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The effect of the chamber bitter (*Phyllanthus amarus*) extract on the quality of the snakehead (*Channa striata*) fillets during ice storage

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ABSTRACT

The current research was conducted to investigate the effect of chamber bitter (*Phyllanthus amarus*) extract on the quality of snakehead (*Channa striata*) fillets during iced storage. The study included three treatments, viz. soaking snakehead fillets in cold water; in *P. amarus* extract solutions with concentrations of 7.71 µg/mL and 156 µg/mL for 30 minutes at 4℃. The samples were stored for 12 days and sampling was done in 1, 4, 8, and 12 days. Evaluated parameters included temperature, total viable counts, sensory property, pH, moisture, water holding capacity, texture, total volatile base nitrogen, peroxide value, thiobarbituric acid reactive substances and colour measurement. Results showed that treating snakehead fillet with *P. amarus* extract enhanced the sensory values compared to that of the untreated sample during ice storage. In addition, *P. amarus* extract was effective at delaying lipid oxidation. Based on the sensory properties and total viable count, snakehead fillets treated with chamber bitter can be used up to 8 days whilst less than 8 days for control treatment.

INTRODUCTION

Snakehead fish (*Channa striata*) is a freshwater fish in the family Channidae **(Nelson, 1994)**. This fish species is widely consumed in Malaysia and other Southeast Asian countries for its agreable flavour and medicinal properties, which promote wound healing and reduce postoperative pain and discomfort **(Song *et al.*, 2013)**. They have a wide range of habitats, including rivers, swamps, ponds, canals, lakes and rice paddies **(Song *et al.*, 2013)**. Snakehead is considered the most popular fish for consumption in Vietnam **(Sinh *et al.*, 2014)**. It is a predatory fish with high nutritional value suitable for human food, especially for children **(Mustafa *et al.*, 2012)**. The spoilage of fish during storage is usually caused by biochemical reaction such as oxidation of lipids, protein degradation, microbial growth and metabolic activities, resulting in the short shelf life and the decrease in flesh quality **(Arashisara *et al.*, 2004)**.

Therefore, taking some measures to delay the deterioration of snakehead quality and extend its preservation life are worthwhile.

Phyllanthus amarus Schum. & Thonn. (*P. amarus*) is one of the herbs that show a wide spectrum of pharmacological effects, viz. antioxidant, antimicrobial, anticancer, anti-inflammatory, antiviral and antidiabetic activities. It contains various bioactive compounds e.g. lignans, flavonoids, hydrolysable tannins, triterpenes and alkaloids **(Patel *et al.*, 2011)**. High amounts of phenolic compounds detected in the methanolic extract of *P. amarus* possess potential antioxidant activity, including lipid peroxidation inhibition capacity and free radical scavenging ability **(Guha *et al.*, 2010)**. In addition, the extract of *P. amarus* showed significant antimicrobial activity against *Shigella* spp., *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. The antibacterial action was mainly due to the isolated phyllanthin **(Mazumder *et al.*, 2006)**. Variety studies were conducted to investigate the change of fish quality during ice storage, for example, salmon **(Duun & Rustad, 2007; Bahuaud *et al.*, 2009)**, tilapia **(Rong *et al.*, 2009; Liu *et al.*, 2010; Thiansilakul *et al.*, 2010)**, large yellow croaker **(Li *et al.*, 2012)** and catfish **(Viji *et al.*, 2014)**. However, little work has been organized on the effects of *P. amarus* extract on the quality of seafood. Therefore, this study was conducted to evaluate the effect of *Phyllanthus amarus* extract on the quality of snakehead (*Channa striata*) fillets during ice storage to provide comprehensive data for a better preservation method.

MATERIALS AND METHODS

1. Research materials

Snakeheads (300-400 g) were purchased from fish retailer in Can Tho city, Vietnam originated from one culture pond to guarantee the same physiological and biological characteristics of fish under study. Fish were anaesthetized by ice, killed and bled for 10 minutes. Scales were manually removed, filleted and washed to be prepared for the experiment.

The plant was collected from various areas in Mekong Delta, Vietnam. The dried-powder (100 g) was soaked in ethanol of 96% (800 mL) for at least 24 hours at room temperature with frequent agitation. The solvent-containing extracts were then decanted and filtered. The ground samples were further extracted for 4 times with ethanol 96%. The filtrates from each extraction were combined and the solvent was evaporated under reduced pressure using a rotary evaporator to give crude ethanolic extracts. All the well-dried crude ethanol extracts were stored in the refrigerator until further use.

The concentration of *P. amarus* extract selected in this experiment was 7.71 μg/mL, corresponding with concentration of 50% DPPH exhibition (IC_{50}) and 156 μg/mL as a minimum inhibitory concentration (MIC), following the method of **Le Anh Dao *et al.* (2020)**.

2. Research methods

2.1. Experimental design

The 96 fish fillets (130-150 g/fillet) specimens were randomly subjected to three treatments, such as soaking in ice tap water (control), in solution of 7.71 μg/mL and 156 μg/mL (weight/volume) of *P. amarus* ethanolic extract. Soaking solutions were maintained below 4°C by adding ice, and soaking time was 30 minutes. Ratio of fish weight and solution was 1:1 (w:v). After that, fish fillets were drained for 5 minutes before packing in polyethylene (PE) bags (8 fillets per bag). Fish fillets were then placed in insulated box (100 L), with fish and ice ratio of 1:1 (w:w). Ice was added and water in the box was removed every storage day to ascertain the temperature below 4°C during storage.

Sampling was taken on the 1^{st}, 4^{th}, 8^{th}, and 12^{th} day of ice storage. At each sampling time and for each treatment, eight fillets were collected. Four fillets from each treatment were individually used for sampling of total viable count (TVC) and sensory evaluation. In the other four fillets, the top part of the fillets was used to measure the texture property; the middle part was for colour measurement, and the remaining arts of fillets were individually minced for measurement of pH, moisture, water holding capacity (WHC), total volatile base nitrogen (TVB–N), peroxide value (PV), and thiobarbituric acid reactive substances (TBARs). Sampling and analysis were performed at the same sampling times.

2.2. Analytical methods

Temperature

On the days of sampling, fillet temperature (°C) was measured in four fillets in each treatment by thermometer (Ebro, Germany).

pH evaluation

The pH was determined in duplicate in a 1:1 (w:v) mixture of minced muscle and 0.15 M KCl by a digital pH meter (C1020, Consort, Germany), equipped with a combined glass-electrode according to the method of **Hultmann *et al.* (2012)**.

