# **Research Article**



Effects of ascorbic acid and sodium citrate treatments on the sensory quality and lipid stability of fresh snakehead fish (*Channa striata*) fillets during 14 days chilled storage at 2-4°C

Chawafambira T.A.<sup>1</sup>; Dang H.T.T.<sup>1</sup>; Nguyen D.T.<sup>2</sup>; Nguyen M.V.<sup>3</sup>; Nguyen M.V.<sup>1\*</sup>

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#### **Abstract**

Snakehead fish (Channa striata) is one of the most common and important freshwater species in tropical Asia. Lipid oxidation is one of the major deteriorative reactions taking place in fish muscle during processing and storage, affecting the physicochemical properties and limiting the shelf-life of fishery products. The objective of this study was to investigate the effects of antioxidants on lipid stability, sensory quality and physicochemical properties of snakehead fish fillets during chilled storage. The results stipulated that ascorbic acid and sodium citrate treatments significantly interrupted lipid hydrolysis and lipid oxidation progression in the fish muscle, emanating in lower FFA, PV and TBARS values and higher PL content obtained in the treated samples. The samples treated with ascorbic acid and sodium citrate had significantly higher whiteness values and lower yellowish  $(b^*)$  values compared to the untreated samples throughout the storage period. The development of lipid oxidation was in high correlations with sensory quality (QI and Torry scores). Based on the QIM and Torry scores, the shelf lives of the untreated, 0.80% sodium citrate treated, 0.25% ascorbic acid-treated and 0.50% ascorbic acid-treated samples were of 10 days, 11 days, 13 days and 14 days, respectively.

**Keywords**: Snakehead fish, Antioxidant, Lipid oxidation, Sensory quality, Chilled storage

<sup>1-</sup>Faculty of Food Technology, Nha Trang University, 02 Nguyen Dinh Chieu, Nha Trang, Khanh Hoa, Vietnam

<sup>2-</sup>Faculty of Chemical and Food Technology, HCMC University of Technology and Education, 01 Vo Van Ngan, Thu Duc district, Ho Chi Minh city, Vietnam

<sup>3-</sup>College of Agriculture, Can Tho University, University Campus II, 3/2 street, Ninh Kieu district, Can Tho city, Vietnam

<sup>\*</sup>Corresponding author's Email: minhnv@ntu.edu.vn

#### Introduction

Snakehead fish (Channa striata) is a species of freshwater fish of the family Chanidae, native to South and South-East Asia. In Vietnam, it's locally called "Cá lóc". It's one of the most common and important freshwater fish species in Asia that is considered tropical delicious. Globally, it provides about 6 percent of dietary protein in our diet in comparison to animal protein intake (Waite et al., 2014). Besides being a protein source, fish is also a source of long-chain polyunsaturated fatty acids (PUFAs, both  $\omega$ -3 and  $\omega$ -6), such as linolenic acid (LA), y-linolenic acid (GLA), α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are associated with a wide range of human health benefits (Abedi and Sahari. 2014).

Lipid oxidation is one of the major deteriorative reactions taking place in fish muscle during processing and storage, shortening the shelf-life of fishery products. Lipid oxidation is dependent on different factors, such as the lipid quantity present, the degree of unsaturated fatty acids in the muscle, the packaging method, and conditions during processing and storage (Nguyen et al., 2012; Taheri et al., 2012). Since PUFAs are highly prone to oxidation, the lipid fraction is an issue of a great deal of attention (Pérez-Alonso et al., 2003). Reactions between the byproducts derived from lipid oxidation and proteins cause undesirable changes in food properties including protein denaturation, loss of protein solubility,

alteration of texture and functional properties of the protein, and destruction of nutrient components (Serdaroğlu and Felekoğlu, 2005). It is very important to develop efficient approaches to reduce lipid and protein oxidation to maximize the use of Snakehead fish (*Channa striata*) resources.

The addition of antioxidants is a valid method to prevent/minimize lipid and protein oxidation. Lipid degradation in foods can be controlled by the use of synthetic antioxidants such as butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT), and tertiary butylated hydroquinone (TBHQ) (Hettiarachchy et al., 1996). Reports have shown that synthetic antioxidants may act as mutagenic and carcinogenic agents, while natural ones may provide nutritional and therapeutic effects. Recent efforts have been focused on the replacement of synthetic antioxidants with natural ones. Natural antioxidants have been successfully employed with filleted fish (Aubourg, 2000).

Amid natural antioxidants, ascorbic acid (AA) is known to have a strong antioxidative effect, acting as a metal chelator and oxygen scavenger agent and playing a reducing role. It is an organic acid with low molecular weight, which is easily available, has a low commercial cost and has a wide range of permitted concentrations for its use thus making it a good choice (Santos-Sánchez, 2019). A positive effect on increased shelf-life has been described for marine oils (Hamilton *et al.*, 1998), minced fish (Hwang and Regenstein, 1989), fish fillets (Kilinc *et al.*, 2009)

and whole fish (Aubourg et al., 2004). Sodium citrate (Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>) is the sodium salt of citric acid. Sodium citrate is also used as an antioxidant in food as well as to improve the effects of other antioxidants. It is also used as an acidity regulator and sequestrant. A sequestrant forms chelate with complexes with polyvalent metal ions especially copper, iron, and nickel, which can prevent the oxidation of the fats in the food (Lindsay, 2017). The present study focused on the retention of the freshness of the Channa striata fillets when commercialised as a chilled product. This research will contribute to preserve fish fillets and unveil the effects of ascorbic acid treatments and sodium citrate on the product quality of fresh fish fillets during chilled storage.

