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Effects of guava (*Psidium guajava*) leaf extract on the quality of striped catfish (*Pangasianodon hypophthalmus*) fillets during frozen storage

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Abstract

This study aimed to investigate the effects of guava (*Psidium guajava*) leaf extract on the quality of striped catfish (*Pangasianodon hypophthalmus*) fillets during 18 months of frozen storage (-20±2°C). The study included three treatments, soaking striped catfish fillets in cold tap water as a control treatment, in 5.04 µg/mL *Psidium guajava* extract and in 313 µg/mL *Psidium guajava* extract. The samples were stored for 18 months and sampling was done at 0, 1, 3, 6, 9, 12 and 18 months. Evaluated parameters included total viable count (TVC), physicochemical parameters (water holding capacity (WHC), total volatile basic nitrogen (TVB-N), peroxide value (PV), thiobarbituric acid reactive substances (TBARs), moisture and pH), sensory properties and colour measurement. Results showed that striped catfish fillets treated with *Psidium guajava* extract reduced lipid oxidation inhibited bacteria growth, and enhanced sensory properties compared to untreated samples. In addition, treatment of *Psidium guajava* extract did not affect the pH, moisture, WHC, TVB-N and colour of fillets during frozen storage. Based on the total viable count, physicochemical parameters and sensory quality, it can be concluded that striped catfish fillets untreated or treated with *Psidium guajava* (5.04 and 313 µg/mL) extracts can be used for up to 18 months.

1. Introduction

Striped catfish (*Pangasianodon hypophthalmus*), a freshwater fish, has been widely commercial cultured in the Mekong Delta, Vietnam with the production of 1.5 million tons in 2020 (Vietnam Association of Seafood Exporters and Producers (VASEP), 2021). Striped catfish products containing high nutritional quality (Orban *et al*., 2008) are mainly exported to more than 140 countries over the world and were marketed roughly 10% in Vietnam. However, striped catfish is an easily perishable product because of its high water activity and nutrient content (Orban *et al*., 2008). The spoilage of fish during storage is usually caused by biochemical reactions such as lipid oxidation, protein degradation, microbial growth and metabolic activities, which result in shorter shelf-life and decrease the flesh quality (Arashisara *et al*., 2004). Therefore, taking some measures to delay the deterioration of striped catfish quality and extend its preservation is economically worthwhile. The preservation of fish for longer periods can be obtained by freezing it at a temperature of -18°C or below. This method is efficient in minimizing microbial contamination; however, the enzymatic activity can continue at a slow rate in frozen fish. Many parameters can affect the survival of spoilage bacteria during freezing such as microorganisms and fish species, initial fish quality, catching methods and the handling and storage processes aboard the fishing vessel (Ghaly *et al*., 2010).

Recently, natural antioxidants from plant extracts have been studied to improve fish product preservation. Different natural bioactive compounds have been used in fish preservation for their capacity to delay lipid oxidation and microbial growth and enhance the quality of fish flesh, thus the shelf-life of fish was extended. Metwally *et al.* (2010) reported that *Psidium guajava* exhibits 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. Chen and Yen (2007) reported the antioxidant activity of *P. guajava* extract and the use of plant extracts with known antioxidant and antimicrobial properties can be important for food preservation.

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A large variety of studies have been undertaken to investigate the changes in fish quality during frozen storage (Sathivel *et al.*, 2007; Sahari *et al.*, 2009; Rodezno *et al.*, 2013; Sriket and La-ongnual, 2018). Akter *et al*. (2014) revealed that pangas catfish (*Pangasianodon hypophthalmus*) fillet could be stored for up to 120 days at -20°C whereas after 150 days became inedible. Besides, recent studies showed the positive effect of natural antioxidant compounds on fish quality during frozen storage (Yerlikaya and Gokoglu, 2010; Adeyemi *et al*., 2013; Viji *et al*., 2017; Chanraborty *et al*., 2017; Özen and Soyer, 2018). *Psidium guajava* extract was used as a soaking solution for mackerel fish (*Rastrelliger* sp.) (Riyanto and Sitorus, 2020) and giant freshwater prawn (Macrobrachium rosenbergii) (Karim *et a*l., 2018) under cold conditions, showed extended shelf-life to 10 days for the prawn while only two days for the fish. However, little work has been conducted on the effects of *P. guajava* extract on the quality of seafood as well as the frozen storage time of striped catfish fillets. Therefore, this study was conducted to evaluate the effects of *P. guajava* extracts on the quality of striped catfish (*Pangasianodon hypophthalmus*) fillets during frozen storage with the aim to determine the maximum frozen storage time and alternative methods for frozen preservation.

