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# Effects of *Phyllanthus amarus* and *Euphorbia hirta* Dip Treatments on the Protection of Striped Catfish (*Pangasianodon hypophthalmus*) Fillets against Spoilage during Ice Storage

Nguyen Le Anh Dao [a,b], Tran Minh Phu [a], Caroline Douny [b], Joëlle Quetin-Leclercq [c], Bui Thi Buu Hue [d], Le Thi Bach [d], Truong Quynh Nhu [a], Bui Thi Bich Hang [a], Do Thi Thanh Huong [a], Nguyen Thanh Phuong [a], Patrick Kestemont [e], and Marie-Louise Scippo [b]

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**ABSTRACT**

This study was conducted to evaluate the effects of herbal extracts on the quality of striped catfish (*Pangasianodon hypophthalmus*) fillets throughout the storage period. Quality changes during ice storage of striped catfish fillets were studied after dip treatments in aqueous solutions of ethanolic extract of *Phyllanthus amarus* (0.02% and 0.04%, w/v) or *Euphorbia hirta* (0.06% and 0.02%, w/v). The control (dipped in tap water) and the treated fish samples were analyzed periodically for total viable counts (TVC), peroxide value, physicochemical parameters (pH, texture), and sensory properties. Results indicated that *Pseudomonas* spp. and *Listeria monocytogenes* were absent in the raw fish fillets, reflecting the safety of raw materials regarding specific spoilage and psychrotrophic pathogenic microorganisms. Fish fillets dipped in 0.04% *P. amarus* extract solution displayed lower TVC values (4.76 $\log_{10}$ CFU/g) during the initial storage period compared to treatment with *E. hirta* extracts. Dip treatments in water containing *P. amarus* or *E. hirta* extracts significantly reduced the primary lipid oxidation in fish samples. In conclusion, the dip treatment in water containing 0.04% *P. amarus* or 0.06% *E. hirta* extract was effective to maintain a good sensory quality of the fish fillets and to prolong their shelf life up to 8 days under ice storage.

**KEYWORDS**

Dip treatments; *Euphorbia hirta*; ice storage; *Pangasianodon hypophthalmus*; *Phyllanthus amarus*

## Introduction

Striped catfish (*Pangasianodon hypophthalmus*), a freshwater fish species, is the main commercial cultured fish in the Mekong delta, Vietnam (Phan et al. 2009). Striped catfish production reached 1.42 million tons in 2019 (VASEP 2019). It is mainly exported to more than 132 countries in the world, and about 10% is marketed in Vietnam.

However, as is generally the case for fish, striped catfish fillet is an easily perishable product, mainly because of its high water activity. The spoilage of fish during storage is usually caused by the microbial growth and metabolic activities, protein degradation and lipid oxidation and results in short shelf life and the decrease in flesh quality (Olatunde and Benjakul 2018).

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In the modern seafood industry, quality loss of the end products could be prevented or delayed by using chemical preservatives to control microbiological, enzymatical, or chemical changes, and prolonging the shelf life of seafood. However, these synthetic chemicals can be at risk for public health due to possible adverse effects, such as carcinogenicity, acute toxicity, and teratogenicity (Embuscado 2015; Faleiro 2011; Yang et al. 2017). Consequently, pressure exists on the agrifood industry in general, and seafood industry in particular, to replace synthetic preservative chemicals with natural alternatives having antioxidant and antimicrobial activities (Embuscado 2015; Yashin et al. 2017). Among natural products, plant extracts are good candidates as potential successful means to extend the shelf life of seafood (Erkan et al. 2011; Falguera et al. 2011). In recent years, many studies have been conducted with herbals possessing antioxidant and antimicrobial capacity, which are used to improve the quality and to maintain shelf life of perishable foods in general and of striped catfish in particular (Greeshma et al. 2019; Jeyakumari et al. 2017; Rao et al. 2017).

*Phyllanthus* and *Euphorbia* genera, belonging to the family of the Euphorbiaceae, are usually found in tropical and subtropical countries, such as India, Malaysia, Thailand, China, Vietnam, and Nigeria. These plants have been shown to display pharmacological properties (Kumar et al. 2010; Kumar Sarangi and Padhi 2017; Nguyen et al. 2015) and have been extensively used in folk medicine for treatments of a broad spectrum of diseases for thousands of years. In Vietnam, *P. amarus* and *E. hirta* were studied for their use in aquaculture for prophylaxis and treatment of fish and shrimp diseases against *Aeromonas hydrophila* and *Edwardsiella ictaluri* (Direkbusarakom 2004; Nhu et al. 2020a, 2020b, 2019a, 2019b).

*Phyllanthus amarus* Schum. et Thonn. (Mitra and Jain 1985) *(P. amarus)* is one of the herbs that shows a wide spectrum of pharmacological effects, including antioxidant, antimicrobial, anticancer, anti-inflammatory, antiviral, and anti-diabetic activities (Devi et al. 2017; Sen and Batra 2013; Tan et al. 2013; Ushie et al. 2013). It contains various bioactive compounds, including lignans, flavonoids, hydrolysable tannins, triterpenes, and alkaloids (Patel et al. 2011). The methanolic extract of *P. amarus* was found to contain high amounts of phenolic compounds. It was also shown to have free radical scavenging and to inhibit lipid peroxidation and consecutive cellular damages induced by chromium (VI) (Guha et al. 2010; Nguyen et al. 2017). In addition, the extract of *P. amarus* also showed significant antimicrobial activity against *Shigella* spp., *Escherichia coli, Staphylococcus aureus, Bacillus subtilis*, and *Pseudomonas aeruginosa*. The antibacterial action was shown to be mainly due to the phyllanthin isolated from *P. amarus* (Mazumder et al. 2006; Oluboyo et al. 2016; Senjobi et al. 2017).