#### Material and methods

#### Materials

All snakehead fish samples used for this research were bought from the local fish farm in Nha Trang city, Khanh Hoa province. The average weight of the snakehead fish was 700-800 g. The fish were transported to the labs alive in water containers.

#### Chemicals

All pure and analytical grade chemicals (Sigma-Aldrich, USA) were purchased from Asia Laboratory Instruments Company Limited, 594/23 Au Co Street, Tan Binh District, Ho Chi Minh City, Vietnam.

Sample preparation and sampling

Fish were rested for 2 h before bleeding and filleting in following the procedure described in Nguyen et al. (2021a). The fillets were divided into four groups (35 fillets in each group): control group (Control), 0.25% ascorbic acid-treated group (0.25% AA), 0.50% ascorbic treated group (0.50% AA), and 0.80% sodium citrate treated group (0.80% SC). For antioxidant treatments, fish fillets were immersed in the ascorbic acid and sodium citrate solutions at a ratio of 1:1.3 (fish: solution, w/w) for 10 minutes. After that, fish fillets were drained for 5 minutes on a plastic grid to remove excess water. Fish fillets of each group were packaged individually in a Styrofoam tray covered by plastic film. Samples were stored in a refrigerator at 2-4°C for 14 days, the temperature was controlled by using a temperature controller (Conotec FOX-1004, Korea).

At each sampling point (0, 3, 6, 8, 10, 12 and 14 days of storage), five fillets were randomly taken for determinations of colour, cooking yield, share strength, lipid degradation and soluble protein contents and sensory evaluations (QIM and Torry). All measurements were done in three replications.

Sensory analysis using QIM scheme

Sensory analyses were carried out by five panellists from the Faculty of Food Technology of the Nha Trang University. The ages of the panellists ranged from 30-35 years and consisted of three females and two males. These panellists were selected for their expertise in the descriptive analysis of

food sensory parameters. Before the main evaluation, several training sessions were conducted to train the panellists on how to use the QIM and Torry Schemes developed for freshness analysis of snakehead fish fillets (Nguyen *et al.*, 2021b).

# Evaluation of cooked snakehead fillets with Torry scheme

For the analysis of the odour and flavour of the SHF fillets, six slices (2×6 cm) were cut from two fillets, wrapped in aluminium foil paper, placed in a perforated stainless-steel pan, steam-cooked for 10 minutes at 95-100°C. After cooking, the samples were blind coded with a 3-digit random number and served to the panellists for evaluation and grading using Torry Scheme developed by Shewan et al. (1953) with some modifications made by Martinsdottir et al. (2001) for medium fatty fish.

## Colour measurement

The intensity of the flesh side colour was measured by using the Minolta CR-400 chromameter (Minolta Camera Co., Ltd; Osaka, Japan) in Lab\* system (CIE, 1976) with CIE Illuminant C as described in Nguyen  $et\ al.\ (2011)$ . Three positions of the fillets (n=3) were measured. The average  $L^*$ ,  $a^*$ , and  $b^*$  value of three measurements for each fillet was used to calculate the mean and standard deviation for each group. The whiteness was calculated using followed equation (Park, 1994):

Whiteness =  $L^* - 3 \times b^*$ 

# Total lipid determination

Lipids of fish muscle were extracted from a sample with methanol/chloroform/0.88% KCl (at 1/1/0.5, v/v/v) according to the Bligh and Dyer (1959) method. The lipid content was determined gravimetrically after evaporation of all chloroform and the results were expressed as a percentage of the wet weight samples.

# Lipid hydrolysis determinations

Free fatty acid (FFA) content was determined on lipid extract according to the method of Bernardez *et al.* (2005), based on complex formation with cupric acetate-pyridine, followed by absorbance reading at 715 nm (Libra S50 UV/VIS spectrophotometer, Biochrom, UK). The results were expressed as grams FFA/100g of lipid using a standard curve prepared from oleic acid.

Phospholipid content of the fish muscle was determined according to the method of Stewart (1980), based on the complex formation of phospholipid with ammonium ferrothiocyanate, followed by absorbance reading at 488 nm (Libra S50 UV/VIS spectrophotometer, Biochrom, UK). The results were expressed as a percentage of total lipid content and calculated using a standard curve prepared from phosphatidylcholine.

## Lipid oxidation measurements

Lipid hydroperoxide was determined by the ferric thiocyanate method of Shantha and Decker (1994). The results were expressed as  $\mu$ mol lipid hydroperoxides per g of sample ( $\mu$ M CPO/g).

Thiobarbituric acid-reactive substances (TBARS) was determined according to the method of Lemon (1975) with adjustments described by Nguyen and Phan (2018). The results expressed as µmol malondialdehyde per kg (µM MDA/kg) were calculated using a standard curve constructed from MDA equivalence in tetraethoxypropane (TEP).

## Fatty acid profile analysis

The fatty acid composition of the samples was determined by gas chromatography (Shimadzu, Tokyo, Japan) following derivatization of extracted lipids to fatty acid methyl esters (FAME), according to the AOCS (1998). The programme was based on AOAC (2000). The result of each fatty acid was expressed as g per 100 g TL.