2. Materials and methods

2.1 Preparation of fish and plant extracts

Striped catfish fillets (100-120 g) at the stage of trimming after skinning were obtained from a processing company in Can Tho City, Vietnam. No protective treatment was applied by the company to the fish fillets used in this study. Fillets were then transported in ice to the laboratory at Can Tho University, Can Tho City, Vietnam.

The *P. guajava* leaves were collected from various areas in the Mekong Delta, Vietnam. They were identified and prepared following the description of Bach *et al.* (2018). The leaves were washed in tap water to remove mud and dust. Samples were air-dried in the shade for three days and dried in an oven at 60°C until well-dried, and then ground into a fine powder. The dried powder (100 g) was soaked in ethanol 96% (800 mL) for 24 hrs at room temperature with frequent agitation. The extracts were then decanted and filtered. The extraction was further repeated three times. The filtrates from each extraction were combined and the solvent was evaporated using a rotary evaporator to produce crude ethanolic extracts. All the well-dried crude ethanol extracts after freeze-drying were stored at 4°C until use.

The concentration of *P. guajava* extracts selected in this experiment is 5.04 mg/mL, which corresponds to the concentration of 50% DPPH exhibition (IC_{50}) and 313 mg/mL as the minimum inhibitory concentration (MIC), following the description in Dao *et al*. (2020).

2.2 Experimental design

The 126 fish fillets (100-120 g/fillet) were randomly assigned to three treatments, soaked in iced tap water (control), soaked in a solution of 5.04 mg/mL and soaked in a solution of 313 mg/mL of *P. guajava* extract. Soaking solutions were maintained below 4°C by adding ice and the soaking time was 30 mins. The ratio of fish weight and the solution was 1:1 (w:v). Thereafter, fillets were drained for 5 mins and quickly frozen under liquid nitrogen. The fillets were then packed (6 fillets/PA bag) and stored at -20±2°C. The temperature in the centre of frozen fillets was recorded, approximated at -18±2°C after 3 hours of freezing.

Sampling was undertaken after 0, 1, 3, 6, 9, 12 and 18 months of frozen storage. At each sampling time and for each treatment, six fillets were collected. Three fillets from each treatment were used individually for a sampling of total viable count (TVC) and sensory evaluation. For the other three fillets, the middle part of the fillets was used for colour measurement and the rest of the fillets were minced for measurement of pH, moisture, WHC, TVB–N, PV and TBARs.

2.3 Proximate composition analyses

The proximate composition of striped catfish fillets (moisture, protein, lipid and total ash content) was determined according to the AOAC Official Method (AOAC, 2016) on the first day of storage.

2.4 Total viable counts

Striped catfish fillets (25 g) were transferred to a sterile tube and homogenized with 225 mL of sterile normal saline water for 60 s and then diluted to decimal dilutions. The diluted solutions (1 mL) were pipetted into sterile Petri dishes and 15 mL of PCA medium was added. TVC was determined by counting the number of colony-forming units after incubation at 30°C for 48 hrs. Petri dishes containing between 25 to 250 colonies were selected for the counting according to the Nordic Committee for Food Analysis (NMKL 86, 2006).

2.5 pH value

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

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2.6 Moisture content

The moisture content was determined by drying the samples at 105°C until a constant weight was achieved.

2.7 Water holding capacity

WHC was determined by using the centrifugation method described by Ofstad *et al.* (1993). Minced muscle (1.5 g) was weighed in a 15 mL centrifugal tube and centrifuged at 4°C for 10 mins at 300×*g* using a Mikro 22-R centrifuge (Hettich zentrifugen, Germany). WHC is given as the fraction of water bound after centrifugation (% of total water).

2.8 Total volatile basic nitrogen

TVB-N was measured following the method described by Velho (2001). Five grams of fish sample were loaded into a Kjeldahl tube, followed by 2 g MgO and 50 mL distilled water. Each tube was then agitated and placed in the Kjeldahl distillation system. The distillation was performed for 5 mins and the distillate was collected in a flask containing 25 mL boric acid 1% (mixed with the indicator of methyl red/methylene blue 2:1). Afterwards, the boric acid solution was titrated with a 0.1 N sulfuric acid solution.