Similarly, *Euphorbia hirta* L. (*E. hirta*) displays biological activities such as antioxidant, antibacterial, antifungal, antihistaminic, antiasthmatic, and anticancer (Ahmad et al. 2017; Al-Snafi 2017). Perumal et al. (2012) reported that *E. hirta* holds potential antimicrobial effects against a wide spectrum of pathogenic microorganisms and therefore can be used as a safe, reliable, economical, and natural antimicrobial source for therapeutics (Perumal et al. 2012). These findings may also be useful in the food industry, as the plant extracts could be used as preservatives to protect food from spoilage and food-borne pathogen contamination. However, there have been few studies on the preservative effect of *P. amarus* and *E. hirta* in fish flesh during ice storage, which is the research subject of the present paper.

## Materials and methods

### *Preparation of P. amarus and E. hirta extracts*

Twenty fresh plants were collected from various areas in Mekong Delta, Vietnam. The plants were authenticated by the Department of Biology, College of Natural Science, Can Tho University, and compared to the literature. Plant extracts were prepared as described by Bach et al. (2018). All collected parts of plants were then washed to remove mud and dust; the rotten and damaged parts were also discarded. Samples were air-dried in shade for some days and then put in an oven at about 50°C until

well-dried. After that, they were ground into a fine powder with a blender and stored in sealed containers in a dry and cool place. The dried-powder (100 g) was soaked in 96% ethanol (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 4 times with 96% ethanol. The filtrates from each extraction were combined, and the solvent was evaporated under reduced pressure using a rotary evaporator to provide crude ethanolic extracts. All extracted samples were lyophilized until dryness and stored at −20°C before use.

### Experimental design

Striped catfish fillets (450 fish fillet weighting about 80–100 g) were obtained from a striped catfish processing company (Can Tho, Vietnam) at the stage of trimming after skinning and divided into two batches of 225 fillets (one batch per treatment). No protective treatment was applied by the company to the fish fillets used in this study, and the fish fillets were used for the experiments the same day they were produced from the company. Striped catfish originated from the same pond, same harvest age, and were fairly similar in weight before catching.

In *P. amarus* experiment, fish fillets (225 fillets) were given a dip treatment for 30 minutes at 4°C in tap water containing 0% (negative control), 0.02%, and 0.04% (weight/volume) of *P. amarus* ethanolic extract. The ratio between fish fillet and aqueous solution of plant extract was 1:1 (w:v). After that, fish fillets were drained well for 5 minutes before packing in sterile polyethylene (PE) bags (five fillets per bag). Fish fillets were then placed into insulated box (100 L) with fish to ice ratio of 1:1 (w:w). Ice was added and water in the box was removed every day of storage. The temperature of fish fillets was recorded at the time of sampling.

The *E. hirta* experiment was done in the same way as the *P. amarus* experiment, except that *E. hirta* ethanolic extract was used at concentrations of 0% (negative control), 0.06%, and 0.2% in tap water.

Both experiments were replicated three times: three bags containing five fish fillets were prepared for each sampling time and each treatment. In total, in each experiment, 45 bags containing 5 fish fillets each were prepared.

### Samplings

Sampling was done on the day of the experimental setup (day 0) and after 4, 8, 12, 16 days of ice storage. At each sampling time and for each treatment, three bags were collected. The sampling at day 0 was done after 1 hour of ice storage. From each bag, two fish fillets were used for sensory analysis and to measure the texture property, one fish fillet was used for a duplicate total viable counts determination, and the two remaining fillets were minced together for duplicate measurement of pH and peroxide value (PV).

### Proximate composition analyses

A proximate composition analysis (moisture, protein, ash, and lipid content) was performed on six fish fillets taken from the same big batch of 450 fillets before any treatment, according to the procedures of the AOAC (2016).

### Texture analysis

The texture profile analysis (TPA) indices of fillets were determined using a texture analyzer (Model TA.XTplus Texture Analyzer, Stable Micro Systems, Godalming, UK). The conditions of the texture analyzer 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, 5 g. 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).

### Sensory property

The sensory quality of striped catfish fillets was evaluated using the quality index method (QIM) by a panel of seven trained members (Sveinsdottir et al. 2003). QIM is based on significant, well-defined changes of appearance attributes that occur in raw fish after storage, such as odor, texture, color, gaping, and surface. A score from 0 to 3 demerit (index) points was given for five quality parameters according to the specific parameter descriptions (Appendix A). 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 five 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 14 score (very bad) (Bao 2006).

Sensory evaluation of cooked striped catfish fillets in terms of taste was conducted according to Simeonidou et al. (1997). The taste of cooked fillet samples was scored using a nine-point scale. A sensory score of five was taken as the threshold of acceptability (Appendix B). The samples of fish fillet were not less than 50 g per person. Fish fillets were cooked in a steam oven at 100°C for 10 minutes. The samples were coded before serving.

### Pseudomonas spp. and Listeria monocytogenes determination

The determination of *Pseudomonas* spp. and *Listeria monocytogenes* was conducted on six striped catfish fillets before carrying out any treatment at the microbiological laboratory of Intertek Vietnam Limited Company-Total Quality Assurance provider. The tests were performed according to methods of ISO 13720:2010 and ISO 11290–2:2017, respectively.

### Total viable counts (TVC)

Striped catfish fillets were taken aseptically in a vertical flow hood, and 1 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 45°C were added. TVC were determined by counting the number of colony-forming units after incubation at 30°C for 72 h. Petri dishes containing from 25 to 250 colonies were selected for the counting, according to the Nordic Committee on Food Analysis (2006).

### Determination of pH

A 20 g sample of fish fillet was homogenized in 20 mL KCl 0.15 M. The pH was measured using a digital pH meter according to Hultmann et al. (2012).

### Peroxide value

Peroxide values were determined according to the spectrophotometric ferric thiocyanate method of International IDF standards (1991). Five grams of fish sample were added to twenty mL of chloroform: methanol mixture (2:1) (v:v), shaken for 3 hours, and extracted solution was centrifuged at 4000 rpm at 25°C for 5 minutes. The lower phase was collected for determination of fat content and considered as the sample extract for the latter analysis.