## Statistical Analysis

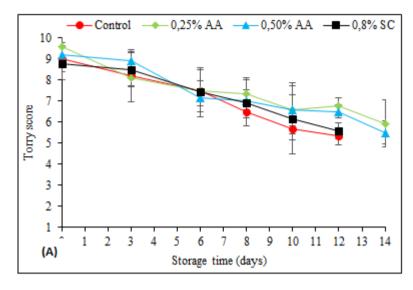
Microsoft Excel 2021 was used to generate graphs and tables (Microsoft Corporation, USA). The results were analysed using one-way ANOVA and the mean was statistically evaluated using Duncan's multiple range test (DMRT) to obtain the conservative differences with multiple comparisons with the level of significance set at P<0.05. All the statistical analyses were carried out using the SPSS (version 26) software (SPSS Inc., Chicago, Illinois). results were presented means±SD. A multivariate analysis on weighted principal component analysis (PCA) was performed on all data

obtained using XLSTAT (ADDINSOFT, New York, USA). PCA was performed to detect the most important factors of variability and to describe the relationship between variables and observations. All data were mean centred and scaled to equal unit variance before PCA.

#### **Results**

Sensory evaluation

The results of assessing the quality change of cooked snakehead fish of Torry scores are shown in Figure 1A. Torry scores of snakehead fish fillet samples decreased with an increase in storage time ( $r^2=-0.950$ ). The following changes were noted: 9.00, 9.60, 9.20, and 8.75 at day 0 and 5.67, 6.57, 6.58 and 6.17 at day 10, for control, 0.25% AA. 0.50% AA and 0.80% respectively. No significant difference (p>0.05) between treatment groups was detected. The Torry score limit for fish fillets suitable for human consumption is  $\geq$  5.5 (Martinsdottir *et al.*, 2001). Based on the Torry score limit point, the shelflives of untreated (control), 0.80% SC, 0.50% AA and 0.25% AA samples were of 10, 11, 13 and 14 days, respectively. At the rejected storage date, the characteristic smell of fish muscle was a slight alcohol smell, the colour of the fish muscle was uneven, turning pale pink, the surface of the fish muscle appears to be sticky meat. Torry scores had significant correlations of  $r^2=-0.898$ , -0.707, 0.806, -0.799 and 0.725 with QI, FFA, PL, and PV, respectively. From these results, it is likely to be affected by lipid hydrolysis and lipid oxidation.



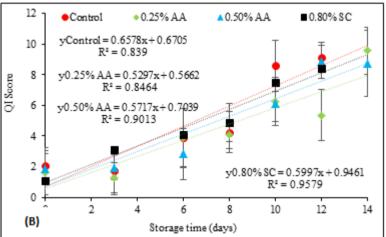


Figure 1: Changes in Torry score (A) and QI score (B) of snakehead fish fillets during chilled storage as functions antioxidant treatments [Control (•), 0.25% ascorbic acid treated (♦), 0.50% ascorbic acid treated (▲) and 0.80% sodium citrate treated (■)].

Changes in QI score of snakehead fish fillets at chilled storage at 2-4°C are shown in Figure 1B. The QI scores increased with storage time ( $r^2$ =0.907) for all samples indicating that attributes gradually deteriorated with time and obeyed the QIM scheme. This was supported by a significant positive correlation ( $r^2$ =0.907) between QI and storage time and can be evidenced by Figure 1B. The following correlations were noted,  $r^2$ =0.839, 0.8464, 0.9013,

and 0.9579 for untreated (control), 0.25% AA, 0.50% AA, and 0.80% SC, respectively throughout the storage time. The obtained regression equations were completely suitable to evaluate the freshness and determine the remaining shelf-life of the following, untreated sample, ascorbic acid treatments (i.e., 0.25% AA, 0.50% AA) and sodium citrate treatment (0.80% SC) samples of snakehead fish fillets during chilled stored at 2-4°C. QI also exhibited a

negative correlation ( $r^2$ =-0.704) with PL. It can be deduced from the correlation values that QI scores are likely to be affected by lipid hydrolysis and lipid oxidation.

Changes in the colour of snakehead fish fillets

Changes in whiteness,  $b^*$  (yellowness) and  $a^*$  (redness) values of the flesh side of snakehead fish fillets are shown in Fig. 2A, 2B and 2C, respectively. At day 0 of storage, the following whiteness values were recorded of 53.14, 53.37, 54.71 and 54.18, and 43.98, 49.84, 45.00 and 51.24 on day 10 for the control, 0.25% AA, 0.50% AA and 0.80% SC samples, respectively (Fig. 2A). There were significant differences (p < 0.05) between the sodium citrate treatment (0.80% SC) and ascorbic acid treatments (i.e., 0.25% AA and 0.50% AA) and untreated sample (control). The sodium citrate treated samples (0.80% SC) had higher whiteness values compared to all other treatments throughout the storage period. A strong negative correlation  $(r^2=-0.970)$  existed between whiteness and the  $b^*$  value, which can be depicted in Figure 2A and Figure 2B.