2.9 Peroxide value

PV was determined through the spectrophotometric ferric thiocyanate method of International Dairy Federation (1991). Fish samples (7.5 g) were extracted by 30 mL of chloroform: methanol mixture (2:1, v:v) for 3 hrs. After centrifugation at 700×*g* and at 25°C for 5 mins, the lower phase was collected for the determination of fat content and considered as the sample extract for the latter analysis. 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 and later with 50 µL NH_4SCN 30%. The solution was stirred in a vortex 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 a regression line (y = ax + b) and the fat content of the fish samples.

2.10 Thiobarbituric acid reactive substances

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

2.11 Sensory property

The sensory quality of striped catfish fillets was evaluated by a panel of seven trained members using the quality index method (QIM) (Bao, 2006). The fillets were given demerit scores of 0–2 or 0–3 points for the different attributes (colour, odour, gaping, texture and surface) according to the specific parameter descriptions. In particular, the odour was evaluated as fresh, neutral/ slightly fishy, fishy or ammonia/sour, giving 0, 1, 2 or 3 points, respectively. The other attributes evaluated were gaping (0: no gaping – 3: gaping over 75% of fillet), colour (0: homogeneous white – 3: pink or yellow), surface (0: very shiny – 2: wrinkled), and texture (0: firm and elastic – 3: very soft). The five scores were then 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).

Furthermore, 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 scale from 1 to 9, where 1 is no intensity (sharp 'off-flavour' of amines, rotten, defective fish fillets) and 9 is clear intensity (fresh sweet taste for striped catfish fillets). Three fillets from each group were used for 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.

2.12 Colour measurements

Fish samples were measured for colour at a fixed position in the middle of the fish fillet (7 cm from the head of the fish fillet) using a colorimeter (C160) according to the principle of the 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 three times. The values of L*, a*, b* were recorded (Pathare *et al.*, 2013).

2.13 Statistical analysis

All data were expressed as mean±standard deviation by Microsoft Excel software. The analysis of variance (ANOVA) was performed by using SPSS 20.0 software. The Duncan procedure was used to test for the difference between treatments (significance was defined at $p<0.05$).

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3. Results and discussion

3.1 Proximate composition of striped catfish fillets

The chemical composition of striped catfish fillets was characterized by high moisture (81.1±0.1%), relatively low protein (15.9±0.04%), lipid (1.97±0.15%) and total ash (0.79±0.02%) contents which is similar to the analysis done by Orban *et al.* (2008).

3.2 Total viable counts

Values of TVC of striped catfish fillets during the 18 -month frozen storage are presented in Figure 1. The maximum acceptable count for freshwater fish is 7 $\log_{10}$ CFU/g as recommended by the International Commission on Microbiological Specification for Foods (ICMSF, 1986) and the Vietnam Ministry of Public Health (2012) proposes that 6 $\log_{10}$ CFU/g is the microbiological acceptability limit value for human consumption. The TVC of all treatments slightly increased during frozen storage but they were all below 10^5 CFU/g. Therefore, it is acceptable for human consumption after 18 months of storage. As can be seen in Figure 1, the TVC values in the control treatment were significantly higher than TVC values in the treatments with *P. guajava* extract (313 µg/mL) in the first 9 months, and *P. guajava* extracts (5.04 µg/mL) at month 3 and month 9 of frozen storage ($p<0.05$). Moreover, the TVC values of striped catfish fillets treated with *P. guajava* 313 µg/mL extract were significantly lower than the TVC in the treatment with *P. guajava* 5.04 µg/mL extract at months 0, 1 and 9 of storage ($p<0.05$). However, after 12 months of storage, there was no significant difference in TVC values between treatments. In the present study, an inhibitory effect of the *P. guajava* extract on TVC formation was evident, especially in the case of the highest concentration tested during frozen storage. *P. guajava* exhibits potential antimicrobial effects against a wide spectrum of pathogenic microorganisms due to containing several antimicrobial compounds e.g. Quercetin-3-O-β-D-arabinopyranoside (Metwally *et al*., 2010). The decrease in the total bacterial counts during frozen storage was also reported in studies by Adeyemi *et al.* (2013) and Chanraborty *et al.* (2017) treated products with *Moringa oleifera* leaf extract.