For calibration, a set of solutions of increasing $Fe^{3+}$ concentration in the range 0–4 μg/mL was prepared by successive dilutions of the working solution. The calibration curve was obtained by plotting absorbance (at 480 nm) with $Fe^{3+}$ concentration.

To determine the peroxide value, the sample extract (1 mL) was mixed with 3.9 mL chloroform: methanol (2:1). Then, 50 µL of $Fe^{2+}$ solution (0.018 M) was added, followed by 50 µL $NH_4SCN$ 30%. The solution was stirred on a vortex mixer for 15 s. The absorbance of the sample was measured at 480 nm against a blank that contained 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 regression line (y = ax + b) and the fat content of fish samples.

### Statistical analysis

All data were expressed as mean ± standard deviation and calculated using Microsoft Excel software. The data of all parameters analyzed at each sampling time were subjected to analysis of variance (one-way ANOVA) using SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

## Results and discussion

### Proximate composition

Proximate analysis was determined in the initial fish fillets used in each experiment before any treatment with plant extracts. The proximate composition of striped catfish fillets is given in Table 1. The chemical composition of striped catfish fillets used in both experiments was characterized by high moisture (83.0%) and relatively low protein (14.9%) and lipid (0.44%) contents. In this study, the percentages of moisture and protein are similar with the results of Vietnamese striped catfish fillets reported by Orban et al. (2008) (moisture 83.6% and protein 13.6%) and Karl et al. (2010) (moisture 82.1% and protein 15.7%), whereas the lipid content is much lower than 1.84% in the study of Orban et al. (2008) and 1.4% in the research of Karl et al. (2010).

### Temperature

The internal temperature of fillets collected during storage time in both experiments of dip treatment with *P. amarus* and *E. hirta* extracts were under 4°C (from 0.85 to 2.03°C, data not shown). There was no significant difference between fish fillet temperature at each sampling day. Icing is one of the most prevalent techniques for fresh fish preservation (Viji et al. 2015), which could exert increase of psychrophilic strains along with the expense of mesophilic population (Roberts et al. 2005). At the point of spoilage of iced fresh-water fish, *Pseudomonas* spp. is the dominant specific spoilage Gram-negative bacteria (Gram et al. 1989). This species has been determined as the dominant bacteria identified at the end of the shelf life of defrosted Vietnamese Pangasius products (Noseda et al. 2012). Among a few psychrotrophic pathogenic bacteria in striped catfish fillets that are of concern, *L. monocytogenes* is a recognized foodborne pathogen (RASFF 2011; Donnelly et al. 1992) that can grow below 5°C. Thus, checking the presence of *Pseudomonas* spp. and *L. monocytogenes* in the raw fish flesh is necessary, although Roberts et al. (2005) suggested that maintaining chilled storage temperatures as low as ≤2°C could inhibit the development of psychrophilic pathogens in fresh products.

**Table 1.** Proximate composition of striped catfish fillets.

| Proximate composition (%) | | | |
|---|---|---|---|
| Moisture | Ash | Lipid | Protein |
| 83.0 | 0.87 | 0.44 | 14.9 |
| (82.7–83.2) | (0.74–1.09) | (0.14–0.81) | (14.5–15.3) |

Data expressed as mean (min-max) (n = 6)

### pH

According to Huss (1988), a decrease of pH in fish muscle within the first day of death is due to the post-mortem anaerobic formation of lactic acid. During the later post-mortem changes, the production of basic compounds results in a pH more or less stable or slightly increased. However, other authors describe a post-mortem increase of the pH due to fish stress during harvesting or to the post-mortem accumulation of microbial metabolites like biogenic amines (Abbas et al. 2008; Gill 1983).

Changes in the pH values of striped catfish fillets during ice storage observed in this study are shown in Table 2. pH values obtained in both dip treatment experiments with *P. amarus* and *E. hirta* extracts during the time storage ranged from 6.38 to 6.71 and from 6.51 to 6.60, respectively. The pH values remained under 7, corresponding to a slightly increasing trend with no significant differences. These results are not surprising as, in both experiments, total volatile basic-nitrogen (TVB-N) levels in fish fillets remained below the maximum acceptable limit of 25–30 mg/100 g in ice-stored *Pangasianodon hypophthalmus* (Viji et al. 2015) (data not shown), showing that the products were not affected by the spoilage (Abbas et al. 2008; Ashie et al. 1996). These low pH values are in agreement with the study of Azam et al. (2005), showing fresh *Pangasius hypophthalmus* with pH ranging from 6.57 to 7.30 during 18 days stored on ice. In the study of Jeyakumari et al. (2017), pH values of pangasius chunks treated with spice extracts were observed at an interval of 6.25 and 6.65. Other studies reported variations of pH values between 6.2 and 6.6 for gilthead sea beam and from 6.34 to 6.69 for European sea bass muscle (Abbas et al. 2008). The differences of species, diet, season, level of stress during the catch, and type of muscle have been reported to influence post-mortem pH in fish muscle (Huss 1988).

In this study, at the beginning of storage (day 0), the fish flesh dipped with 0.04% *P. amarus* showed significantly lower ($p < .05$) pH values compared to the control and 0.02% plant extract treated fish fillets (Table 2). A similar trend was observed from day 4 to day 12, where the lower pH values of 0.04% *P. amarus* treated samples might be due to the acidic nature of *P. amarus* extract. Low pH values obtained in this study were analogous to those reported in the dip treatment of *P. hypophthalmus* with 10% *Moringa oleifera* leaf aqueous extract (Greeshma et al. 2019). The author documented that the higher the concentration used, the lower the pH values were obtained. The increase of pH values during storage was postulated to be associated with the production of volatile basic components, such as ammonia and trimethylamine from the decomposition of nitrogenous compounds by either the microbial enzymes (Ruiz-Capillas and Moral 2001) or muscle endogenous protease (Liu et al. 2018).

In the *E. hirta* extract dip treatment group, pH values of the three groups did not show much variation during the entire period of storage (Table 2). No statistically significant differences were found between pH values of the control fish fillets and of *E. hirta* extract treated fish fillets ($p > .05$), except after 4 days of storage, where the pH of the control filets was found to be significantly higher. In this study, the treatment of 0.04% *P. amarus* was seen to be able to reduce pH values (compared to control) better than *E. hirta* treatments.