The  $b^*$  (– blue or + yellow) values were as follows -2.04, -1.77, -1.95 and -2.08 on day 0 and 0.92, -0.84, 0.33, and -1.45 on day 12 for control, 025% AA, 0.50% AA, and 0.80% SC treatments respectively (Fig. 2B). There were significant differences (p<0.05) between sodium citrate treatment (0.80% SC) and ascorbic acid treatments (i.e., 0.25% AA and 0.50% AA) and untreated (control)

treatment. The samples treated with 0.80% SC had the lowest  $b^*$  values throughout the storage time compared to those of other samples (Fig. 2B). The treated samples had significantly higher whiteness values and lower yellowish  $(b^*)$  values compared to the control samples. The  $b^*$  values were negatively correlated to PL content ( $r^2 = -0.537$ ). revealing that a decrease in PL corresponded to an increase in  $b^*$  value. The colour values of redness  $(a^*)$  are shown in Figure 2C. A positive value is red colour and a negative value is green. Generally, the  $a^*$  values of all samples decreased with increased storage time. There was no significant difference (p>0.05) between groups.

## Changes in lipid content

Changes in total lipid (TL) content of fresh snakehead fish fillets during chilled (2-4°C) storage are shown in Table 1. A general decrease in lipid content was observed with increased storage time ( $r^2=-0.639$ ) for all samples. The treated samples (0.25% AA, 0.50% AA and 0.80% SC) tended to have higher TL values than the control samples, but no significant difference (p>0.05) between groups was observed. A negative correlation  $(r^2=-0.840)$ existed between TL and FFA. A positive correlation (r<sup>2</sup>=0.744) between TL and PL denotes an increase in TL proceeds to an increase in PL (Fig. 3B). Lipid oxidation affects TL, this is supported by a correlation (r<sup>2</sup>=-0.699) between TL and PV obtained in this study.

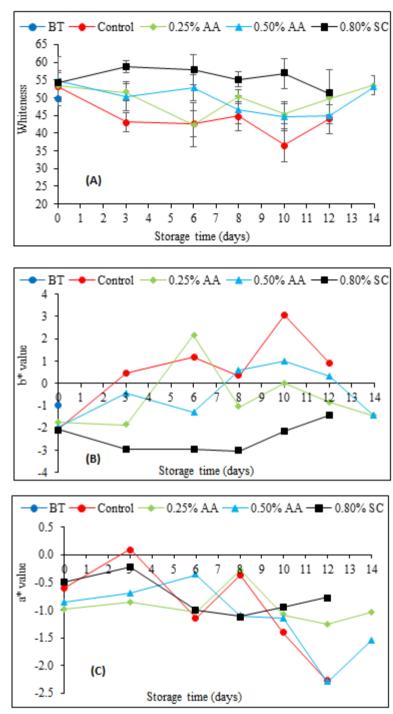


Figure 2: Changes in whiteness (A), b\* value (B) and a\* value (C) of snakehead fish fillets during chilled storage as functions antioxidant treatments [BT: before treatment, Control (•), 0.25% ascorbic acid treated (◆), 0.50% ascorbic acid treated (▲) and 0.80% sodium citrate treated (■)].

Table 1: Changes in total lipid (TL) content of snakehead muscle during 14 days chilled storage at 2-4°C as functions of antioxidant treatments.

Treatments	Storage time (days)							
	0	3	6	8	10	12	14	
Control	2.74±0.05 <sup>a</sup>	2.70±0.13 <sup>a</sup>	2.71±0.23 <sup>a</sup>	2.63±0.05a	2.63±0.06 <sup>a</sup>	2.43±0.18°		
0.25% AA	$2.75\pm0.02^{a}$	$2.83\pm0.16^{a}$	$2.70\pm0.06^{a}$	$2.78\pm0.09^{a}$	$2.70\pm0.16^{a}$	$2.74\pm0.08^{a}$	$2.59\pm0.13^a$	
0.50% AA	$2.77\pm0.22^{a}$	$2.85\pm0.09^{a}$	$2.59\pm0.09^{b}$	$2.73\pm0.05^{a}$	$2.69\pm0.14^{a}$	$2.59\pm0.10^{b}$	$2.51\pm0.09^a$	
0.80% SC	$2.74\pm0.09^{a}$	2.82±0.31a	$2.65\pm0.04^{ab}$	$2.69\pm0.23^{a}$	2.62±0.01a	$2.49\pm0.06^{bc}$		

0.25% AA: samples treated with 0.25% ascorbic acid; 0.50% AA: samples treated with 0.50% ascorbic acid; 0.80% SC: samples treated with 0.80% sodium citrate; Control: untreated sample. Different lowercase letters (superscript) in the same column indicate a significant difference between treatment groups (p<0.05).

# Lipid hydrolysis development

The free fatty acids (FFA) phospholipid (PL) contents of the muscle were measured to evaluate lipid hydrolysis during chilled storage. Generally, the amount of FFA of all samples increased with the storage time, but to different extents (Fig. 3A). The treated samples had significantly lower FFA values compared to the untreated samples. The FFA content of the control samples increased significantly from 1.10 g/100 g to 2.51 g/100 g after 10 days of storage. The FFA content of the 0.80% SC treated samples was rather stable during the first 6 days of storage and increased significantly from day 6 to day 12 of the storage period. Whereas, the FFA content of the 0.25% AA and 0.50% AA treated samples was slightly (p>0.05) increased throughout the storage time and no significant (p>0.05) difference was observed between 0.25% AA and 0.50% AA groups. The FFA exhibited a progressive increase during fish storage, the PL content decreased with increased chilled storage time for all samples ( $r^2$ =-0.726) (Fig. 3B). A negative correlation ( $r^2=-0.715$ ) between FFA and PL was observed in the present study. The untreated samples had significantly (p<0.05) lower PL values compared to the treated samples (e.g., 0.25% AA, 0.50% AA and 0.80% SC) during the storage time.

