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FOOD TECHNOLOGY

Camellia sinensis extract delays microbiological and oxidative changes in striped catfish fillets during frozen storage

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ABSTRACT: *This study investigated the effects of tea leaf (Camellia sinensis) extract on the quality of striped catfish (Pangasianodon hypophthalmus) fillets during 18-months of frozen storage (-20 ± 2 °C). Fillet samples were submitted to the treatments Control (cold tap water), CS 7.63 (C. sinensis extract solution 7.63 µg / mL) and CS 625 (C. sinensis extract 625 µg / mL) and stored for 18 months, with collections performed at 0, 1, 3, 6, 9, 12 and 18 months. Total viable count, physicochemical parameters (water holding capacity, total volatile basic nitrogen, peroxide value, thiobarbituric acid reactive substances, moisture and pH), sensory properties and color measurement were evaluated. Results showed that fillets treated with C.a sinensis extracts slightly reduced lipid oxidation, inhibited bacterial growth and improved sensory properties compared to untreated samples, without causing significant changes in the other quality indicators. The findings indicated that the green tea leaf extract immersion treatments, contributed to the improved quality preservation of striped catfish fillets during frozen storage.*
Key words: *Camellia sinensis, extract, frozen storage, striped catfish.*

Extrato de chá verde age sobre a qualidade de filés de pescado durante o armazenamento por congelamento

RESUMO: *O objetivo deste estudo foi investigar os efeitos do extrato de folhas de chá verde (Camellia sinensis) na qualidade de filés de bagre listrado (Pangasianodon hypophthalmus) durante 18 meses de armazenamento congelado (-20 ± 2 ° C). O estudo incluiu três tratamentos: imersão dos filés de bagre listrado em água fria da torneira como um tratamento de controle, em solução de extrato de Camellia sinensis 7,63 µg / mL e em solução de extrato de Camellia sinensis 625 µg / mL. As amostras foram armazenadas por 18 meses e as coletas foram feitas aos zero, um, três, seis, nove, 12 e 18 meses. Os parâmetros avaliados incluíram contagem viável total, parâmetros físico-químicos (capacidade de retenção de água, nitrogênio básico volátil total, valor de peróxido, substâncias reativas ao ácido tiobarbitúrico, umidade e pH), propriedades sensoriais e medição de cor. Os resultados mostraram que os filés de bagre listrado tratados com extratos de Camellia sinensis reduziram ligeiramente a oxidação de lipídios, inibiram o crescimento de bactérias e aumentaram as propriedades sensoriais em comparação com as amostras não tratadas. Além disso, o tratamento do extrato de Camellia sinensis não afetou o pH, a umidade, a capacidade de retenção de água, o nitrogênio básico volátil total e a cor do filé durante o armazenamento congelado. Com base na contagem viável total, parâmetros físico-químicos e qualidade sensorial, pode-se concluir que filés de bagre listrados não tratados ou tratados com extratos de Camellia sinensis (7,63 e 625 µg / mL) podem ser usados por até 18 meses.*
Palavras-chave: *Camellia sinensis, extrato, armazenamento congelado, bagre listrado.*

INTRODUCTION

Striped catfish (*Pangasianodon hypophthalmus*), a freshwater fish, have been widely commercial cultured in the Mekong delta, Vietnam with the production of 1.5 million tons in 2020 (VASEP, 2021). Striped catfish products containing high nutritional quality (ORBAN et al., 2008) are exported to more than 140 countries and roughly 10% marketed 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 (ARASHISAR et al., 2004). Therefore, taking some measures to delay the

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deterioration of striped catfish quality and extend its preservation, which 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 to minimize 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 handling, and storage processes aboard the fishing vessel (GHALY et al., 2010).