Moisture content

The moisture content was determined by drying minced muscle (in quadruplicate) at 105°C until constant weight.

Sensory property

The sensory quality of snakehead fillets was evaluated using the quality index method (QIM) by a panel of seven trained members **(Bao, 2006)**. QIM is based on significant, well-defined changes of appearance attributes occuring in raw fish after storage, viz. odour, texture, colour, gaping and surface. A score from 0 to 3 demerit points was given for 5 quality parameters according to specific parameter descriptions (Table 1). A value of 0 corresponded to very fresh fillets. The scores increased according to spoilage, with a maximum score of 3 for each parameter. The 5 scores are summed to

give an overall sensory score referred to as the Quality Index (QI), which can vary from 0 (very fresh) to a maximum score of 14 (very bad).

Table 1. Description and explanation of the properties in sensory analysis

Quality parameter	Description	Score
Texture	Firm and elastic	0
	Somewhat soft	1
	Soft	2
	Very soft	3
Surface	Very shiny	0
	Rather wrinkled and dried	1
	Wrinkled, dried	2
Odour	Fresh, seaweed	0
	Neutral, slightly fishy	1
	Fishy	2
	Sour, ammonia smell	3
Colour	Cloudy white, bright	0
	Pinkish	1
	Yellowish	2
	Overall pink or yellow	3
Gaping	No Gaping	0
	Gaping, less than 25% of fillet	1
	Gaping, 25-75% of fillet	2
	Gaping, over 75% of fillet	3
Quality index (0-14)		

Sensory evaluation of cooked snakehead fillets in terms of taste was carried out according to the study of **Simeonidou (1997)**. A trained sensory panel of seven persons analysed the samples and classified property on a scale from 1 to 9, where 1 is no intensity and 9 is clear intensity. A sensory score of five was taken as the threshold of acceptability (Table 2). Four fillets from each group were used for sensory analysis. Each evaluator got one sample from each of the fillets. The fillets were kept in ice until the sensory analysis. On the day of analysis, the fillets, without skin and bones, were steamed and served to the evaluators in randomized order at the time of testing.

Total viable counts

Snakehead fillets were taken aseptically in a vertical laminar and 1.0 g was transferred to a sterile tube and homogenized with 9 mL of sterile normal saline water for 60 s. From this first 10^{-1} dilution, other decimal dilutions were prepared. A portion (1 mL) of these dilutions was pipetted into sterile petri dishes, and 15 mL PCA medium at 45ºC were added. Total viable count were determined by counting the number of colony-forming units after incubation at 30°C for 48h. Petri dishes containing colonies from 25

to 250 were selected for the counting according to Nordic Committee for Food Analyses **(NMKL 86, 2006)**.

Table 2. Sensory evaluation of cooked snakehead fillets

		Attribute score	Taste
Acceptable levels	No off-flavour	9	Typically fresh sweet taste for snakehead fillets
		8	Some loss of sweetness
		7	Loss of the characteristic taste of snakehead fillets
		6	Neutral taste, no off-flavours, slightly meaty
	Slight off-flavour	5	Trace of 'off-flavours', slightly putrid, slightly bitter
Reject levels	Severe off-flavour	4	Bitter, sour (citric)
		3	Sharp bitter taste, slight amine taste
		2	Sharp bitterness, strong sour
		1	Sharp 'off-flavour' of amines, rotten, defective fish fillets

Texture analysis

The texture profile analysis (TPA) of fillets was performed using a texture analyser (Model TA.XTplus Texture Analyser, Stable Micro Systems, Godalming, UK). The conditions of the texture analyser were as follows: pretest speed, 1.0 mm/s; posttest speed, 10.0 mm/s; distance, 5.0 mm; trigger type, auto; and trigger force, 5g. The calculation of TPA values was obtained by graphing a curve using force and distance. Penetration values (peak force of the compression cycle) of fish fillets were measured using a P/5S probe (5 mm spherical stainless, Stable Micro Systems).

Water holding capacity

The water holding capacity (WHC) was determined in each fish using the centrifugation method of **Ofstad *et al.* (1993)**. Minced muscle (1.5 g) was weighed in a centrifugal tube (15 mL) and centrifuged at 4ºC for 10 minutes at 300*g* using a Mikro 22-R centrifuge (Hettich centrifuge, Germany). WHC is given as fraction of water bound after centrifugation (% of total water). The analyses were run in quadruplicate.

Total volatile base nitrogen

Total volatile basic nitrogen (TVB-N) was measured following the method of **Velho (2001)**. Fish sample (5 g) was loaded into a Kjeldahl tube, followed by 2g MgO and 50 mL distilled water. The tube was then agitated and placed in the Kjeldahl distillation system. The distillation was performed for 5 minutes, and then the distillate

was collected in a flask containing 25mL boric acid 1% (mixed indicator of methyl red/methylene blue 2:1). Afterwards, the boric acid solution was titrated with a 0.1 N sulphuric acid solution.

Colour measurements

Fish samples are measured in terms of colour at a fixed position in the middle of the piece, 10 cm from the head of the fish, using a spectrophotometer (PCE-CSM 2, PCE Instrument, UK) according to the principle of CIE Lab system (L* a* b*) with L* indicating the lightness within the scale range of 0 – 100 points from black to white, a* indicating the position between red (+) and green (–), and b* indicating the position between yellow (+) and blue (–). Each treatment was repeated four times **(Pathare *et al.*, 2013)**. The values of L *, a * and b * were recorded.

Peroxide value

Peroxide values (PV) were determined by the spectrophotometric ferric thiocyanate method **(International IDF Standards, 1991)**. Fish samples (5 g) were extracted by 20 mL of chloroform: methanol mixture (2:1) (v:v) for 3 hours. After centrifugation of 700 *g* at 25°C for 5 minutes, the lower phase was collected for the determination of fat content and was considered as the sample extract for the latter analysis. The sample extract (1.0 mL) was mixed with 3.9 mL chloroform: methanol (2:1). Then, a volume of 50µL of Fe^{2+} solution (0.018 M) was added, and later 50µL NH_4SCN 30% was adjusted. The solution was stirred on a vortex mixer for 15s. The absorbance of the sample was measured at 480 nm against a blank containing all the reagents except the sample. Peroxide values, expressed as milliequivalents (meq) peroxide/kg fish fat, were calculated based on the concentration of Fe^{3+} determined from the regression line ($y = ax + b$) and the fat content of fish samples.