# Lipid oxidation development

Lipid hydroperoxides (PV) were measured to evaluate the formation of primary lipid oxidation products. The changes in PV of snakehead fish fillets during chilled storage are given in Figure 4A. The PV in the samples increased with storage time ( $r^2=0.707$ ), especially between day 6 and day 10. The PV content of the control samples increased significantly (p<0.05) from an initial value of 0.55 µM CPO/g to 2.49 μM CPO/g by the end of the 10<sup>th</sup> day. The treated samples showed an increase of 0.24 to 1.13 µM CPO/g, 0.35 to 0.84 uM CPO/g and 0.44 to 1.21 uM CPO/g for 0.25% AA, 0.50% AA and 0.80% SC treatments, respectively from day 0 to the  $10^{th}$  day. No significant (p>0.05)difference existed in PV values between the groups during the first 6 days of storage. During the subsequent storage time, the PV contents of the untreated and 0.80% SC treated samples increased significantly (p<0.05), whereas the PV contents of ascorbic acid-treated groups were rather stable (Fig. 4A). A positive correlation ( $r^2$ =0.899) existed between PV and FFA and a negative correlation of ( $r^2$ =-0.765) existed between PL and PV contents. The results of the present study indicate that ascorbic acid

treatments (i.e., 0.25% AA and 0.50% AA) and sodium citrate treatment (0.80% SC) were effective in retarding the production of PV in snakehead fish fillets during chilled storage at 2-4°C.

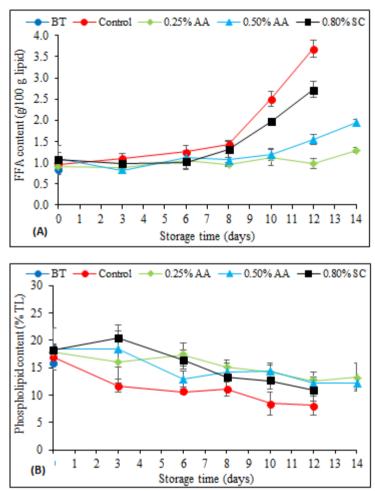


Figure 3: Changes in free fatty acids (FFA) (A) and phospholipid content (PL) (B) of snakehead fish fillets during chilled storage as functions antioxidant treatments [BT: before treatment, Control (●), 0.25% ascorbic acid treated (●), 0.50% ascorbic acid treated (▲) and 0.80% sodium citrate treated (■)].

The TBARS index is widely used as an indicator for the assessment of the degree of the secondary lipid oxidation product. Variations in values of TBARS during storage are depicted in Figure 4B. Generally, an increase in TBARS with increased storage time was observed ( $r^2$ =0.412). Treated samples had lower TBARS values compared to untreated

samples. In this study, the TBARS values reached 13.62, 4.28, 3.93, and 9.60  $\mu$ M MDA/kg on the 10<sup>th</sup> day from initial values of 4.54, 4.28, 4.0 and 6.40  $\mu$ M MDA/kg for control, 0.25% AA, 0.50% AA, and 0.80% SC samples, respectively. Significant differences (p<0.05) in TBARS contents between groups were noted between the sodium

citrate treatment (0.80% SC) and ascorbic acid treatments (i.e., 0.25% AA and 0.50% AA) as well as between control and ascorbic acid treatments. The sodium citrate treatment (0.80% SC) had significant (p<0.05) higher TBARS values compared to the ascorbic

acid treatments (i.e., 0.25% AA and 0.50% AA). The results indicate that ascorbic acid treatments (i.e., 0.25% AA and 0.50% AA) demonstrated a greater lipid antioxidative activity compared to sodium citrate treatment (0.80% SC).

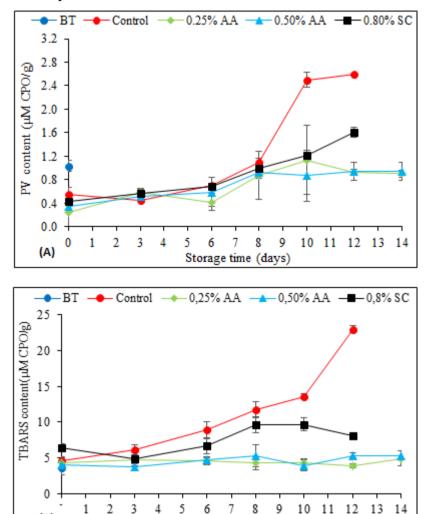


Figure 4: Changes in PV (A) and TBARS (B) of snakehead fish fillets during chilled storage as functions antioxidant treatments [BT: before treatment, Control (•), 0.25% ascorbic acid treated (♠), 0.50% ascorbic acid treated (♠) and 0.80% sodium citrate treated (■)].

Storage time (days)

## Fatty acid composition

Habitually, fatty acid profiles are closely related to lipid oxidation, and changes in the content of different fatty acids in snakehead fish muscles might reflect the extent of lipid oxidation. The changes in

(B)

fatty acid composition (g/100 g TL) of total lipids extracted from snakehead muscle samples are listed in Table 2. The SFAs, MUFAs and PUFAs content of fresh snakehead muscle was 26.49, 46.49 and 27.03 (g/100 g TL) of total

lipid, respectively. After storage, the untreated snakehead muscle contained 43.70, 39.33, and 16.98 (g/100 g TL). In contrast, the SFAs, MUFAs and PUFAs contents of the treated samples; 0.25% AA and 0.8% SC were 32.71, 42.52, 24.77 (g/100 g TL) and 32.70, 45.50, 21.80 (g/100 g TL), respectively. As expected, the untreated samples had

significantly lower PUFAs and higher SFAs contents compared to that of fresh snakehead muscle and treated samples. Similarly, higher DHA (C22:6*n*–3) and EPA (C20:5*n*–3) values were noted in the 0.25% AA samples compared to those of 0.80% SC and untreated samples after storage at 2-4°C.

