these species in the spoilage of tilapia fillets examined. The initial *Pseudomonas* count ($\log_{10}$ CFU/g) in this study ranged from 2.34 ± 0.06 in S to 5.03 ± 0.02 in control samples. According to Gram & Dalgaard (2002), respiratory Gram-negative bacteria are typically inhibited in fish products preserved by the addition of low levels of NaCl.

By day 15 of storage period, *Pseudomonas* counts in all treatments were in excess of 2-3-logs lower than that of control fillets. In this result, only treatment of STNaA was able to lengthen the shelf life of refrigerated tilapia fillets up to 12 days and the rest of tilapia treatments were spoiled at 9 days compared to that of control at 6 days of refrigerated storage.

Psychotropic bacteria count (PTC)

Figure 1c shows a significant difference between all the treatments and all time intervals for PTC. By day-9 and day-12, PTCs in tilapia fillets for STNaA and SCNaA were 7 $\log_{10}$ CFU/g, while other treatment gave higher PTCs. In this study, with treatment STNaA has extended the shelf life of refrigerated tilapia fillets up to 12 days, while treatment with SCNaA, ST and SC has increased the shelf life of refrigerated tilapia fillets up to 9 days. Fonseca *et al*. (2013) found that Nile tilapia fillets treated with sodium chloride shown 4.57 log CFU/g of *Staphylococcus* sp. at eighth day storage.

Total volatile base nitrogen

Figure 2a shows there was no significant difference in TVBN values between all treatments. The TVBN may be considered as a quality index for fish. TVBN increase is correlated to the activity of spoilage bacteria and endogenous enzymes (Ozogul *et al*., 2004). The initial day of storage value was range of 8.11–11.38 mg N/100 g for all treatment and 12.31 in control sample. It was similar or lower than the values reported for fresh sea bream by other authors: 20 mg N/100 g after 1 day of storage at 2 ± 1ºC (Tejada & Huidobro, 2002).

TVBN values of the ST, SC, S and control samples reached the value range of 50.39 ± 0.19 – 87.00 ± 0.01 mg N/100 g after 10 days of storage, exceeding the upper acceptability limit set by the EU for TVB-N values of fish (35 mg N/100 g of fish flesh) after 15–16 days of storage. The significantly (p<0.05) lower TVBN values of two treatments of tilapia fillets (STNaA and SCNaA) containing chilli powder, turmeric powder and sodium acetate may be attributed to the antibacterial properties of the spices.

Trimethylamine

Figure 2b shows there was no significant difference in TMA values between CO, STNaA and SCNaA treatments. TMA in marine fish in the form of trimethylamine oxide (TMAO) which derived from bacterial enzyme activity is contributed to unpleasant “fishy” odour (Sivertsvik *et al*., 2002). The initial TMA content of tilapia fillets was in the low range of 0.42 ± 0.04 -0.59 ± 0.17 mg N/100 g of flesh while the control was 0.67 ± 0.01 mg N/100 g of flesh indicating the freshness of the samples as reported by Tejada & Huidobro (2002) for the initial TMA content of sea bream and fresh cod normally has less than 3 mg N/l00 g TMA.