**Table 2.** pH of striped catfish fillets during ice storage.

| | pH | | | | |
|---|---|---|---|---|---|
| Treatment group | Day 0 | Day 4 | Day 8 | Day 12 | Day 16 |
| *P. amarus* | | | | | |
| Control (0%) | $6.58 \pm 0.11^{b}$ | $6.67 \pm 0.05^{b}$ | $6.68 \pm 0.04^{b}$ | $6.71 \pm 0.02^{b}$ | $6.66 \pm 0.02^{b}$ |
| 0.02% | $6.62 \pm 0.03^{b}$ | $6.58 \pm 0.07^{b}$ | $6.59 \pm 0.07^{ab}$ | $6.70 \pm 0.07^{b}$ | $6.55 \pm 0.02^{a}$ |
| 0.04% | $6.38 \pm 0.04^{a}$ | $6.43 \pm 0.07^{a}$ | $6.57 \pm 0.08^{a}$ | $6.56 \pm 0.00^{a}$ | $6.56 \pm 0.06^{a}$ |
| *E. hirta* | | | | | |
| Control (0%) | $6.56 \pm 0.13^{a}$ | $6.60 \pm 0.04^{b}$ | $6.56 \pm 0.10^{a}$ | $6.55 \pm 0.10^{a}$ | $6.49 \pm 0.02^{a}$ |
| 0.06% | $6.55 \pm 0.04^{a}$ | $6.52 \pm 0.04^{a}$ | $6.55 \pm 0.06^{a}$ | $6.56 \pm 0.04^{a}$ | $6.59 \pm 0.15^{a}$ |
| 0.2% | $6.57 \pm 0.02^{a}$ | $6.51 \pm 0.02^{a}$ | $6.52 \pm 0.06^{a}$ | $6.59 \pm 0.06^{a}$ | $6.51 \pm 0.11^{a}$ |

Values are mean ± SD (n = 6). Mean values within a column with the same letter are not significantly different ($p > 0.05$).

### Microbiological quality

The presence of *Pseudomonas* spp. and *Listeria monocytogenes* in raw materials of striped catfish fillets was tested, and results showed that both bacteria were not detected in 25 grams of fish samples (Table 3). *Pseudomonas* spp. are acknowledged as specific spoilage microorganisms of iced tropical fresh water fish, in general (Ghaly et al. 2010; Gram 1993; ICMSF 2005) and defrosted Vietnamese striped catfish products at the end of their shelf-life, in particular (Noseda et al. 2012). According to Bagge-Ravn et al. (2003), *Pseudomonas* spp. was demonstrated as environmentally opportunistic bacteria that can contaminate fish fillets due to the adherence of these species on contact surfaces. Additionally, *L. monocytogenes* is a foodborne infection agent causing listeriosis in humans. It is capable of growth under refrigerated conditions (Fernandes et al. 1998) and may represent the main contamination risk of chilling fresh catfish fillets (Chen et al. 2010). Duffes (1999) found that *L. monocytogenes* could be transmitted from the raw products to processing surfaces and equipment during filleting, leading to a potential source of contamination. In this study, striped catfish fillets were not contaminated by *Pseudomonas* spp. and *L. monocytogenes*. This can reflect a good hygienic status of the processing environment and good personal hygiene (Novoslavskij et al. 2016). Furthermore, several findings revealed that the potential use of plant extracts was considered as an effective type of active coating to protect fish fillets against the microorganism hazards during cold storage. Zhuang et al. (2019) reported that using ethanolic pomegranate peel extract (0.5 mg of phenolic compounds/mL) induced the reduction of *Pseudomonas* counts in bighead carp (*Aristichthys nobilis*) fillets during chilled storage (4°C). Also, the application on cod fillets of water-soluble oregano (*Origanum vulgare*) and cranberry (*Vaccinium macrocarpon*) phenolic compound mixture (0.1 mg of phenolic compounds/mL) inhibited and inactivated *L. monocytogenes* (Lin et al. 2004).

In Vietnam, TVC is one of the standardized parameters required for evaluation of the quality of striped catfish fillets (Vietnam Standard TCVN8338: 2010). Values of TVC of striped catfish fillets during the 16-day ice storage are presented in Figure 1. The TVC values of all treatments showed an increasing trend during the 16 days storage time, although the usual errors on bacterial enumeration were seen within the range of 0.3–0.5 $\log_{10}$ CFU/g, suggesting significant difference was not obviously found among the experimental treatments. The increase of TVC in striped catfish during ice storage has been shown in various studies. Rao et al. (2017) reported that the total viable counts of striped catfish fillets coated with plant (clove, thyme) oil incorporated alginate gels increased from 4 $\log_{10}$ CFU/g to less than 5 $\log_{10}$ CFU/g during 15 days of chilled storage. In a study of striped catfish fillets dip treated with *Moringa oleifera* leaf extract, TVC of the control and treated samples increased from 4.2 log10 CFU/g to above 6 $\log_{10}$ CFU/g during 15 days stored at 2 ± 1°C (Greeshma et al. 2019).