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 which enhances the quality of fish flesh, thus the shelf-life of fish is able to be extended (NGUYEN et al., 2021; HUYNH et al., 2022). *Camellia sinensis* (*C. sinensis*) is a herb that shows a wide spectrum of pharmacological properties including antioxidant, antimicrobial, anticancer, anti-inflammatory, antiviral, and anti-obesity activities (KRIS-ETHERTON & KEEN, 2002; YUAN, 2013, HUANG et al., 2014). Many investigations indicated tea leaves (*C. sinensis*) as a potential antioxidant, which contains a rich source of antioxidative compounds (HARBOWY et al., 1997; GRAMZA & KORCZAK, 2005). The occurrence of antioxidant action of tea catechin was described by the donation of hydrogen atom as an acceptor of free radicals, interrupter of oxidation reaction chains, or by chelating metals (GRAMZA & KORCZAK, 2005). Thus, antioxidants in tea leaves can protect food components from oxidation in similar antioxidative mechanisms of action by vitamin C and E (BREWER, 2011). Besides, the preservative effect of tea polyphenols is mainly due to the inhibition of several enzymes. They can prevent fat oxidation, which is useful as a preservative and antioxidant in the food industry (FAN et al., 2008; SONG et al., 2011). Due to its strong antibacterial and antioxidant character, tea polyphenol is also commonly used to preserve meat and fish (YANG et al., 2009).

A large variety of studies have been undertaken to investigate the changes of fish quality during frozen storage (SATHIVEL et al., 2007; SAHARI et al., 2009; RODEZNO et al., 2013; SRIKET & LA-ONGNUAL, 2018). Recently, studies have showed the positive effect of natural antioxidant compounds in fish quality during frozen storage (YERLIKAYA & GOKOGLU, 2010; VIJI et al., 2017; CHANRABORTY et al., 2017; ÖZEN & SOYER, 2018). It is essential to carry out the study on utilization of tea polyphenol as an alternative to commercial preservatives in fish storage. However, little research has been conducted on the effects of *C. sinensis* 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 *C. sinensis* extracts on the quality of striped catfish (*Pangasianodon hypophthalmus*) fillets during frozen storage to determine the maximum frozen storage time and alternative method for frozen preservation.

MATERIALS AND METHODS

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.

The *C. sinensis* 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 h 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.

DPPH (2,2′-diphenyl-1-picrylhydrazyl) radical scavenging activity of *C. sinensis* extracts and minimum inhibitory concentration (MIC) of *C. sinensis* against *Aeromonas hydrophila* were determined following the methods described in NGUYEN et al. (2020). The concentration of 50% DPPH exhibition (IC_{50}) and MIC of *C. sinensis* extract were 7.63 µg/mL and 625 µg/mL, respectively, and used as soaking concentrations.

Experimental design

The 126 fish fillets (100–120 g/fillet) were randomly assigned into three treatments: soaked in iced tap water (control), soaked in a solution of 7.63 µg/mL, and soaked in a solution of 625 µg/mL of *C. sinensis* extract. Soaking solutions were maintained below 4 °C by adding ice and having a soaking time of 30 min. The ratio of fish weight and solution was 1:1 (w:v). Thereafter, fillets were drained in baskets for 5 min and quickly frozen under liquid nitrogen. The fillets were then packed (6 fillets/bag) and stored at -20 ± 2 °C.

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 sampling of total viable count (TVC) and sensory evaluation. For the other three fillets, the middle part of the fillets was used for color measurement and the rest of the fillets was minced for measurement of pH, moisture, protein and ash content, water holding capacity (WHC), total volatile basic nitrogen (TVB–N), peroxide value (PV), and thiobarbituric acid reactive substances (TBARs).

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) at the first day of storage.

Total viable counts (TVC)

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 (Merck, Germany) was added. Total viable counts (TVC) were determined by counting the number of colony-forming units after incubation at 30 °C for 48 h. Petri dishes containing between 25 to 250 colonies were selected for the counting according to the Nordic Committee for Food Analyses (NMKL 86, 2006).

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).

Moisture content

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

Water holding capacity

The water holding capacity (WHC) was determined by using the centrifugation method described in OFSTAD et al. (1993). Minced muscle (1.5 g) was weighed in a 15 mL centrifugal tube and centrifuged at 4 °C for 10 min 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).