Table 2: Fatty acid composition (g/100 g lipid) of total lipids extraction from fresh snakehead muscle and treated samples after storage for 14 days at 2-4°C.

Fotty acid (c/100 c linid)	Fresh	After chilled storage at 2-4 °C			
Fatty acid (g/100 g lipid)	snakehead	0.25% AA	0.80% SC	Control	
C16:0	22.16 <sup>b</sup>	23.47 <sup>b</sup>	23.22 <sup>b</sup>	30.59a	
C16:1n9	3.24	1.88	1.90	2.91	
C17:0	0.00	0.47	0.00	0.00	
C18:0	4.32	8.92	9.48	13.11	
C18:1n9	41.62 <sup>a</sup>	39.91 <sup>b</sup>	42.65 <sup>a</sup>	34.96 <sup>c</sup>	
C18:2n6	15.68 <sup>b</sup>	17.37 <sup>a</sup>	16.59 <sup>b</sup>	8.74 <sup>c</sup>	
C18:3n6	2.16 <sup>a</sup>	1.88 <sup>b</sup>	$1.90^{b}$	2.41a	
C20:1n9	1.62	0.94	0.95	1.46	
C20:2n6	1.08	0.94	0.00	1.46	
C20:3n6	1.62	0.94	0.95	1.46	
C20:4n6	1.08	0.94	0.95	1.46	
C20:5n3 (EPA)	1.08 <sup>a</sup>	$0.47^{\rm b}$	$0.00^{c}$	$0.00^{c}$	
C22:6n3 (DHA)	4.32 <sup>a</sup>	2.35 <sup>b</sup>	1.42°	1.46 <sup>c</sup>	
SFAs	26.49°	32.71 <sup>b</sup>	$32.70^{b}$	43.70 <sup>a</sup>	
MUFAs	46.49a	$42.52^{b}$	45.50 <sup>a</sup>	39.33 <sup>c</sup>	
PUFAs	27.03 <sup>a</sup>	$24.77^{b}$	21.80 <sup>c</sup>	16.98 <sup>d</sup>	

0.25% AA: samples treated with 0.25% ascorbic acid; 0.80% SC: samples treated with 0.80% sodium citrate; Control: untreated sample. Different lowercase letters (superscript) in the same row indicate a significant difference between treatments and fresh snakehead fish fillet (p<0.05).

# Multivariate analysis

In order to achieve an overview of the similarities and differences among the quality parameters, a principal component analysis (PCA) was carried out. All the quality parameters were represented by mean values, given antioxidant treatments and storage time. It was found that the first two principal components (PC1 and PC2) explained 69.6% of the total variations in the data set. The correlation loadings of the first

two PCs, representing 54% and 15.6% of the total variations, can be seen in Figure 5A. The correlation loadings indicate that the variations in QI score, Torry score, TL-, PL-, FFA-, PV-, a\*- and TBARS- values were mainly described by PC1, and that these quality parameters were strongly correlated to the storage time. The fresh snakehead fish fillets were characterised by total lipid (TL), phospholipid (PL), Torry score, cooking yield (CY), and soluble

protein (SP) contents as well as redness value (a\*). Whereas, spoiled snakehead fish fillets were characterised by PV, TBARS and FFA contents as well as QI score. Oxidation products (PV and TBARS) and FFA content significantly correlated with QI score. PC2 accounted for 15.6% of the total variations between the samples, which was due to the

different antioxidant treatments (Fig. 5A). Whiteness-, L\*- and b\* values are influenced by antioxidant treatments rather than the storage time. The score plots in Figure 5B show that Group-0.25% AA and Group-0.50% AA were located close to each other.

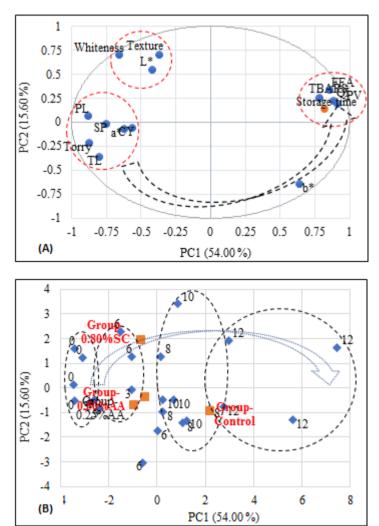


Figure 5: Principal component analysis (PCA) of scores for different parameters measured during chilled storage of snakehead fish fillets at 2-4°C. PC1 describes 15.60% of variation and PC2 describes 54%.

This indicates that no significant difference was observed between 0.25% AA and 0.50% AA treated snakehead fish fillets. The Group-0.80% SC was located far away from ascorbic acid

treatments, revealing significant difference was noted between ascorbic acid treatments and sodium citrate treatment. Sodium citrate treatment resulted in increased whiteness and L\*

values. All antioxidant treated groups (i.e., Group-0.25% AA, Group-0.50% AA and Group-0.80% SC) were located on the left side of PC1-axis, whereas untreated group (Group-control) was located on the right side of the PC1-axis. This means that at the end of storage the quality of untreated period. snakehead fish fillets was lower compared to treated samples.