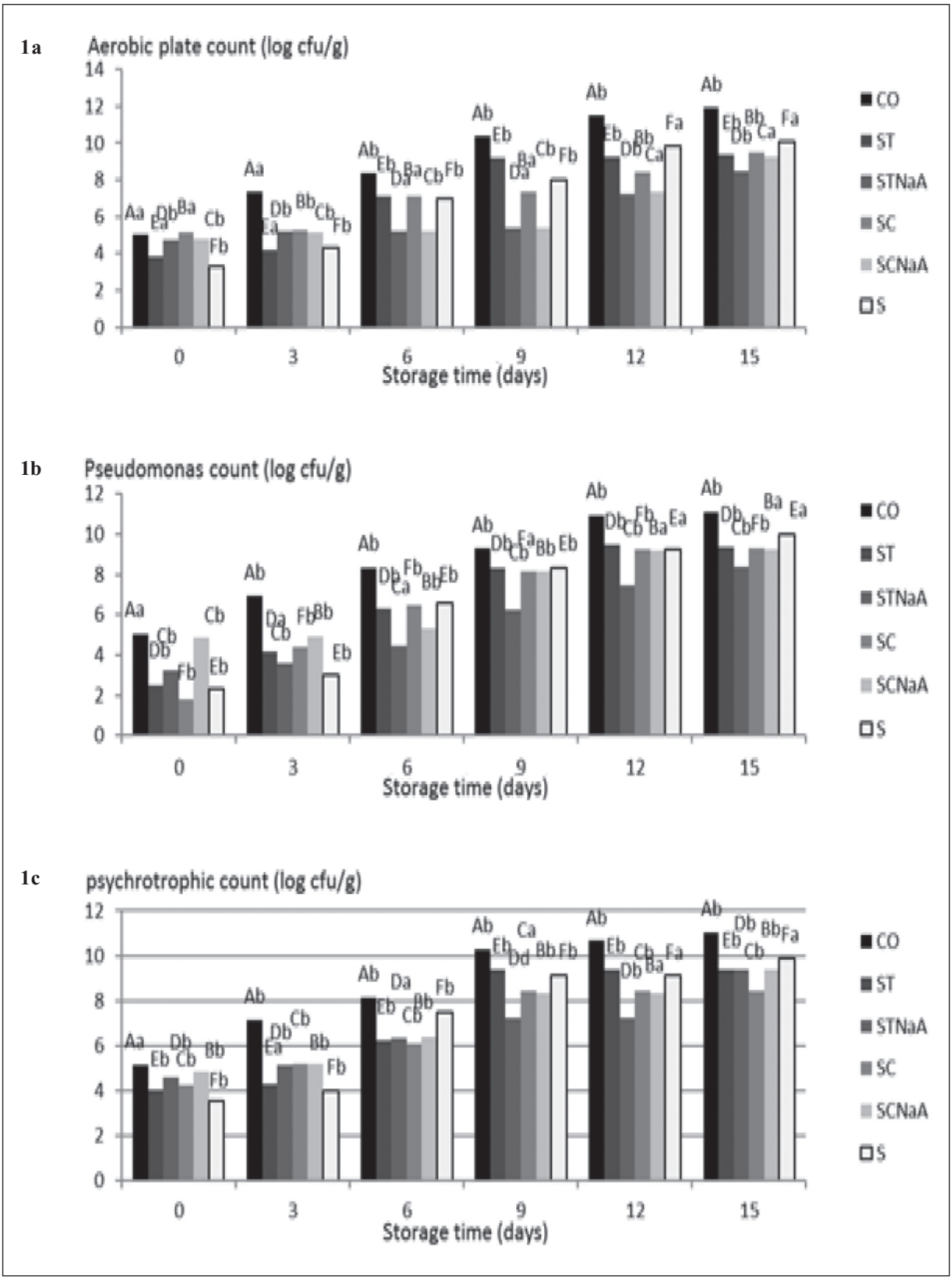

Fig. 1a-1c. Aerobic count, *Pseudomonas* count and psychtrophic count of marinated tilapia fillets during 15 days of refrigerated storage. The capital letters indicate significant difference between means of samples using different marination treatments at the same storage time interval, while the small letters indicate significant differences between the means of samples at different storage time intervals for the same marination treatment.