Thiobarbituric acid reactive substances

The TBARS were determined according to the spectrophotometric method of **Raharjo *et al.* (1992)**. Fish sample was homogenized and extracted with duplicate in TCA 5%. After centrifugation at 1050*g* for 15 minutes at 4°C, the supernatant was collected and filled up to 50.0mL in volumetric flask. In the test tubes, an amount of 2.0mL of extracted sample and TEP standard solution were added, followed with the addition of 2.0mL of TBA reagent 80 mM. The solution was stirred on a vortex mixer for 15s and placed in a water bath at 94°C for 5 minutes. Samples were cooled in a cold-water bath and the absorbance was mmeasured with the spectrophotometer at 530 nm.

3. Statistical analysis

All data were expressed as mean ± standard deviation by Microsoft Excel software. The data of all parameters analysed at each sampling time were subjected to the analysis of variances (one-way ANOVA) using SPSS 16.0. Differences at $p<0.05$ were considered significant.

RESULTS

1. Temperature

The core temperature of the fillets recorded during ice storage was below 4°C (1.53 - 3.20°C). There was no significant difference in core temperature of the fillets between treatments during each sampling times ($p>0.05$). Icing is one of the most prevalent technique for fresh fish preservation **(Roberts *et al.*, 2005)**. Thus, fillet stored in ice in this experiment satisfy the requirements of the cryopreservation.

2. Muscle pH

Changes in the pH values of snakehead fillets during ice storage are shown in Table (3). In this experiment, pH values of three groups did not highly vary during the storage, ranging from 6.38 to 6.56. There was no significant difference between treatments during sampling times ($p>0.05$). Thus, *P. amarus* extract did not affect the pH values. It agrees with the observations of **Feng *et al.* (2012)** with respect to black sea bream (*Sparus macrocephalus*) and **Li *et al*. (2012)** regarding yellow grouper (*Epinephelus awoara*).

Table 3. pH values of snakehead fillets under treatment of *Phyllanthus amarus* extract during ice storage

Treatment	Storage time (days)			
	1	4	8	12
Control	6.43±0.08[a]	6.49±0.09[a]	6.50±0.07[a]	6.56±0.03[a]
Phy7.71 μg/mL	6.45±0.08[a]	6.38±0.03[a]	6.49±0.05[a]	6.52±0.06[a]
Phy156 μg/mL	6.40±0.06[a]	6.45±0.14[a]	6.51±0.04[a]	6.55±0.11[a]

Values in the same sampling day followed by different letters present significant differences between treatments ($p<0.05$). Phy 7.71 μg/mL and Phy 156 μg/mL: fillets treated with Phyllanthus amarus extract at concentrations of 7.71 μg/mL and 156 μg/mL

3. Water holding capacity

Changes in the water holding capacity (WHC) values of snakehead fillets during ice storage are shown in Fig. (1). No significant difference in WHC between treatments was detected during storage time ($p>0.05$), except on day 1. After one day of storage, the WHC of treatments treated with *P. amarus* was significantly higher than that of the control treatment ($p<0.05$). The WHC values slightly increased following storage period, ranging from 87.5 to 94.3%. The increase of WHC could be explained by the liquid loss during storage, presented through the decrease in moisture of the fillet. The results showed that *P. amarus* extract did not significantly affect the WHC of snakehead fillets during chilled storage.

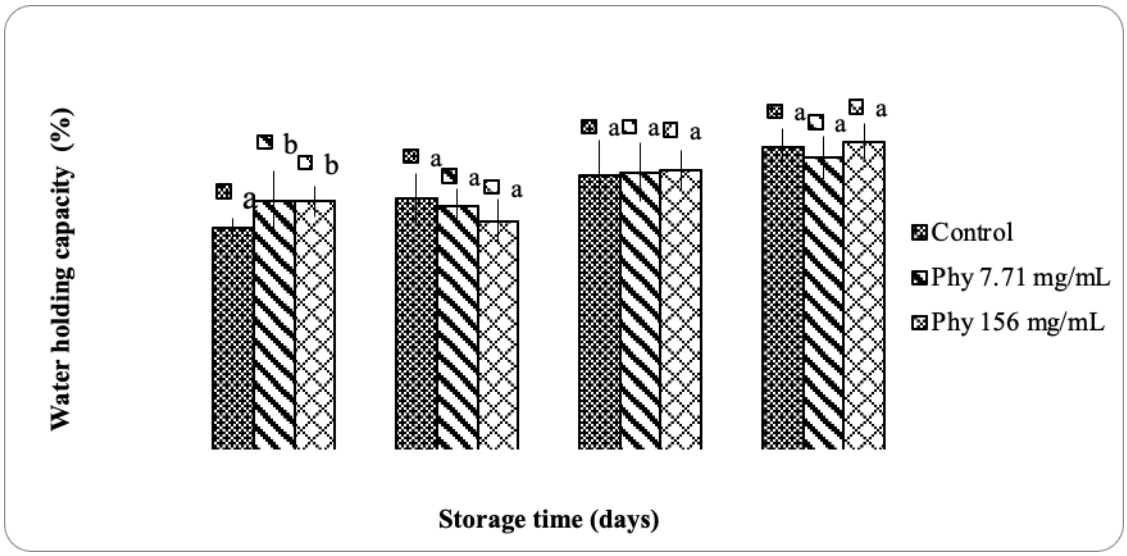

Fig. 1. Water holding capacity of snakehead fillets under treatment of *Phyllanthus amarus* extract during ice storage

Values in the same sampling day followed by different letters present significant differences between treatments ($p<0.05$). Phy7.71 μg/mL andPhy156 μg/mL: fillets treated with Phyllanthus amarus extract at concentrations of 7.71 μg/mL and 156 μg/mL

4. Moisture

Moisture of snakehead fillets during ice storage are depicted in Table (4). Treating snakehead fillet by *Phyllanthus amarus* did not affect the moisture of the fillet during ice storage ($p>0.05$). The moisture content of fillets slightly varied from 78.8% to 80.3%.