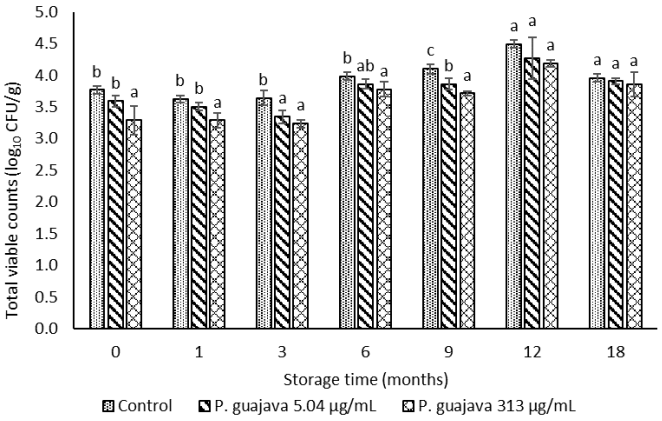

Figure 1. Total viable counts of striped catfish fillets under treatment of *P. guajava* extract during frozen storage. Values are presented as mean±SD (n = 3). Bars with different notations are statistically significantly different ($p<0.05$) between treatments of the sample sampling time analyzed using Duncan test.

3.3 pH value

pH is an important indicator used to assess fish quality. Changes in pH values of striped catfish fillets soaked in *P. guajava* solutions and control fillets after 18 months of frozen storage are shown in Table 1. In this experiment, there was no significant difference among treatments during sampling times ($p>0.05$), apart from month 0. The pH values the of three groups did not greatly vary during the storage, ranging from 6.47 to 6.98. The increase in pH is assumed to be due to an increase in the volatile basic compounds produced by either endogenous or microbial enzymes (Benjakul *et al*., 2002), associated with the TVB-N values in this study.

Table 1. pH value and total volatile basic nitrogen of striped catfish fillets under treatment of *P. guajava* extract during frozen storage.

Storage time (months)	pH value			Total volatile basic nitrogen (TVB-N; mg N/100 g)		
	Control	*P. guajava* 5.04 µg/mL	*P. guajava* 313 µg/mL	Control	*P. guajava* 5.04 µg/mL	*P. guajava* 313 µg/mL
0	6.74±0.10^{b}	6.47±0.17^{a}	6.68±0.07^{ab}	12.2±0.58^{a}	11.8±0.28^{a}	12.2±0.81^{a}
1	6.81±0.04^{a}	6.64±0.13^{a}	6.66±0.10^{a}	12.8±0.90^{a}	12.0±0.48^{a}	12.3±0.28^{a}
3	6.84±0.15^{a}	6.55±0.05^{a}	6.60±0.22^{a}	12.9±0.56^{a}	12.0±0.29^{a}	12.6±0.49^{a}
6	6.82±0.18^{a}	6.76±0.19^{a}	6.73±0.16^{a}	13.4±0.86^{a}	12.7±0.65^{a}	13.1±0.70^{a}
9	6.85±0.31^{a}	6.80±0.16^{a}	6.75±0.07^{a}	14.2±1.19^{a}	14.6±1.00^{a}	15.1±0.65^{a}
12	6.98±0.01^{a}	6.92±0.11^{a}	6.81±0.05^{a}	15.6±1.12^{a}	15.7±0.40^{a}	15.9±1.16^{a}
18	6.80±0.19^{a}	6.70±0.16^{a}	6.68±0.13^{a}	12.9±0.46^{a}	13.4±0.34^{a}	13.0±0.58^{a}

Values are presented as mean±SD (n = 3). Values with different superscripts within the same column of the same sampling time are statistically significantly different ($p<0.05$) analyzed using Duncan test.

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3.4 Total volatile basic nitrogen

TVB-N, which is mainly composed of trimethylamine, dimethylamine and ammonia, as well as other volatile basic nitrogenous compounds, is produced by spoilage bacteria, auto enzymes during preservation and the deamination of amino acids and nucleotide catabolites (Huss, 1995). Changes in the mean TVB-N values of striped catfish samples during frozen storage are shown in Table 1. Overall, the TVB-N values of all fillet samples showed a gradual increase from month 0 to month 12 (from 11.8 to 15.9 mg N/100 g), then a decrease at month 18 of storage time. The decrease of TVB-N after 18 months of storage could be due to the decomposition and evaporation of nitrogen-based compounds. However, no significant difference in TVB-N levels of striped catfish was observed in all treatments during 18 months of storage ($p>0.05$). Thus, it can be concluded that using *P. guajava* (5.04 µg/mL and 313 µg/mL) extract did not significantly affect the TVB-N of fillets over the storage period. The maximum acceptable limit proposed by Huss (1995) is 35 mg N/100 g while Lakshmanan (2000) suggested using a limit of 35-40 mg N/100 g. In this study, TVB-N values of striped catfish fillets in three treatments were lower than those limits after 18 months of storage and were then acceptable for human consumption. TVBN values presented here were also lower than those reported by Akter *et al.* (2014) and Chanraborty *et al.* (2017) on catfish after 5 months of frozen storage. This can be explained by the fact that TVB-N values depend on the species, season, catching methods, age and sex of fish (Nasopoulou *et al.*, 2012).