In the *P. amarus* group, the TVC in untreated and 0.02% plant extract treated fish fillets showed an increasing trend that was not observed in the 0.04% plant extract treated fish fillets, at days 0 (i.e. after one hour of iced storage after dip treatment) and 4 (Figure 1a). This increasing trend was not observed for the TVC in the *E. hirta* group (Figure 1b). These results could be due to an inhibiting effect of the 0.04% *P. amarus* ethanolic extract treatment on microorganism development after the dip treatment, whereas this was not observed in *E. hirta* extract treatment. These findings may indicate a good correlation with the results of the *in vitro* antimicrobial activity testing, where the ethanolic extract of *P. amarus* showed a higher activity against *Aeromonas hydrophila*, one of the most active specific spoilage bacteria in chilled fish, with MIC of 156 µg/mL compared to *E. hirta* extract with MIC of 625 µg/mL (Nguyen et al. 2020). On the other hand, pH is one of the main factors that might impact the antimicrobial growth. The reciprocal relationship between studied antimicrobial plant extracts and pH were indicated in the results of TVC. It can be shown that the lower pH values of 0.04% *P. amarus*

**Table 3.** Determination of *Pseudomonas* spp. and *Listeria monocytogenes* in 25 grams of striped catfish fillets (n = 6).

| Raw materials | *Pseudomonas* spp. | *Listeria monocytogenes* |
|---|---|---|
| Striped catfish fillet | Not detected | Not detected |

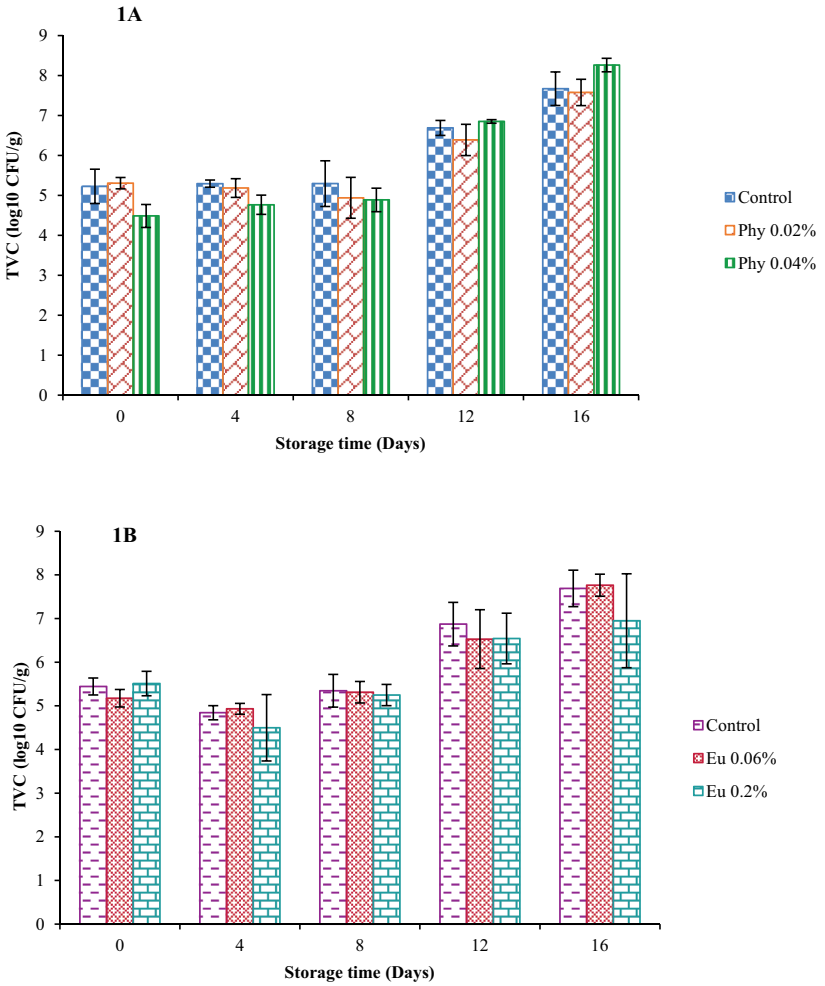


**Figure 1.** Total viable count of striped catfish fillets after *P. amarus* (Phy) (a) or *E. hirta* (Eu) (b) dip treatment during ice storage (mean ± SD, n = 6).

treatment might restrain microbial growth as well as contribute to the prolonging of the fish fillet preservation. In the group of dip treatment with *E. hirta*, no difference was observed between TVC of control and treated during the whole ice storage time (Figure 1b). At day 16, fish treated with 0.06% and 0.2% *E. hirta* displayed TVC of 7.76 and 6.95 $\log_{10}$ CFU/g, respectively, while the TVC of the control fish was 7.69 $\log_{10}$ CFU/g.

The fish samples of all treatments achieved TVC values higher than 6 $\log_{10}$ CFU/g on day 12 and reached more than 7 $\log_{10}$ CFU/g after 16 days of storage. Thus, the microbiological shelf life was about 8 days for the striped catfish fillets during ice storage, according to the microbiological acceptability limit value (<6 $\log_{10}$ CFU/g) for raw fish of the Vietnam Ministry of Public Health (2012). When comparing with the TVC acceptable level of 7 $\log_{10}$ CFU/g proposed by the International Commission on Microbiological Specifications for Foods (ICMSF 1986), the shelf life of fish fillets stored in ice would be 12 days.

In the present study, the results indicated that the use of *P. amarus* extract immersion exhibited the capacity to prevent the growth of bacteria during the initial storage period, i.e. the first hour stored in ice and on the fourth day of storage; whereas *E. hirta* extract did not show any effect on microbiological inhibition during the entire storage period.

### Peroxide value

The peroxide value allows for the determination of the fatty acid hydroperoxides, which are fatty acid primary oxidation products (Olafsdottir et al. 1997). The effect of dip treatment with *P. amarus* and *E. hirta* extracts on changes in PV of fish fillets is presented in Figure 2.

In the *P. amarus* experiment (Figure 2a), the PV of control fish fillets was 3.3 and 4.9 meq peroxide/kg fat at the beginning and the end of the storage period, respectively, while in fish fillets dipped in water containing *P. amarus* extract, PV was 3.1 meq peroxide/kg fat for both day 0 and day 16 after treatment with 0.02% extract, and 2.4 and 2.3 meq peroxide/kg fat, respectively, after treatment with 0.04% extract. After 8 and 16 days of ice storage, PV was significantly lower ($p < .05$) in fish fillets dipped in water containing 0.02% or 0.04% *P. amarus* extract than in control fish. Moreover, 0.04% *P. amarus* extract treatment seemed more effective to delay the fatty acid oxidation than 0.02%, as PV was significantly lower in fillets dipped in a solution containing 0.04% *P. amarus* extract. For some unexplained reason, the PV seems to increase after 12 days of storage in *P. amarus* extract treated fish fillets, while it remained stable in control fillets.