Table 4. Moisture (%) of snakehead fillets under treatment of *Phyllanthus amarus* extract during ice storage

Sample	Storage time (days)			
	1	4	8	12
Control	79.6±0.54^{a}	79.8±0.19^{a}	79.1± 0.67^{a}	78.8± 0.74^{a}
Phy7.71 μg/mL	80.1±0.17^{b}	79.7±0.21^{a}	79.1± 0.66^{a}	79.1± 0.42^{a}
Phy156 μg/mL	80.3±0.78^{b}	79.3± 0.34^{a}	79.5±0.42^{a}	79.1± 0.42^{a}

Values in the same sampling day followed by different letters present significant differences between treatments ($p<0.05$). Phy 7.71 μg/mL and Phy 156 μg/mL: fillets treated with Phyllanthus amarus extract at concentrations of 7.71 μg/mL and 156 μg/mL

5. Texture property

Toughness is one of the key textural parameter presented through penetration work. The changes in the penetration values of snakehead fillet during iced storage are illustrated in Table (5).

In general, toughness of fillet in all treatments ranged from 112-199 (g*cm) during storage, and there was no significant difference in penetration values between treatments

during sampling times ($p>0.05$). The increase of toughness of fillets after 4 days of ice storage resulted from the stage of rigor mortis. After that, the toughness decreased because of the activity of autolytic enzymes (such as collagenase and ATPase), which degrades proteins from the connective tissue and the spoilage by bacteria **(Laksmanan & Piggott, 2003)**. Texture softening during storage observed in this study is consistent to previous report of catfish storage in ice **(Viji *et al.*, 2015)**. The variation of textural properties of fish muscle depends on factors, viz. the structure of contractile protein, the framework of connective tissue and lipid oxidation **(Aussanasuwannakul *et al.*, 2012)**. In addition, texture properties depend on physical structure of the fillets **(Sigurgisladottir *et al.*, 1999)**. Thus, it can be concluded that the dip treatment of *P. amarus* extract did not affect the texture of fillets, compared to the control.

Table 5. Penetration values (g*cm) of snakehead fillets under treatment of *Phyllanthus amarus* extract during ice storage

Treatment	Storage time (days)			
	1	4	8	12
Control	166±4.43[a]	189±11.3[a]	141±10.7[a]	112±6.78[a]
Phy7.71 μg/mL	160±6.55[a]	190±15.3[a]	144±6.83[a]	116±9.29[a]
Phy156 μg/mL	163±12.0[a]	199±8.19[a]	148±4.51[a]	124±7.18[a]

Values in the same sampling day followed by different letters present significant differences between treatments ($p<0.05$). Phy 7.71 μg/mL and Phy 156 μg/mL: fillets treated with Phyllanthus amarus extract at concentrations of 7.71 μg/mL and 156 μg/mL

6. Total volatile base nitrogen

Total volatile basic nitrogen (TVB-N), mainly composed of trimethylamine, dimethylamine and ammonia, as well as other volatile basic nitrogenous compounds, is produced by spoilage bacteria, auto enzymes during iced preservation and deamination of amino acids and nucleotide catabolites. The TVB-N value is considered an indicator of fish deterioration **(Olafsdottir *et al.*, 1997)**. Changes in the mean TVB-N values of snakehead samples during iced storage are shown in the Fig. (2).

TVB-N values of snakehead fillets increased slightly from the initial values of 16.6, 15.2, and 14.5 mg N/100 g to the final values of 18.1, 17.4, and 17.3 mg N/100 g on the 12th day for the fillets in control treatment, fillet treated with *P. amarus* at concentration of 7.71 μg/mL and 156 μg/mL, respectively. The TVB-N values of fillets treated with *P. amarus* extract was significantly higher than those of the control treatments from day 1 to day 8 ($p<0.05$). Treating fillet with *P. amarus* extract significantly reduced the TVB-N of snakehead fillets in its early stage of ice storage. After 12 days of storage, TVB-N values of snakehead fillets in all treatments was acceptable for human consumption since they were

lower than 35 mgN/100 g, a maximum acceptable limit proposed in the study of **Huss (1995)**.

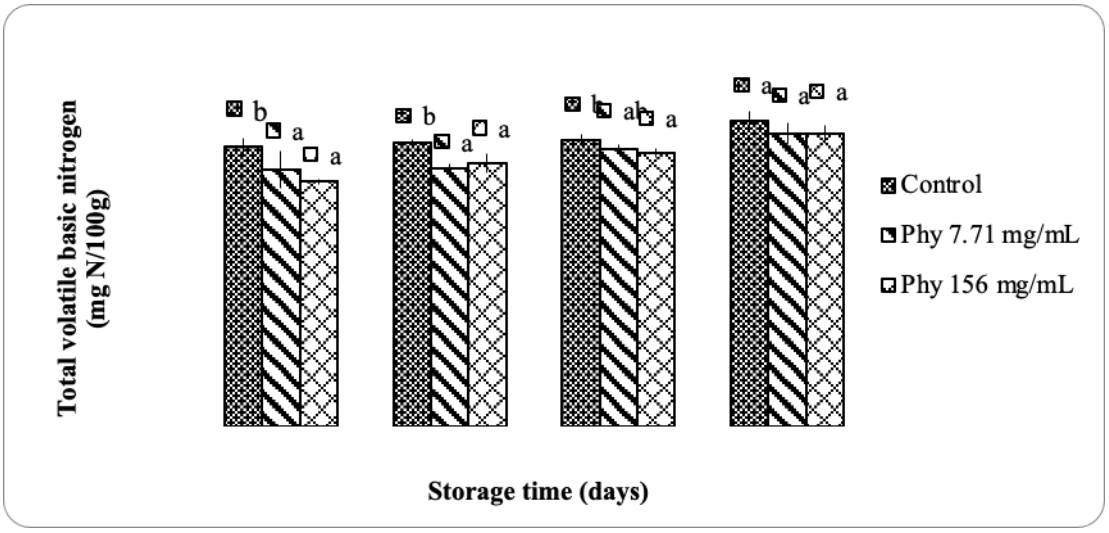

Fig. 2. Total volatile basic nitrogen of snakehead fillets under treatment of *Phyllanthus amarus* extract during ice storage

Values in the same sampling day followed by different letters present significant differences between treatments ($p<0.05$). Phy7.71 μg/mL andPhy156 μg/mL: fillets treated with Phyllanthus amarus extract at concentrations of 7.71 μg/mL and 156 μg/mL