3.5 Water holding capacity

WHC is given as the amount of water retained after centrifugation in percentage of the original total water in the sample. Changes in WHC values of striped catfish fillets during the storage period of 18 months are depicted in Table 2. The WHC ranged from 88.0% to 95.2% during storage time. No statistically significant differences were found between the WHC of the control and the other treatments during 18 months of frozen storage ($p>0.05$). The results of the present study reveal that soaking *P. guajava* (5.04 µg/mL and 313 µg/mL) extract did not significantly impact the WHC of striped catfish fillets during the frozen storage period.

3.6 Moisture

Moisture content influences the quality of the products. The moisture of striped catfish fillets during frozen storage is presented in Table 2. The moisture content of all samples varied between 78.6% and 80.9%. It was reported that the denaturation of muscle protein in combination with the increase of degraded enzyme activities leads to free water being released out of fish muscle tissue (Tsuchiya *et al*., 1992). Overall, no significant difference in the moisture of striped catfish was observed among the three treatments over storage time ($p>0.05$). The present study indicates that treating striped catfish fillets with *P. guajava* (5.04 µg/mL and 313 µg/mL) extract did not affect the moisture of the fillets during the storage period. Similar observations of the decrease of the moisture content during frozen storage have been reported in shark (*Carcharhinus Dussumieri*) fillets (Sahari *et al*., 2009) and catfish fillets (Rodezno *et al*., 2013).

3.7 Peroxide value

During frozen storage, striped catfish can be submitted to lipid oxidation resulting in the formation of hydroperoxides as primary oxidation products. Changes in PV of striped catfish fillets soaked in *P. guajava* extract solutions and the control during frozen storage are depicted in Table 3.

Overall, PV increased in the three treatments until three months of storage, then declined until the end of the storage experiment. Similar results were reported by Özen and Soyer (2018) about using green tea, grape seed and pomegranate rind extracts in frozen storage of

Table 2. Water holding capacity and moisture of striped catfish fillets under treatment of *P. guajava* extract during frozen storage.

Storage time (months)	Water holding capacity (WHC; %)			Moisture (%)		
	Control	*P. guajava* 5.04 µg/mL	*P. guajava* 313 µg/mL	Control	*P. guajava* 5.04 µg/mL	*P. guajava* 313 µg/mL
0	90.8±0.60[a]	89.9±1.52[a]	90.6±2.34[a]	80.8±1.22[a]	79.2±1.14[a]	80.9±0.47[a]
1	94.0±1.31[a]	94.5±0.62[a]	92.9±0.04[a]	80.9±0.49[a]	80.8±1.07[a]	80.4±0.86[a]
3	95.0±0.87[a]	95.2±0.17[a]	94.3±2.23[a]	80.4±0.93[a]	80.0±1.08[a]	80.0±1.24[a]
6	90.4±0.01[a]	91.8±0.36[a]	89.7±2.16[a]	80.7±2.30[a]	80.1±0.55[a]	80.9±0.97[a]
9	91.1±0.17[a]	89.9±3.54[a]	90.8±1.46[a]	80.9±0.20[a]	79.6±0.86[a]	80.3±0.79[a]
12	94.3±0.42[a]	94.4±2.54[a]	94.6±2.19[a]	80.0±0.84[a]	79.6±0.99[a]	78.6±1.50[a]
18	90.4±2.30[a]	89.9±1.01[a]	88.0±4.40[a]	79.0±1.28[a]	79.4±0.64[a]	79.4±1.21[a]

Values are presented as mean±SD (n = 3). Values with different superscripts within the same column of the same sampling time are statistically significantly different ($p<0.05$) analyzed using Duncan test.

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mackerel (*Scomber scombrus*), where PV increased until month 4 and then gradually decreased until the end of the frozen storage period. The reduction of PV observed with extended storage time is due to the decomposition of hydroperoxide and formation of secondary oxidation products (Undeland, 2001). From month 3 onwards, the PV of the control treatment was significantly higher than those of fish treated with *P. guajava* extract at 313 µg/mL ($p<0.05$). Besides, PV was significantly lower in fillets soaked in *P. guajava* extract solution at the concentration 5.04 µg/mL compared to control fish at the stages of 9, 12 and 18th months of frozen storage ($p<0.05$). The results showed that lipid oxidation in striped catfish fillets after storage months could be delayed by soaking treatment of the fillets in *P. guajava* (5.04 and 313 µg/mL) extract solution before frozen storage. Hraš *et al*. (2000) demonstrated that the presence of phenolic compounds in plant extracts could inhibit the production of free radicals and delay the initiation of the autoxidative processes in fat. Dao *et al.* (2020) reported a high phenolic content in *P. guajava* extract with 14.5 mg gallic acid equivalent/100 mg plant extract. Generally, this PV is below the acceptable threshold of PV content for fat oxidation of 8-10 meq/kg (Linhartová *et al*., 2019) and 10-20 meq/kg (Huss, 1995; Lakshmanan, 2000). This observation was similar to the results from Viji *et al*. (2017) who reported the inhibition of hydroperoxide during frozen storage when treating products with plant extract before storage.