## **Discussion**

The average Torry score of 5.5 is used as the limit for human consumption (Martinsdottir et al., 2001). Based on the Torry score limit point, the shelf-lives of untreated (control), 0.80% SC, 0.50% AA and 0.25% AA samples were 10, 11, 13 and 14 days, respectively. The results of Nguyen et al. (2013) showed that the shelf-life of cod fillets stored at -1±0.5°C is 13-14 days. Margeirsson et al. (2010) reported a shelf-life of 11-12 days for fresh cod fillets. Variations in the shelf-life of aquatic products may arise due to differences in species, resulting in differences in biochemical composition. Besides, geographical region, diet, packaging methods and storage conditions also have a notable influence on the shelf-life of aquatic products. Nonetheless, the shelf-life can be extended to 17 or 21 days if cod fillets are modified atmosphere packaged and super chilled stored (Lauzon et al., 2009). Research results of Cyprian et al. (2013) exhibited that the shelf-life of air packed tilapia fillets stored at -1°C is 21 days. The results of this study confirmed that the QIM method is suitable for assessing the freshness of seafood products. The results of this study were under the guidelines for evaluation of snakehead fillets previously published by Nguyen *et al.* (2021b) for snakehead fillets, Cyprian *et al.* (2013) for tilapia fillets, Bonilla *et al.* (2007) for cod fillets and Sveinsdottir *et al.* (2003) for salmon fillets.

The decrease in whiteness (Fig. 2A) and increase in b\* values (Fig. 2B) might result from lipid hydrolysis, lipid and protein oxidation, which changed the light reflectance and produce some substances that affect colour (Mohan et al., 2012). Ascorbic acid treatments (i.e., 0.25% AA and 0.50% AA) and sodium citrate (0.80% SC) generally improved fish muscle, snakehead probably because they distorted or broke protein covalent bonds, and to a certain extent, the structure of protein got denatured and agglutinated (Feng et al., 2017). Higher whiteness values obtained in the 0.80% SC treated fillets could be due to the water absorption during treatment, leading to a change in light refraction in the fish muscle.

The mean lipid content ranged between 2.49% and 2.85% (Table 1) and was similar to changes in total lipid (TL) contents of salted dried snakehead fish during refrigerated storage (2.59%-2.86%) (Nitipong *et al.*, 2014). The general decrease of lipid content with increased storage time (r<sup>2</sup>=-0.639) for all samples, maybe due to the lipid degradation (i.e., lipid hydrolysis and lipid oxidation) that occurred during storage. The slight increase in lipid content in some groups at some

sampling points during storage time may probably be due to increased recovery of the extraction of lipids caused by changes in fish tissues. It might also have resulted from the different fillets. which could be difficult to harmonize when working with biological samples such as fish. The lower lipid content and higher lipid oxidation products (i.e., PV and TBARS) were obtained in the untreated samples. Lipid oxidation affects TL, this is supported by a correlation (r<sup>2</sup>=-0.699) between TL and PV. From the correlations noted above, it can be deduced the reduction of lipid content is due to lipid hydrolysis and lipid oxidation. Moreover, the decrease in lipid content is thought to be due to the degradation of lipid and the incorporation of proteins, leading to non-extractable lipids (Nlelsen et al., 1985).

Generally, the amount of FFA of all samples increased with the storage time, but to different extents (Fig. 3A). Similar results were reported by Miranda et al. (2018)the study of quality enhancement of chilled lean fish by previous active dipping in Bifurcaria bifurcata alga extract and also by Sveinsdottir et al. (2020) in the study of effects of antioxidants for Atlantic during mackerel frozen storage. Previous studies have shown that microbial activity also contributed to the increase of FFA content and was mostly responsible for lipid hydrolysis after 9 days of storage (Miranda et al., 2018). Whilst, FFA exhibited a progressive increase during fish storage, the PL content decreased with increased chilled storage time for all samples ( $r^2=-0.726$ ) (Fig. 3B). The increased FFA values in the snakehead fillets during chilled storage were in a negative correlation  $(r^2=-0.715)$  with the decrease in PL content, suggesting the development of FFA was chiefly due to PL degradation. The changes in FFA and PL contents may be due to the reaction of phospholipase enzyme in the fish muscle during storage (Jin et al., 2010). These results (FFA, PL) revealed that lipolysis occurred in snakehead fish fillets during chilled storage at 2-4°C for all samples. Literature has suggested that most of the FFA evolving in fish during storage come from PL (Lopez-Amaya and 2000). It Marangoni, has documented lipids in the fish muscle, especially glycerol-fatty acid esters and phospholipids during storage, are chiefly hydrolysed by microbial enzyme activity such as lipase and phospholipase and non-microbial enzyme activity (i.e., natural lipase and phospholipase present in the fish muscle) as well as spontaneous lipid hydrolysis (Jin et al., 2010; Nguyen et al., 2012). As a result of endogenous enzyme activity (lipases and phospholipases), FFAs have been reported to be produced during the first stage of the chilling process (Ortea et al., 2010). An accumulation of free fatty acids (FFA) produced bv hydrolysis could result in acceleration of lipid oxidation, which brings in muscle texture changes, and off-odour development, leading shelf-life reduction (Vázquez et al., 2013).