7. Total viable counts

Changes of total viable counts (TVC) of snakehead fillets during iced storage are presented in Fig. (3). The TVC values in the control treatment was significantly higher than TVC in the treatments with *P. amarus* extract on day 4 and 8 of storage time ($p<0.05$). The differences could prove the inhibiting effect of *P. amarus* extract on microorganisms after the dip treatment. The TVC values of the three treatments increased gradually during the 12- day storage. On day 8 of storage, the TVC values reached more 6 $\log_{10}$ cfu/g for the treatment control; while, the TVC of treatments with plant extract reached a value plus 6 $\log_{10}$ cfu/g only after 12 days of storage time (6.34 and 6.53 $\log_{10}$ cfu/g for treatment *P. amarus* 7.71 μg/mL and *P. amarus* 156 μg/mL, respectively). Thus, TVC values of all treatments on day 12 exceeded 6 $\log_{10}$ cfu/g, which is the microbiological acceptability limit value for raw fish following the **Vietnam Ministry of Public Health (2012)**. Thus, a microbiological shelf-life was about 8 days for the treated *P. amarus* extract and less than 8 days in the control for the snakehead fillets during ice storage. The increasing of TVC during storage in ice was reported in the research of **Feng *et al.* (2012)**, who used tea polyphenol combined with ozone water washing in the storage of black sea bream (*Sparus macrocephalus*).

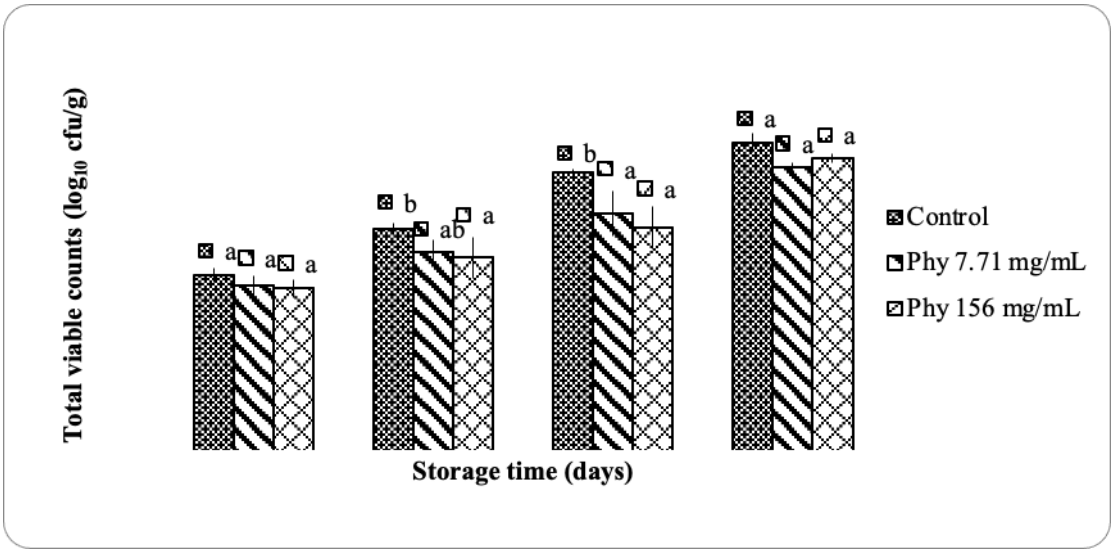

Fig. 3. Total viable counts of snakehead fillets under treatment of *Phyllanthus amarus* extract during ice storage

Values in the same sampling day followed by different letters present significant differences between treatments ($p<0.05$). Phy7.71 μg/mL andPhy156 μg/mL: fillets treated with Phyllanthus amarus extract at concentrations of 7.71 μg/mL and 156 μg/mL

8. Peroxide value

The effect of dip treatment with *P. amarus* extract on changes of peroxide value (PV) of snakehead fillets are shown in Table (6). Fish lipids contain polyunsaturated fatty acids which are highly sensitive to oxidation. During storage time, snakehead underwent lipid oxidation resulting in the formation of hydroperoxides. The peroxide value (PV) allows determining the fatty acid hydroperoxides, which are fatty acid primary oxidation products **(Olafsdottir *et al.*, 1997)**. The PV values of three treatments showed an increasing trend during the 12 days storage; from 4.72 to 9.72 meq/kg. The PV values of the control treatment was significantly higher than those of *P. amarus* treated treatments during 12 days of storage ($p < 0.05$), except day "1". Generally, these PV values are below the acceptable limit range of PV content for fat oxidation of 10-20 meq/kg **(Huss, 1995)**. The results showed that lipid oxidation in snakehead fillets after storage days could be delayed by dipping *P. amarus* extract before ice storage. The presence of high amounts of phenolic compounds in the methanolic extract of *P. amarus* was has potential of antioxidant activity in the forms of lipid peroxidation inhibition capacity and free radical scavenging ability **(Guha *et al.*, 2010)**. This finding coincides with that of **Bensid *et al.* (2014)** who added natural extracts to fish before ice storage. **Haghparast *et al.* (2011)** revealed that green tea extract and onion juice could effectively delay the peroxidation in Persian sturgeon fillets and maintain the PV at a lower level compared to the control sample.

Table 6. Peroxide value (PV; meq/kg) and thiobarbituric acid reactive substances (TBARs; mg MDA/kg) of snakehead fillets under treatment of *Phyllanthus amarus* extract during ice storage

Day	Peroxide value; PV, meq/kg			Thiobarbituric acid reactive substances; TBARs, mg MDA/kg		
	Control	Phy 7.71 μg/mL	Phy 156 μg/mL	Control	Phy 7.71 μg/mL	Phy 156 μg/mL
1	4.47±0.95[a]	4.42±0.86[a]	4.56±0.92[a]	0.288±0.10[b]	0.128±0.04[a]	0.159±0.03[a]
4	6.99±1.06[b]	5.38±0.92[a]	5.81±0.94[a]	0.628±0.15[b]	0.421±0.08[a]	0.263±0.06[a]
8	7.86±0.90[b]	6.64±0.99[a]	6.02±0.89[a]	0.736±0.07[b]	0.625±0.05[a]	0.325±0.10[a]
12	9.72±0.93[b]	7.02±0.80[a]	7.18±0.77[a]	1.088±0.29[b]	0.572±0.19[a]	0.373±0.06[a]

Values in the same sampling day followed by different letters present significant differences between treatments ($p<0.05$). Phy7.71 μg/mL andPhy156 μg/mL: fillets treated with Phyllanthus amarus extract at concentrations of 7.71 μg/mL and 156 μg/mL. meq: milliequivalents MDA: malonaldehydes