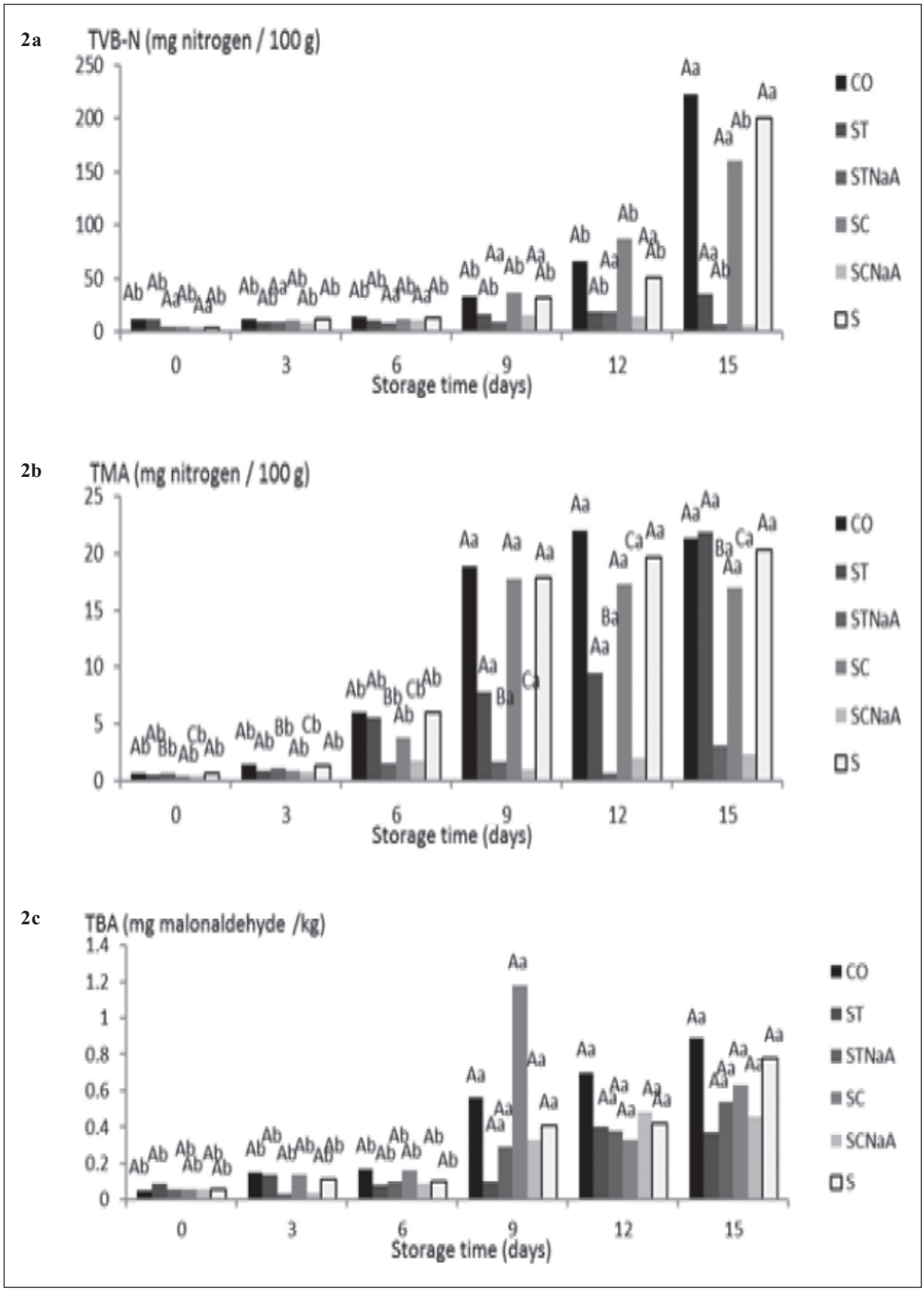

Fig. 2a-2c. TVB-N, TMA and TBA of marinated tilapia fillets during 15 days of refrigerated storage. The capital letters indicate significant difference between means of samples using different marination treatments at the same storage time interval, while the small letters indicate significant differences between the means of samples at different storage time intervals for the same marination treatment.

Day-9 onwards, however, the TMA value of control fish samples increased gradually, attaining a final value of 21.28 ± 0.60 mg/100 g flesh by the end of the storage period (day 15), whereas significantly (p<0.05) lower values of 3.10 ± 0.04 and 2.35 ± 0.07 mg/100 g flesh were detected for tilapia fillets STNaA and SCNaA where the fillets were dipped in NaA respectively.

Thiobarbituric acid

Figure 2c shows the effect of different treatments on lipid oxidation (TBA value) of tilapia fillets during storage at 5ºC. This figure shows there was no significant difference in TBA values between all treatments. Arannilewa *et al*. (2005) reported that the fat content in fresh samples of tilapia fish was 9.72 ± 0.25%. TBA value is an index of lipid oxidation by measuring malonaldehyde (MDA) content.

TBA values of the control and treated tilapia fillets were significantly increased (p<0.05) with the storage time. By the end of the storage period (day 15), ST, SC and SCNaA samples achieved significant (p<0.05) different comparison TBA value compared to that of control. TBA values of control and sodium lactate (2%) treated catfish fillets showed a significant effect on storage time especially after 8-days storage at 1ºC (Williams *et al*., 1995). Kapoor & Priyadarsini, (2001) also was reported that curcumin is a powerful antioxidant to repair both oxidative and reductive damage caused to proteins by radiation and antioxidant mechanism of curcumin. From the result, all the treatment showed a low value of malonaldehyde in fish (below than 1.0 mg malonaldehyde/kg). This result shows that in terms of TBA values, all tilapia fillet samples were acceptable up to 15 days.