Total volatile basic nitrogen

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 (Velp, Italy). The distillation was performed for 5 min 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.

Peroxide value

Peroxide values were determined through the spectrophotometric ferric thiocyanate method of International IDF Standards (1991). Fish samples (7.5 g) were extracted by 30 mL of chloroform: methanol mixture (2:1, v:v) for 3 h. After centrifugation at 700 *g* and at 25 °C for 5 min, 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, stirred roughly, and added to 50 μL NH_4SCN 30%. The solution was stirred on 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.

Thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances 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 min 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 on a vortex for 15 s and placed in a water bath at 94 °C for 5 min. Samples were cooled in a cold-water bath and the absorbance was measured with the spectrophotometer at 530 nm.

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 (color, odor, gaping, texture, and surface) according to the specific parameter descriptions. In particular, the odor 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), color (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-flavor' 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.

Color measurements

Fish samples were measured for color at a fixed position in the middle of the fish fillet (7 cm from the head of the fish fillet) using a spectrophotometer (PCE-CSM 2, PCE Instrument, UK) 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.

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 $P < 0.05$). The interactions between tea leave extracts concentration and time storage on investigated parameters were analyzed by a two-way ANOVA.

RESULTS AND DISCUSSION

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).

Changes in total viable counts of striped catfish fillets during frozen storage

Values of total viable counts (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 Vietnam Ministry of Public Health (2012) which proposed 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 it can be seen in figure 1, the TVC values in the control treatment were significantly higher than TVC values in the treatments with *C. sinensis* extract (625 µg/mL) at months 1, 3, and 12, and *C. sinensis* extract (7.63 µg/mL) at months 3 and 12 of frozen storage ($P < 0.05$). However, there was no significant difference in TVC values of striped catfish fillets between the two concentrations of extract, except on month 3. In the present study, the result indicated that soaking *C. sinensis* extract before storage slightly inhibits bacteria growth. TVC of all treatments were below 10^5 cfu/g while CHANRABORTY et al. (2017) revealed high values of TVC (above 10^6 cfu/g) after 5 months frozen storage of minced catfish treated with *Moringa oleifera* leaf extract 15%.

No interactions between tea leave extracts concentrations and time of storage were reported in TVC or other investigated parameters. Storage time seemed to negatively affect the quality of products whether they were treated with plant extracts or not.

Changes in the physicochemical parameters of striped catfish fillets during frozen storage

pH value

pH is an important indicator used to assess fish quality. Changes in pH values of striped catfish fillets soaked in *C. sinensis* solutions and control fillets after 18 months of frozen storage are shown in

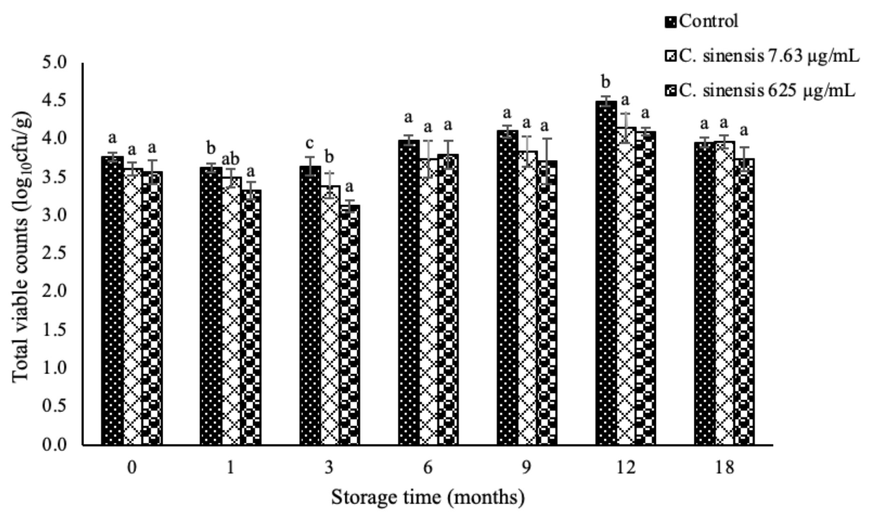

Figure 1 - Total viable counts of striped catfish fillets under treatment of *C. sinensis* extract during frozen storage. Values in the same sampling day followed by same letters indicate insignificant differences between treatments (P > 0.05, Duncan). Values are mean ± SD (n = 3).