3.8 Thiobarbituric acid reactive substances

TBARs values are widely used to describe the degree of lipid oxidation (Sallam, 2007). The presence of TBARs is due to second-stage auto-oxidation, during which peroxides are oxidized to aldehydes and ketones, alcohols, small carboxylic acids and alkanes (Lindsay, 1991). Table 3 depicts the TBARs values measured expressed as mg of malondialdehyde (MDA) per kg of striped catfish fillets during 18 months of frozen storage. The decrease in TBARs values at the end of the storage could be due to the degradation of the secondary oxidation products formed or to the formation of protein polymers. Indeed, De Abreu *et al.* (2011) reported that TBARs could interact with other components such as proteins to form polymers when lipid or fatty acids are oxidized during frozen storage. The formation of secondary oxidation products was significantly impeded in samples treated with *P. guajava* extract. In particular, TBARs values in fish treated with *P. guajava* extract at 313 µg/mL in this experiment were always significantly lower than those of the control treatment during frozen storage ($p<0.05$), apart from month 0. Besides, at months 1, 3, 6 and 18 of storage, the fillets treated with *P. guajava* extract (5.04 µg/mL) exhibited significantly lower TBARs values than the control samples ($p<0.05$). However, no significant difference ($p>0.05$) in TBARs values was found between different concentrations of extract during frozen storage, except in month 6. The TBARs values in all treatments ranged from 0.096 to 0.532 mg malondialdehyde/kg, which is much lower than the acceptable limit for secondary oxidation. Indeed, about 1-2 mg MDA/kg of fish sample is usually taken as the limit of acceptability according to Lakshmanan (2000) or 5-8 mg MDA/kg according to Sallam (2007). The ice layer that developed on the surface of the catfish fillets during the rapid freezing might have acted as a barrier between the fish fillet and its surroundings, thus slowing down the diffusion of oxygen from the surface to the inner part of the fish fillet (Sathivel *et al*., 2007). The results proved that the treatment of *P. guajava* extract could inhibit secondary oxidation of the striped catfish fillets during frozen storage, especially in the case of the highest concentration tested extract (313 µg/mL). The TBARs values in this study were much lower than those reported by Rodezno *et al*. (2013) in frozen storage of catfish fillets, and Viji *et al*. (2017) in Indian mackerel during 8-month of frozen storage. Yerlikaya and Gokoglu (2010) demonstrated that green tea and grape seed extracts

Table 3. Peroxide value and thiobarbituric acid reactive substances of striped catfish fillets under treatment of *P. guajava* extract during frozen storage.

Storage time (months)	Peroxide value (PV; meq/kg)			Thiobarbituric acid reactive substances (TBARs; mg MDA/kg)		
	Control	*P. guajava* 5.04 µg/mL	*P. guajava* 313 µg/mL	Control	*P. guajava* 5.04 µg/mL	*P. guajava* 313 µg/mL
0	4.53±0.74^{a}	4.42±0.53^{a}	4.34±0.38^{a}	0.160±0.03^{a}	0.154±0.02^{a}	0.150±0.02^{a}
1	5.34±0.93^{a}	4.44±0.84^{a}	4.38±0.76^{a}	0.250±0.01^{b}	0.157±0.01^{a}	0.146±0.01^{a}
3	5.43±0.58^{b}	4.57±0.88^{ab}	4.44±0.95^{a}	0.286±0.03^{b}	0.234±0.02^{a}	0.217±0.01^{a}
6	4.53±0.79^{b}	3.93±0.67^{b}	3.11±0.57^{a}	0.348±0.03^{c}	0.305±0.02^{b}	0.210±0.02^{a}
9	4.16±1.18^{b}	3.02±0.59^{a}	2.76±0.32^{a}	0.173±0.01^{b}	0.156±0.02^{ab}	0.137±0.02^{a}
12	3.39±0.90^{b}	2.15±0.52^{a}	2.26±0.82^{a}	0.532±0.06^{b}	0.421±0.13^{ab}	0.293±0.09^{a}
18	3.28±0.26^{b}	1.78±0.47^{a}	1.73±0.32^{a}	0.383±0.13^{b}	0.139±0.04^{a}	0.096±0.01^{a}

Values are presented as mean±SD (n = 3). Values with different superscripts within the same column of the same sampling time are statistically significantly different ($p<0.05$) analyzed using Duncan test.