In the experiment of *E. hirta* dip treatment (Figure 2b), no significant difference was observed between PV of control and treated fish fillet after 0, 12, and 16 days of storage. The PV of fish fillets treated with 0.06% or 0.2% *E. hirta* extract was significantly lower ($p < .05$) than in control after 4 days of ice storage. Surprisingly, after 8 days of storage, only the PV of fish fillets treated with the lowest concentration of *E. hirta* extract (0.06%) was significantly lower ($p < .05$) than the control.

Overall, the PV remained low, around 2.5 to 5 meq peroxide/kg fat, in all samples, including the controls. These PV values are well below the acceptable limit range of PV content for human consumption of 8–10 meq peroxide/kg fat in animal foods (Schormüller 1968 cited by Linhartová et al. 2019). It has been reported that the presence of phenolic compounds in the plant extract could inhibit the production of fatty acid free radicals and postpone the initiation of the autoxidative process in fat (Hraš et al. 2000). In previous studies, researchers evidenced that herbal extracts could help to extend the shelf life of fish products, in regard to their lipid oxidation status. Amoli et al. (2019) successfully conducted a dip experiment of rainbow trout using an alginate coating containing an ethanolic extract and/or essential oil of *Mentha aquatica*. They found that the PV of rainbow trout stored at 4°C was below 7 meq peroxide/kg fish fat. Linhartová et al. (2019) demonstrated that rosemary extract could effectively delay the peroxidation in rainbow trout fillets and maintain the PV in all samples below the maximal PV established for human consumption of 8–10 meq peroxide/kg fat (Schormüller 1968 cited by Linhartová et al. 2019).

In this study, *P. amarus* extract was seen to be more active than *E. hirta* in protecting lipids of striped catfish flesh from oxidation.

### Sensory evaluation

#### Texture of fish fillets

In the present study, the texture of striped catfish fillets was determined by measuring the penetration strength of a probe. Changes in the penetration values of fish fillets during ice storage period are illustrated in Figure 3. In both experiments, no significant difference of texture was observed between control and treated fish (except one unexpected significant decrease of texture in fish treated with 0.02% *P. amarus* after 4 days of storage, Figure 3a), meaning that the plant extracts had no effect on the texture of the fish fillets in this study. Penetration values in both dip treatments tended to increase after 4 days of storage, apart from treatments of 0.02% *P. amarus* and 0.2% *E. hirta*. Afterward, a slight decreasing trend was seen over the storage period. Similar results were observed from the ice storage of sutchi catfish (Viji et al. 2015). The increase of firmness in fillets after 4 days of ice storage resulted

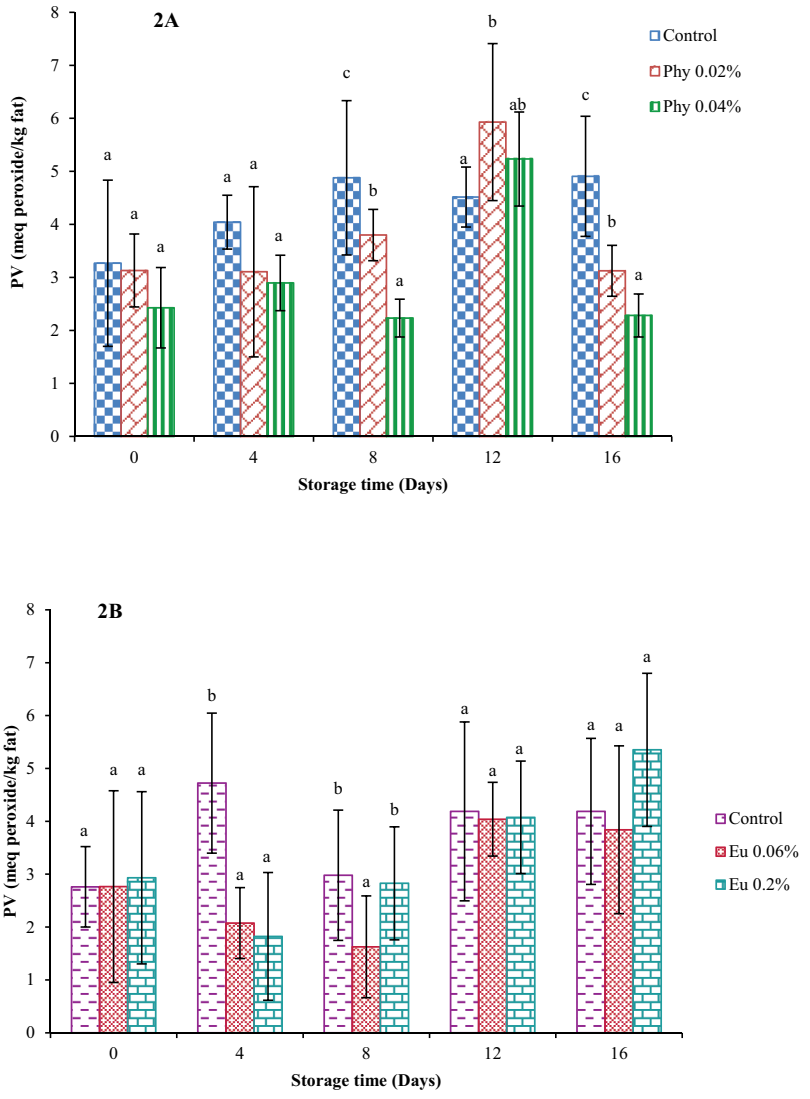


**Figure 2.** PV of striped catfish fillets after *P. amarus* (Phy) (a) or *E. hirta* (Eu) (b) dip treatment during ice storage (mean ± SD, n = 6). For the same day, same letters indicate insignificant differences between treatments ($p > .05$).

from the stage of *rigor mortis* after the fish death. After more than 4 days, the firmness decreases due to the activity of autolytic enzymes (such as collagenase and ATPase) that degrade proteins from the connective tissue and the spoilage by bacteria (Lakshmanan et al. 2003).