table 1. In this experiment, there was no significant difference among treatments during sampling times. The pH values of three groups did not greatly vary during the storage, which ranged from 6.66 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 to the TVB-N values in this study. This agrees with observations reported by VARELTZIS et al. (1997) in frozen storage of horse mackerel (*Trachurus trachurus*).

Total volatile basic nitrogen

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, endogenous 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 12.2 to 16.1 mg N/100 g), then a decrease at month 18 of storage time. The decrease of TVB-N values at month 18 could be due to the long-frozen storage, the evaporation of volatile compounds in combination with interaction between basic compounds. No significant difference in TVB-N levels of striped catfish was observed in all treatments during the 18 months of storage. Thus, it can be concluded that using *C. sinensis* (7.63 µg/mL and 625 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 to use a limit of 35–40 mg N/100 g. In this study, TVB-N values of striped catfish fillets in three treatments were much lower than those limits after 18 months of storage and were then acceptable for human consumption. TVB-N values observed in this study were also lower than those reported in AKTER et al. (2014) and CHANRABORTY et al. (2017) when catfish were frozen for 5 months. 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).

Water holding capacity

Water holding capacity (WHC) is given as the amount of water retained after centrifugation in percent 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 89.4% to 95.6% during storage time. No statistically significant differences were reported between the WHC of the

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

Storage time (months)	----------------------------pH value-----------------------------			---Total volatile basic nitrogen (TVB-N; mg N/100 g)---		
	Control	*C. sinensis* 7.63 µg/mL	*C. sinensis* 625 µg/mL	Control	*C. sinensis* 7.63 µg/mL	*C. sinensis* 625 µg/mL
0	6.74 ± 0.10[a]	6.71 ± 0.06[a]	6.69 ± 0.03[a]	12.2 ± 0.58[a]	12.5 ± 0.64[a]	12.7 ± 1.06[a]
1	6.81 ± 0.04[a]	6.74 ± 0.05[a]	6.79 ± 0.03[a]	12.8 ± 0.90[a]	12.7 ± 0.16[a]	13.0 ± 0.90[a]
3	6.84 ± 0.15[a]	6.77 ± 0.07[a]	6.88 ± 0.09[a]	12.9 ± 0.56[a]	12.6 ± 0.56[a]	12.7 ± 0.65[a]
6	6.82 ± 0.18[a]	6.73 ± 0.14[a]	6.66 ± 0.20[a]	13.4 ± 0.86[a]	13.2 ± 0.28[a]	13.1 ± 0.98[a]
9	6.85 ± 0.31[a]	6.78 ± 0.02[a]	6.87 ± 0.06[a]	14.2 ± 1.19[a]	15.1 ± 0.98[a]	13.7 ± 1.26[a]
12	6.98 ± 0.01[a]	6.86 ± 0.12[a]	6.81 ± 0.12[a]	15.6 ± 1.12[a]	15.7 ± 0.40[a]	16.1 ± 1.00[a]
18	6.80 ± 0.19[a]	6.73 ± 0.07[a]	6.73 ± 0.06[a]	12.9 ± 0.46[a]	12.4 ± 1.11[a]	12.2 ± 1.43[a]

Values in the same sampling time followed by same letters indicate insignificant differences between treatments ($P > 0.05$, Duncan). Values are mean ± SD (n = 3).

control and the other treatments, apart from month 9. The results of the present study reveal that soaking *C. sinensis* (7.63 µg/mL and 625 µg/mL) extract did not significantly impact the WHC of striped catfish fillets during frozen storage period.