9. Thiobarbituric acid reactive substances

Table (6) reveals the formation of malonaldehydes in snakehead fillets during 12 days of refrigerated storage. The formation of malonaldehydes was significantly retarded in samples treated with *P. amarus* extract. TBARs values of *P. amarus* extract treatments were always significantly lower than those of control treatment during chilled storage ($p<0.05$), however, no significant difference ($p > 0.05$) were verified between the two *P. amarus* extract treated samples. The results proved that the treatment of *P. amarus* extract in snakehead fillets are effective at delaying lipid oxidation during ice storage. Accordingly, TBARs values in the range 5-8 mg malonaldehyde/kg in all the samples were within the acceptable limit throughout the storage period **(Sallam, 2007)**. Similar results have been reported by **Cakli *et al.* (2007)** in stored in ice of sea bass and sea bream. TBARs values in this study were much lower than research of **Bensid *et al.* (2014)** about using thyme, oregano and clove extracts on quality anchovy (*Engraulis encrasicholus*) during chilled storage.

10. Colour measurements

Changes in the instrumental colour values of snakehead fillets during iced storage are given in Table "7". Colour of fish and fish products is one of the most important criteria for consumer because it indicates the quality and is also associated with freshness and flavour of seafood product. Overall, lightness (L*) values for all samples decreased slowly during storage and there was no significant difference between control and *P. amarus* treated treatments ($p>0.05$). The redness (a*) values showed gradually increased trend during storage time, whilst the yellowness (b*) slightly reduced with storage period.

The cause of the colour change is due to the changes in the components of fish muscle such as lipid oxidation, enzyme and microbial activity. Lipid oxidation and breakdown of proteins form dark brown complexes, leading to the light colour of fish fillets decrease and red colour to increase. Similar results in the research of **Lu *et al.* (2010)** with L* values were declined trend and a* values rose during 15 days storage at 4°C of snakehead fish fillets.

Table 7. Colour values of snakehead fillets under treatment of *Phyllanthus amarus* extract during ice storage

Storage time (days)	Treatments	L*	a*	b*
1	Control	62.2±0.33^{a}	-2.97±0.28^{a}	5.10±0.43^{b}
	Phy7.71 μg/mL	62.6±0.92^{a}	-2.86±0.39^{a}	4.41±0.38^{a}
	Phy156 μg/mL	62.3±1.22^{a}	-2.46±0.06^{a}	4.75±0.38^{ab}
4	Control	61.2±0.59^{a}	-2.22±0.20^{b}	4.58±0.25^{a}
	Phy7.71 μg/mL	61.1±0.41^{a}	-2.15±0.11^{ab}	4.67±0.27^{a}
	Phy156 μg/mL	61.1±0.70^{a}	-1.86±0.16^{a}	5.02±1.10^{a}
8	Control	60.0±0.65^{a}	-2.13±0.37^{b}	3.67±0.39^{a}
	Phy7.71 μg/mL	60.4±0.70^{a}	-2.05±0.35^{b}	3.53±0.33^{a}
	Phy156 μg/mL	60.7±1.00^{a}	-1.56±0.28^{a}	4.89±0.54^{b}
12	Control	59.5±0.63^{a}	-1.85±0.45^{a}	3.11±0.20^{a}
	Phy7.71 μg/mL	59.6±0.91^{a}	-1.98±0.25^{a}	3.21±0.27^{a}
	Phy156 μg/mL	59.4±0.29^{a}	-1.41±0.29^{b}	3.95±0.09^{a}

Values in the same sampling day followed by different letters presented significant differences between treatments ($p<0.05$).Phy7.71 μg/mL andPhy156 μg/mL: fillets treated with Phyllanthus amarus extract at concentration of 7.71 μg/mL and 156 μg/mL

11. Sensory properties

Results of total quality index (QI) are presented in Figure "4". In general, the sensory scores in both control and treated plant extract declined throughout the 12 days of chilled storage. During storage period, the treated with *P. amarus* extract exhibited a significant lower QI than the control samples in day 8 ($p<0.05$). The results showed that the addition of natural extract improved the sensory quality of fish as in fresh state.

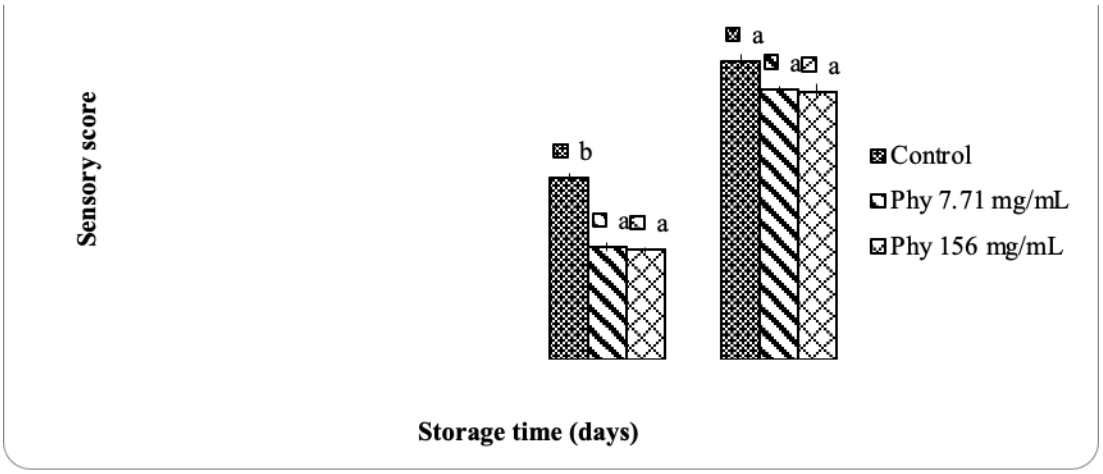

Figure 4. Quality index (QI) of snakehead fillets under treatment of *Phyllanthus amarus* extract during ice storage

Values in the same sampling day followed by different letters presented significant differences between treatments (p<0.05).Phy7.71 µg/mL andPhy156 µg/mL: fillets treated with Phyllanthus amarus extract at concentration of 7.71 µg/mL and 156 µg/mL

Sensory scores of cooked snakehead fillets are shown in Figure "5". The results indicated that sensory scores showed a significant decline in three treatments following the storage period. Fish products were considered to be acceptable for human consumption until the sensory score reached above 5 (**Simeonidou, 1997**). Like sensory evaluation in fresh fillets, the snakehead fillets treated with *P. amarus* extract was significantly higher than that of control samples at days "4" and "8" of storage time ($p<0.05$). It can be concluded that treating snakehead fillet by *P. amarus* extract improved the sensory property during ice storage