### *Sensory properties*

The changes in the total quality index of raw striped catfish fillets in different treated groups over the storage period are presented in Figure 4. For the scale of QI used here, zero represented absolutely fresh fish and 14 defined a completely deteriorated fish (see Appendix A). Overall, the scores of the sensory assessment exhibited a similar tendency of increasing unacceptability of the flesh samples for all groups (control and *P. amarus* and *E. hirta* extract dip treatments) (Figure 4a,b).

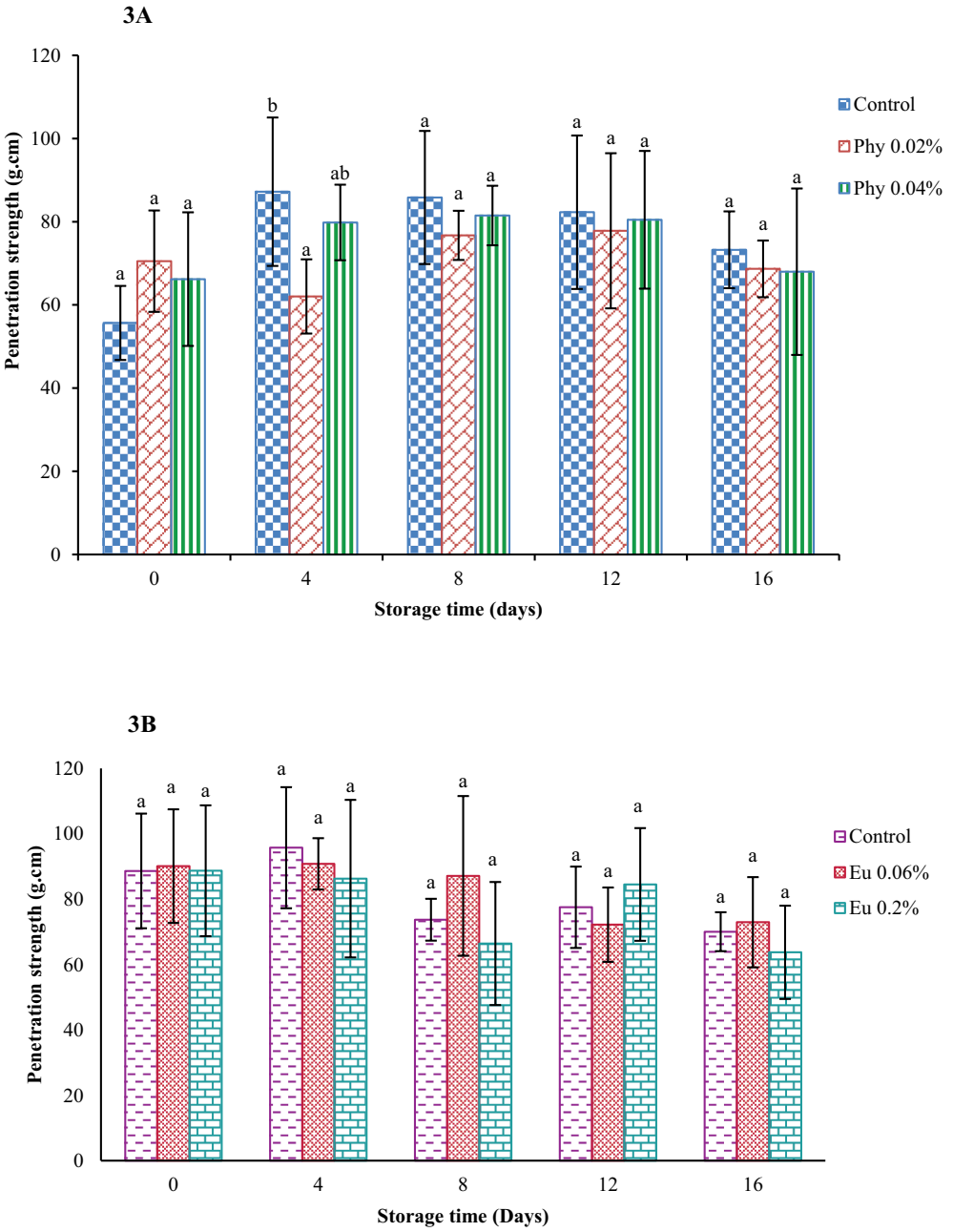


**Figure 3.** Texture property of striped catfish fillets after *P. amarus* (Phy) (a) or *E. hirta* (Eu) (b) dip treatment during ice storage (mean ± SD, n = 6). For the same day, same letters indicate insignificant differences between treatments ($p > .05$).

From day 0 to day 4, control and 0.02% *P. amarus* or 0.06% *E. hirta* treated fish presented the same quality index (of zero), whereas the group of 0.04% *P. amarus* and 0.2% *E. hirta* treated fish displayed a QI of 1, because of the higher concentration of extract that caused a negative effect on the fish fillets color (Figure 4a,b). During the remaining storage period, the groups treated with plant extracts exhibited a significantly lower QI than the control samples ($p < .05$) (Figure 4a,b) and a similar pattern of increasing unacceptability. There was a noticeable change in the control group, with the observable dull color and loss of fresh odor in fillets, compared to the fish samples of plant extract treated groups in both experiments.