Texture analysis

Figure 3 shows no significant differences in firmness of refrigerated tilapia fish between all treatments. However, there were significant differences in firmness of refrigerated tilapia fish between different time intervals. Texture can also be used to predict the fillet freshness (Lawless & Heymann, 1998). The initial value of texture was not significantly different between all treatments. On day-9 of storage, the firmness of tilapia fillets decreased progressively. However, on day-15, the significant difference (p<0.05) was only detected between STNaA and SCNaA with the control samples. The results of firmness indicated that the softening of the samples occurs with the storage time. The shelf life of refrigerated tilapia fillet in terms of texture in decreasing order was given by SCNaA, SC, STNaA, ST, S and CO. According to *He et al*. (2016), protein denaturation was effect the tilapia fillets which is contributed to the acceptance of quality in sensory evaluation.

CONCLUSION

The microbiological quality of tilapia fillets were differ depending on the treatment given. The shelf life of samples treated with STNaA and SCNaA was 12 days, whereas treated with ST, SC and S were 6 days compared to the control was 3 days. In terms

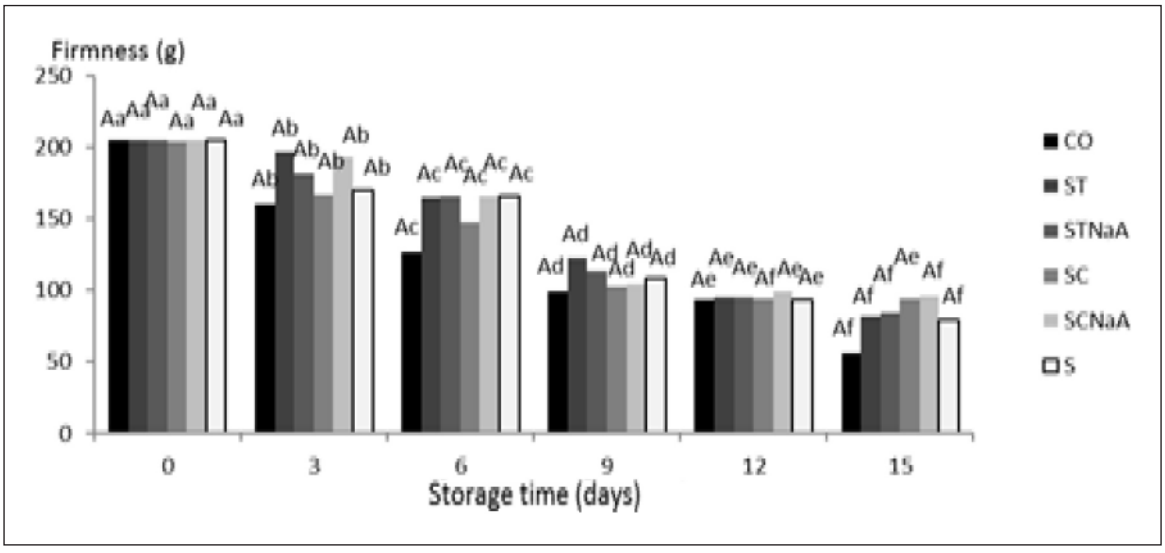

Fig. 3. Firmness of marinated tilapia fillets during 15 days of refrigerated storage. The capital letters indicate significant difference between means of samples using different marination treatments at the same storage time interval, while the small letters indicate significant differences between the means of samples at different storage time intervals for the same marination treatment.

of chemical quality, the study found that treatment with STNaA and SCNaA had prolonged and sustained the shelf life of refrigerated tilapia fillets up to 15 days. On the basis of the chemical and physical analyses in decreasing order, the best treatment of for refrigerated tilapia fillets stored at 5ºC was marinated with salts, turmeric powdered and sodium acetate (2.5% dipping), (STNaA) > marinated with salts, marinated with salt and turmeric powdered (ST) > chilli powdered and sodium acetate (dipping 2.5%), (SCNaA) > marinated with salt and chilli powdered (SC) > marinated tilapia fillets with salts (S) only > control. Generally, the findings of this study will be benefited to the public as a source of fish can be sustained optimally.

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