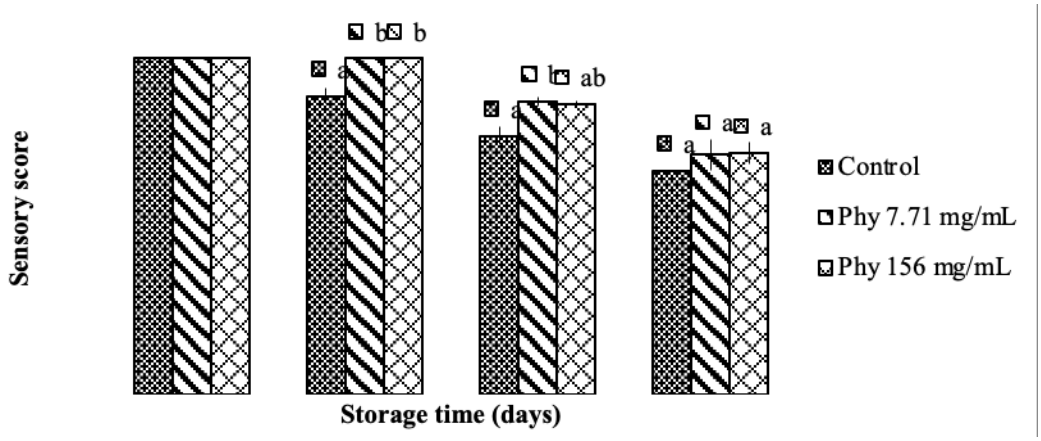

Figure 5. Taste of cooked snakehead fillets under treatment of *Phyllanthus amarus* extract during ice storage

Values in the same sampling day followed by different letters presented significant differences between treatments (p<0.05).Phy7.71 µg/mL andPhy156 µg/mL: fillets treated with Phyllanthus amarus extract at concentration of 7.71 µg/mL and 156 µg/mL

CONCLUSION

Soaking snakehead fillet with *P. amarus* extract at concentration of 7.71 µg/mL and Phy 156 µg/mL significantly reduced total viable count, retarded lipid oxidation, and enhanced sensory property during ice storage. Based on the sensory properties and total viable count, snakehead fillets treated with *P. amarus* extract can be used up to 8 days whilst less than 8 days for control treatment.

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REFERENCES

Arashisara, S.; Hisara, O.; Kayab, M. and Yanik, T. (2004). Effects of modified atmosphere and vacuum packaging on microbiological and chemical properties of rainbow trout (*Oncorynchus mykiss*) fillets. Int. J. Food Microbiol., 97(2): 209–214.

Aussanasuwannakul, A.; Slider, S. D.; Salem, M.; Yao, J. and Brett Kenney, P. (2012). Comparison of Variable- Blade to Allo- Kramer Shear Method in Assessing Rainbow Trout (*Oncorhynchus mykiss*) Fillet Firmness. J. Food Sci., 77(9): S335–S341.

Bahuaud, D.; Mørkøre, T.; Langsrud, Ø.; Sinnes, K.; Veiseth, E.; Ofstad, R. and Thomassen, M.S. (2009). Effects of -1.5 Super-chilling on quality of Atlantic salmon (*Salmo salar* L.) pre rigor fillets: Cathepsin activity, muscle histology, texture and liquid leakage. Food Chem., 111: 329–339.

Bao, H.N.D. (2006). QIM method scores quality, shelf life of Pangasius fillets. Global Aquaculture Advocate, 28–30.

Bensid, A.; Ucar, Y.; Bendeddouche, B. and Özogul, F. (2014). Effect of the icing with thyme, oregano and clove extracts on quality parameters of gutted and beheaded anchovy (*Engraulis encrasicholus*) during chilled storage. Food Chem., 145: 681–686.

Cakli, S.; Kilinc, B.; Cadun, A.; Dincer, T. and Tolasa, S. (2007). Quality differences of whole ungutted sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) while stored in ice. Food Control, 18(5): 391–397.

Duun, A.S. and Rustad, T. (2007). Quality changes during superchilled storage of cod (*Gadus morhua*) fillets. Food Chem., 105(3): 1067–1075.

Feng, L.; Jiang, T.; Wang, Y. and Li, J. (2012). Effects of tea polyphenol coating combined with ozone water washing on the storage quality of black sea bream (*Sparus macrocephalus*). Food chem., 135(4): 2915–2921.

Guha, G.; Rajkumar, V.; Ashok, K.R. and Mathew, L. (2010). Aqueous extract of *Phyllanthus amarus* inhibits chromium (VI)-induced toxicity in MDA-MB-435S cells. Food Chem. Toxicol., 48: 396–401.

Haghparast, S.; Kashiri, H.; Alipour, G. and Shabanpour, B. (2011). Evaluation of Green Tea (*Camellia sinenses*) extract and onion (*Allium cepa* L.) juice effects on lipid degradation and sensory acceptance of Persian sturgeon (*Acipenser persicus*) fillets: a comparative study. J. Agr. Sci. Tech., 855–868.

Hultmann, L.; Phu, T.M.; Tobiassen, T.; Aas-Hansen, Ø. and Rustad, T. (2012). Effects of pre-slaughter stress on proteolytic enzyme activities and muscle quality of farmed Atlantic cod (*Gadus morhua*). Food chem., 134(3): 1399–1408.

Huss, H.H. (1995). Quality and quality changes in fresh fish(Vol. 348), FAO Fisheries Technical Paper. Rome.

International Dairy Federation. (1991). International IDF standards, sec. 74A:1991. IDF-Square Vergote 41, Brussels, Belgium.

Lakshmanan, R.; Piggott, J.R. and Paterson, A. (2003). Potential applications of high pressure for improvement in salmon quality. Trends Food Sci. Technol., 14(9): 354–363.

Li, T.; Hu. W.; Li, J.; Zhang, X.; Zhu, J. and Li, X. (2012). Coating effects of tea pholyphenol and rosemary extract combined with chitosan on the storage quality of large yellow croaker (*Pseudosciaena crocea*). Food control, 25(1): 101–106.