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could also effectively delay lipid oxidation in bonito (*Sarda sarda*) fillets and maintain the TBARs below those of the control samples during 5 months of frozen storage.

3.9 Sensory properties

The results of the quality index (QI) obtained after the application of all treatments to fish over the 18 months of frozen storage are presented in Figure 2A. On the scale of QI used, zero represented absolutely fresh fish and 14 was defined as a completely deteriorated fish. Generally, the scores of the sensory assessment exhibited a similar tendency of increase for the flesh samples of all treatments with increasing storage time. The fillets of the control group exhibited an observable dull colour and loss of fresh odour, compared to the fish samples treated with *P. guajava* extracts leading to a higher QI for the control samples than for the treated samples. The better sensory properties in treated fillets could be due to the antioxidant and antimicrobial activities of the guava leaf extract which delay the oxidation process and inhibit the microorganism. However, there was no significant difference between the two treatments tested during frozen storage ($p>0.05$). It can be concluded that soaking fillets with *P. guajava* extract solutions (5.04 and 313 µg/mL) before storage had better sensory properties during frozen storage compared to the control treatment from month 6 onwards of frozen storage.

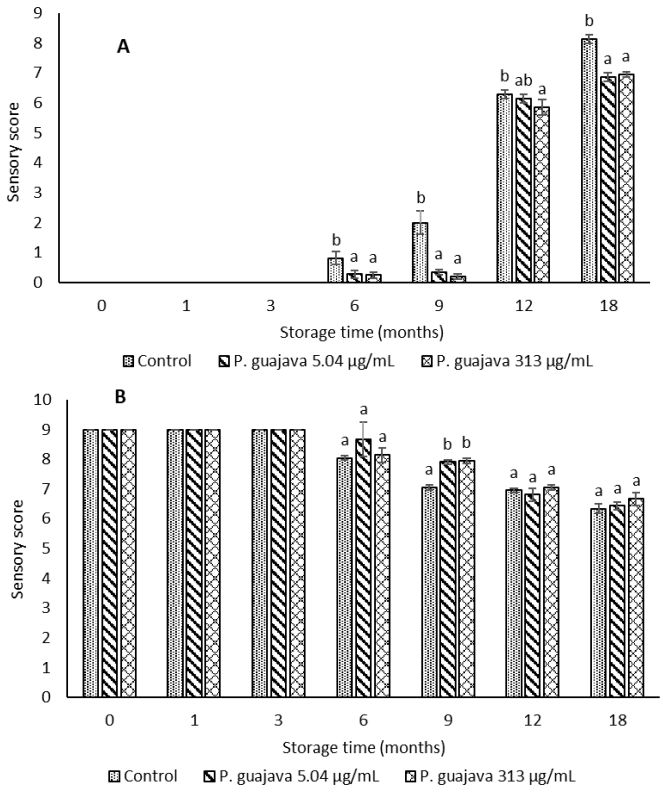

Figure 2. Sensory analysis of striped catfish fillets after no treatment (control) or treatment with *P. guajava* extract during frozen storage; (A) quality index (QI) and (B) tasting of cooked fillets. Values are presented as mean±SD (n = 3). Bars with different notations are statistically significantly different ($p<0.05$) between treatments of the sample sampling time analyzed using Duncan test.

The cooked fish samples were considered to be acceptable for human consumption if the sensory score reached 5 or more (Simeonidou *et al*., 1997). The scores of taste assessment of cooked fish are illustrated in Figure 2B. There was a significant decrease in sensory scores in all treatments following the storage period. No significant difference in sensory scores was observed between the control samples and the fillets treated with *P. guajava* extract (5.04 and 313 µg/mL) ($p>0.05$) during 18-month of storage, except for the values measured after 9 months of storage. The treatment of *P. guajava* extract had better sensory properties through the presence of herbal flavour and taste at month 9 of frozen storage.