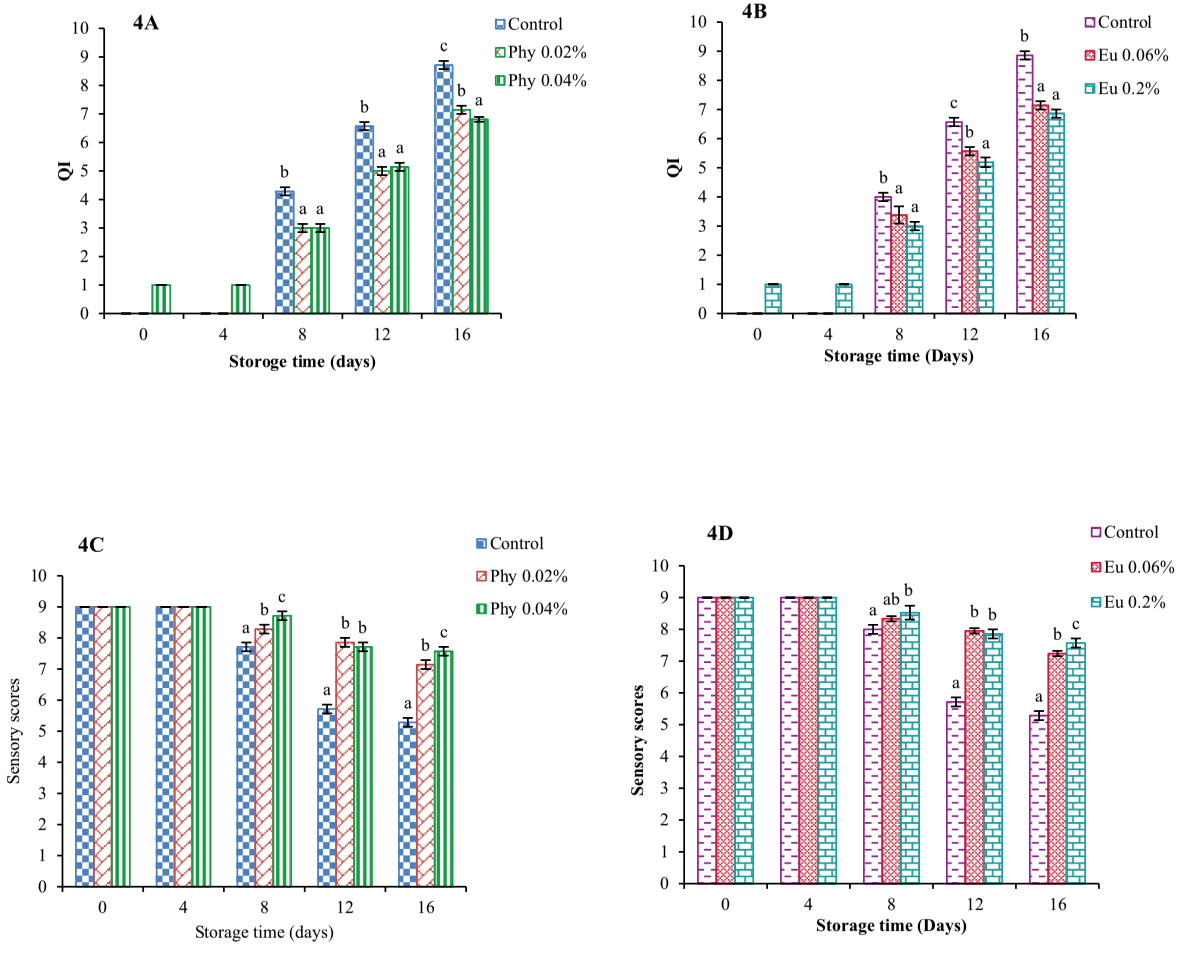


**Figure 4.** Sensory analysis of striped catfish fillets during ice storage after *P. amarus* (Phy) treatment (a, c) or *E. hirta* (Eu) treatment (b, d) (mean ± SD, n = 6). For the same day, same letters indicate insignificant differences between treatments ($p > .05$).

Acceptability score for taste of cooked striped catfish fillets was evaluated using a nine-point scale (see Appendix B). The striped fish samples were considered to be acceptable for human consumption until the sensory score reached 5 (Simeonidou et al. 1997). The scores for taste assessment of cooked fish are illustrated in Figure 4c,d. The results indicate that sensory scores showed a similar pattern of decreasing acceptability for the flesh samples of the control, *P. amarus* extract, and *E. hirta* extract treated groups with increasing storage period. Both treated groups of fish had less fishy smells and the fish with plant extracts treatment were preferred by the panelists from day 8 onwards. In addition, there were no considerable differences between 0.06% and 0.2% *E. hirta* treated samples, but 0.06% could be the best option for the acceptable flavor and color of the fillets.

## Conclusions

The treatment of 0.04% *P. amarus* extract could reduce the pH values during the initial storage period, whereas *E. hirta* extract did not present this property. Neither plant extract dip treatment affected the texture of striped catfish fillets compared to the control group. *Pseudomonas* spp. and *Listeria monocytogenes* were not detected in the fish flesh, reflecting the safety of raw materials with regards to these specific spoilage and psychrotrophic pathogenic bacteria. Based on the TVC value and sensory quality, it was shown that the fish fillets dipped in a solution containing 0.04% *P. amarus* extract or 0.06% *E. hirta* allowed the shelf life of fillets to be prolonged up to 8 days. From the data, it can be concluded that using *P. amarus* and *E. hirta* extract dip treatments on striped catfish fillets could retain their good quality characteristics in terms of sensory assessment. These conclusions were supported by the results for chemical quality analyses (peroxide value).

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## Disclosure statement

No potential conflict of interest was reported by the author(s).

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# Appendices

## Appendix A. Evaluation form for the sensory quality assessment of striped catfish fillets

**Evaluation form for raw quality assessment of striped catfish fillets**

**Name: Date:**

**Code products:**

**Instruction**: Please assess the fish fillets quality placing in front of you by using the list of descriptive terms provided below. Rate the fish on each of the quality parameters and circle the corresponding score on the scale (Table 4).

**Table 4.** Description and explanation of the properties in sensory analysis.

| Quality parameters | 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 |
| Odor | Fresh, seaweed | 0 |
| | Neutral, slightly fishy | 1 |
| | Fishy | 2 |
| | Sour, ammonia smell | 3 |
| Color | 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) | | |

## Appendix B. Evaluation form of cooked striped catfish fillets

**Instruction**: Please test the fish fillets placing in front of you and choose the best description to characterize the code by using the list of descriptive terms provided below. Write the corresponding score on the scale for the description (Table 5).

**Table 5.** Sensory evaluation of cooked striped catfish fillets.

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