Liu, S.; Fan, W.; Zhong, S.; Ma, C.; Li, P.; Zhou, K.; Peng, Z. and Zhu, M. (2010). Quality evaluation of tray-packed tilapia fillets stored at 0^{o}C based on sensory, microbiological, biochemical and physical attributes. Afr. J. Biotechnol., 9(5): 692−701.

Lu, F.; Ding, Y.; Ye, X. and Liu, D. (2010). Cinnamon and nisin in alginate–calcium coating maintain quality of fresh northern snakehead fish fillets. LWT- Food Sci. Technol., 43(9): 1331–1335.

Mazumder, A.; Mahato, A. and Mazumder, R. (2006). Antimicrobial potentiality of *Phyllanthus amarus* against drug resistant pathogens. Nat. Prod. Res., 20(4): 323–326.

Mustafa, A.; Aris Widodo, M. and Kristianto, Y. (2012). Albumin and zinc content of snakehead fish (*Channa striata*) extract and its role in health. IEESE Int. J. Sci. Technol., 1(2):1.

Le Anh Dao, N.; Phu, T. M.; Douny, C.; Quetin-Leclercq, J.; Hue, B. T. B.; Bach, L. T.; Nhu, T. Q.; Hang, B. T. B.; Huong, D. T. T.; Phuong, N. T.; Kestemont, P. and Scippo, M. L. (2020). Screening and comparative study of in vitro antioxidant and antimicrobial activities of ethanolic extracts of selected Vietnamese plants. Int. J. Food Prop., 23(1): 481–496.

Nelson, J.S. (1994). Fishes of the World, third ed. John Wiley & Sons, Inc., New York, 600 pp.

Nordic Committee on Food Analysis. (2006). Aerobic Plate Count in Foods. NMKL method 86, fourth ed. Oslo, Norway.

Ofstad, R.; Kidman, S.; Myklebust, R. and Hermansson, A.M. (1993). Liquid loss capacity and structural changes during heating of fish muscle: Cod (*Gadus morhua* L.) and salmon (*Salmo salar*). Food struct., 12(2): 4.

Olafsdottir, G.; Martinsdóttir, E.; Oehlenschläger, J.; Dalgaard, P.; Jensen, B.; Undeland, I.; Mackie, I. M. and Nilsen, H. (1997). Methods to evaluate fish freshness in research and industry. Trends Food Sci. Technol., 8(8): 258–265.

Patel, J.R.; Tripathi, P.; Sharma, V.; Chauhan, N.S. and Dixit, V.K. (2011). *Phyllanthus amarus*: Ethnomedicinal uses, phytochemistry and pharmacology: A review. J. Ethnopharmacol., 138(2): 286–313.

Pathare, P.B.; Opara, U.L. and Al-Said, F.A.J. (2013). Colour measurement and analysis in fresh and processed foods: a review. Food Bioprocess Technol., 6(1): 36–60.

Roberts, T.; Cordier, J.; Gram, L.; Tompkin, R.; Pitt, J.; Gorris, L. and Swanson, K. (2005). Micro-Organisms in Foods 6: Microbial Ecology of Food Commodities. London, UK: Springer US.

Raharjo S.; Sofo J.N. and Schmidt G.R. (1992). Improved speed, specificity and limit of determination of an aqueous acid-extraction thiobarbituric acid - C18 method for measuring lipid-peroxydation in beef. J. Agr. Food chem., 40(11): 2182–2185.

Rong, C.; Chang-hu, X.; Qi, L. and Bang-zhong, Y. (2009). Microbiological, chemical and sensory assessment of (I) wholeungutted, (II) whole gutted and (III) filleted tilapia (*Oreochromisniloticus*) during refrigerated storage. Int. J. Food Sci. Technol., 44(11): 2243–2248.

Sallam, K.I. (2007). Antimicrobial and antioxidant effects of sodium acetate, sodium lactate, and sodium citrate in refrigerated sliced salmon. Food Control, 18(5): 566–575.

Sigurgisladottir, S.; Hafsteinsson, H.; Jonsson, A.; Lie, Ø.; Nortvedt, R.; Thomassen, M. and Torrissen, O. (1999). Textural properties of raw salmon fillets as related to sampling method. J. Food Sci., 64(1): 99–104.

Simeonidou, S. (1997) Effect of frozen storage on the quality of whole fish and fillets of horse mackerel (*Trachurus trachurus*) and mediterranean hake (*Merluccius mediterraneus*). Z. Lebensm. Unters. Forsch., 204(6): 405–410.

Sinh, L.X.; Navy, H. and Pomeroy, R.S. (2014). Value chain of snakehead fish in the Lower Mekong Basin of Cambodia and Vietnam. Aquac. Econ. Manag., 18(1): 76-96.

Song, L. M.; Munian, K.; Rashid, Z. A. and Bhassu, S. (2013). Characterisation of Asian snakehead murrel *Channa Striata* (Channidae) in Malaysia: An insight into molecular data and morphological approach. Sci. World J., 1–16.

Thiansilakul, Y.; Benjakul, S. and Richards, M.P. (2010). Changes in heme proteins and lipids associated with off-odour of seabass (*Lates calcarifer*) and red tilapia (*Oreochromis mossambicus*×*O. niloticus*) during iced storage. Food Chem., 121(4): 1109–1119.

Velho, N.P.S. (2001). Preparation for obtaining accreditation of analytical methods regarding quality issues as stated in ISO standard ISO/IEC 17025:1999. Final project report.

Vietnam Ministry of Public Health. (2012). National technical regulation of Microbiological contaminants in food (QCVN 8-3:2012/BYT). Retrieved November 15, 2020, from http://www.fsi.org.vn/pic/files/qcvn-8-3_2011-byt-ve-o-nhiem-vi-sinh-vattrong-tp_bia_merged.pdf

Viji, P.; Tanuja, S.; George, N.; Zynudheen, A.A. and Lalitha, K.V. (2014). Quality characteristics and shelf life of Sutchi catfish (*Pangasianodon Hypophthalmus*) steaks during refrigerated storage. Int. J. Agric. Food Sci. Technol., 5(2): 105–116.

Viji, P.; Tanuja, S.; Ninan, G.; Lalitha, K.V.; Zynudheen, A.A.; Binsi, P.K. and Srinivasagopal, T.K. (2015). Biochemical, textural, microbiological and sensory attributes of gutted and ungutted sutchi catfish (*Pangasianodon hypophthalmus*) stored in ice. J. Food Sci. Technol., 52(6): 3312–3321.