The changes in PV are thought to be due to the ratio between the lipid

hydroperoxide formation rate and the lipid hydroperoxides decomposition rate (Nguyen et al., 2012). An increase in PV is believed to be driven by the oxidation of phospholipids. The results were in amity with a decrease in PL in all samples. The PV formation significantly slower (p<0.05) in ascorbic acid treated fillets than in sodium citrate treated fillets, as well as compared to the untreated fillets. The results obtained in this study were similar to the results reported for salmons (Sallam, 2007). A positive correlation (r<sup>2</sup>=0.899) existed between PV and FFA, indicating that hydrolysis advocates lipid lipid oxidation. According to Gokoglu et al. (2012), lipid hydrolysis favours lipid oxidation, the fatty acids formed can be substrates of the oxidation reaction. The lipolytic activity is closely related to the degree of lipid oxidation, as it is directly correlated with the release of FFAs, which are more susceptible to oxidation than the esterified ones (Muriel et al., 2007). Due to the formation secondary and final tertiary products, PV normally rises peak and then drops as many studies have shown (Nguyen et al., 2012). This occurrence was observed on day 3 for the control and day 6 for 0.25% AA treatment and day 10 for 0.50% AA. Such kind of decrease may be related to the secondary reactions of the carbonyl compounds and volatilization (Vidya and Srikar, 1996). A continuous rise in PV of fish without peaking and then dropping during storage has also been shown by other studies (Boran et al., 2006). For **TBARS** content, untreated sample had significantly

higher TBARS values compared to other treatments and the rate of lipid oxidation was higher. This could be a result of products of lipid hydrolysis and primary products of lipid oxidation. The TBARS of the treated samples were much lower throughout the storage time compared to the untreated sample (Control). This clearly depicts that ascorbic acid (i.e., 0.25% AA, 0.50% AA), and sodium citrate (0.80% SC) delayed lipid oxidation process. A negative correlation (r<sup>2</sup>=-0.899) was observed between PV and TBARS. Similar results have been reported by (Taheri et al., 2012). The increase in TBARS values during chilled storage at 2-4°C could be attributed to the decomposition of hydroperoxides in the fish muscle (Chaijan et al., 2006). A positive correlation (r<sup>2</sup>=0.868) was observed between TBARS and FFA. This result could be that the formation of FFA contributes an effect to the decomposition of hydroperoxides to form free radicals (Yoshida et al., 1992). It has been proposed that the maximum level of TBARS value indicating good quality of the fish is 5 mg of MD per kilogram of fish flesh, while the fish may be consumed up to the level of 8 mg of malondialdehyde (MD) per kilogram of fish flesh (Sallam, 2007). In this study, TBARS values for control and treated samples analysed were much lower than such proposed limits throughout the storage period.

Fatty acid composition is affected by different antioxidant treatments which have different effects on lipid oxidation. A lower PUFAs content observed in the untreated samples compared to that of the treated samples (0.25% AA, 080% SC) is expected to sequel from rapid oxidation of PUFAs in the untreated samples. PUFAs are expected to be the easiest oxidised components, and the content of PUFAs decreases due to lipolysis, autoxidation, and catalysis reactions that occur during preservation process especially EPA (C20:5n-3) and DHA (C22:6n-3) that are highly susceptible to oxidation (Masniyom et al., 2005). The results were in agreement with lower EPA (0.00 g/100g TL) and DHA (1.46 g/100g TL) contents obtained in the untreated samples compared to those of 0.25% AA treated samples (0.47 g EPA/100g TL and 2.35 g DHA/100g TL) and 0.8% SC treated samples (0.00% g EPA/100g TL and 1.46 g DHA/100g TL). The lower PUFAs content was in correspondence with the higher SFAs and MUFAs content (Table 2). The same discovery has been reported by Cao et al. (2019) for the inhibitory effect of chlorogenic acid on lipid oxidation of grass carp (Ctenopharyngodon idella) during chilled storage. Among the saturated fatty acids (SFAs), palmitic acid (C16:0) was overridden with a value in the range of 23.22 - 30.59 g/100g TL. Oleic acid (C18:1n-9) was significant among the monounsaturated fatty acids (MUFAs) with a value ranging from 34.96 to 42.65 g/100g TL. From the fatty acid profile, it can be deduced that 0.25% AA performed better antioxidant activity than 0.8% SC.

Implementation of safe and efficient preservation methods for highly

perishable foods including fish and seafood products is of great importance. Fresh fish spoilage is chiefly dominated by microbial activity and chemical changes such as autoxidation, enzymatic hydrolysis of the lipid fraction, and tissue enzyme activity. The current study demonstrates that snakehead fillets treated with ascorbic acid treatments (i.e., 0.25% AA and 0.50% demonstrated greater lipid a antioxidative activity compared to sodium citrate treatment (0.80% SC). For industrial purposes, the use of 0.25% AA would be ideal to reduce processing costs, since there was no significant difference between the 0.25% AA and 0.50% AA treatments. Use of the 0.50% AA would increase processing costs which have a bearing on profits for the industry. The development of lipid hydrolysis and oxidation was in high correlation with the sensory quality and physicochemical properties of snakehead fish fillets. Ascorbic acid and sodium citrate treatments prolonged the shelf-life of snakehead fish fillets during chilled storage. The antioxidants preserved the quality of fish since they reduced the degree of chemical spoilage and enhanced the overall sensory values of snakehead fillets, compared with the control.

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