3.10 Colour

The colour of fish and fish products is one of the most important criteria for consumers, to determine the acceptability and the price of catfish. Changes in the instrumental colour values of striped catfish fillets during frozen storage are given in Table 4. It can be seen that there was no significant difference in lightness (L*) value among control samples and *P. guajava* (5.04 and 313 µg/mL) extract-treated fish fillets during 18 months of storage ($p>0.05$). L* values of all fillet samples showed a gradually decreasing trend from the beginning to the end of the storage period (from 65.1 to 45.5). Besides, no significant difference ($p>0.05$) was observed between treatments of a* value, apart from month 3 and b* value except on month 1 of storage time. The redness (a*) values showed an increasing trend over storage time, whilst the yellowness (b*) was reduced with the storage period. The cause of these colour changes is due to the changes in the components of fish muscle, such as lipid oxidation, and enzymatic and microbial activity. Lipid oxidation and the breakdown of proteins form dark brown complexes, leading to a light colour of fish fillets decreased while the red increased. A similar result was also found by Sriket and La-ongnual (2018), where L* values of *Pangasius bocourti* fillets declined for 20 weeks of storage at -20°C. Furthermore, the decreasing trend in L* and b* values was found in the study by Rodezno *et al*. (2013) in the 6-month frozen storage of catfish fillets.

4. Conclusion

The results indicate that pre-soaking striped catfish fillets with *P. guajava* extract significantly reduced the total viable count, inhibited the formation of primary and secondary lipid oxidation, and improved the sensory

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Table 4. Colour values of striped catfish fillets under treatment of *P. guajava* extract during frozen storage.

Storage time (months)	Treatments	L*	a*	b*
0	Control	64.8±0.42[a]	-1.09±0.28[a]	8.55±0.22[a]
	P. guajava 5.04 µg/mL	64.4±0.43[a]	-1.07±0.29[a]	8.22±0.45[a]
	P. guajava 313 µg/mL	65.1±0.52[a]	-1.04±0.07[a]	8.07±0.12[a]
1	Control	64.5±0.66[a]	-1.12±0.08[a]	8.77±0.12[a]
	P. guajava 5.04 µg/mL	64.8±0.7[a]	-0.98±0.23[a]	8.45±0.03[a]
	P. guajava 313 µg/mL	64.8±0.04[a]	-1.11±0.19[a]	9.82±0.36[b]
3	Control	63.6±0.38[a]	-1.67±0.13[a]	9.41±0.99[a]
	P. guajava 5.04 µg/mL	64.1±0.50[a]	-1.41±0.19[ab]	9.10±0.67[a]
	P. guajava 313 µg/mL	63.7±0.77[a]	-1.27±0.14[b]	9.01±0.19[a]
6	Control	62.7±0.11[a]	-1.83±0.19[a]	8.52±0.74[a]
	P. guajava 5.04 µg/mL	62.5±0.22[a]	-1.49±0.13[a]	9.67±0.50[a]
	P. guajava 313 µg/mL	62.6±0.35[a]	-1.75±0.17[a]	9.53±0.80[a]
9	Control	56.7±1.74[a]	-1.65±0.31[a]	8.71±0.50[a]
	P. guajava 5.04 µg/mL	57.7±0.34[a]	-1.87±0.16[a]	8.23±0.36[a]
	P. guajava 313 µg/mL	57.6±1.72[a]	-1.46±0.23[a]	8.61±0.21[a]
12	Control	55.5±1.64[a]	-1.91±0.32[a]	8.77±0.84[a]
	P. guajava 5.04 µg/mL	55.9±0.66[a]	-1.92±0.08[a]	8.72±0.72[a]
	P. guajava 313 µg/mL	54.7±1.11[a]	-1.86±0.22[a]	8.76±0.34[a]
18	Control	45.5±0.69[a]	1.25±0.05[a]	2.23±0.60[a]
	P. guajava 5.04 µg/mL	45.5±1.37[a]	1.61±0.42[a]	2.41±0.74[a]
	P. guajava 313 µg/mL	45.9±0.55[a]	1.59±0.54[a]	3.21±0.23[a]

Values are presented as mean±SD (n = 3). Values with different superscripts within the same column of the sample sampling time are statistically significantly different ($p<0.05$) analyzed using Duncan test.

properties during frozen storage. In addition, using *P. guajava* extract did not affect the pH, moisture, TVB-N, WHC and colour compared to the control group during frozen storage. Based on the total viable count, physicochemical parameters, and sensory quality, it can be concluded that striped catfish fillets untreated or treated with *P. guajava* (5.04 and 313 µg/mL) extract can be stored for up to 18 months.

Conflict of interest

The authors declare no conflict of interest.

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