Moisture

Moisture content influences the quality of the products. The moisture of striped catfish fillets during frozen storage are presented in Table 2. Moisture content of all samples varied between 79.0% and 81.1%. It was reported that the denaturation of muscle protein in combination with the increase of degraded enzyme activities lead to free water being released out of fish muscle tissue (TSUCHIYA et al., 1992). Overall, no significant difference in moisture of striped catfish was observed among three treatments over storage time. The present study indicated that treating striped catfish fillet with *C. sinensis* (7.63 µg/mL and 625 µg/mL) extract did not affect the moisture of the fillets during the storage period. Similar observations of moisture content decrease during frozen storage have been reported for shark (*Carcharhinus Dussumieri*) fillets (SAHARI et al., 2009) and catfish fillets (RODEZNO et al., 2013).

Peroxide value

During frozen storage, striped catfish can be submitted to lipid oxidation resulting in the formation of hydroperoxides as primary oxidation products (OLAFSDOTTIR et al., 1997). Changes in peroxide value (PV) of striped catfish fillets soaked in *C. sinensis* extract solutions and the control during frozen storage are depicted in table 3.

Overall, PV increased in the three treatments until 3 months of storage, then declined until the end of the storage experiment. Similar results were reported by ÖZEN & SOYER (2018) about using green tea, grape seed, and pomegranate rind extracts in frozen storage of mackerel (*Scomber scombrus*), where PV increased until month 4 and then gradually decreased until the end of 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). In the first 3 months and month 18 of storage, the PV of the control treatment was significantly higher than those of fish treated with *C. sinensis* extract at 625 µg/mL ($P < 0.05$). Besides, no significant difference was reported between control samples and striped catfish fillets treated with *C. sinensis* extract at the concentration of 7.63 µg/mL during 18 months of storage time. Results showed that lipid oxidation in striped catfish fillets after storage months could be delayed by using the soaking treatment in *C. sinensis* extract (625 µg/mL) solution before being frozen in storage. HRAŠ et al. (2000) demonstrated that the presence of phenolic compounds in the plant extracts could inhibit the production of free radicals and delay the initiation of the autoxidative processes in fat. OH et al. (2013) reported a high phenolic content in *C. sinensis* extract with 14.5 mg gallic acid equivalent/100 mg plant extract. Generally, these PV are 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.

Table 2 - Water holding capacity and moisture of striped catfish fillets under treatment of *C. sinensis* extract during frozen storage.

Storage time (months)	--------------Water holding capacity (WHC; %)-------------			--------------------------Moisture (%)----------------------------		
	Control	*C. sinensis* 7.63 µg/mL	*C. sinensis* 625 µg/mL	Control	*C. sinensis* 7.63 µg/mL	*C. sinensis* 625 µg/mL
0	90.8 ± 0.60[a]	92.1 ± 1.43[a]	90.8 ± 1.47[a]	80.8 ± 1.22[a]	79.8 ± 0.27[a]	80.0 ± 0.08[a]
1	94.0 ± 1.31[a]	94.0 ± 0.04[a]	95.6 ± 1.40[a]	80.9 ± 0.49[a]	80.4 ± 0.56[a]	80.6 ± 1.15[a]
3	95.0 ± 0.87[a]	93.1 ± 0.68[a]	93.6 ± 1.86[a]	80.4 ± 0.93[a]	81.1 ± 0.15[a]	81.0 ± 0.91[a]
6	90.4 ± 0.01[a]	89.4 ± 1.75[a]	90.7 ± 1.21[a]	80.7 ± 2.30[a]	80.2 ± 0.48[a]	80.0 ± 1.08[a]
9	91.1 ± 0.16[b]	89.9 ± 0.35[a]	90.8 ± 0.40[b]	80.9 ± 0.20[a]	79.6 ± 0.04[a]	80.7 ± 1.52[a]
12	94.3 ± 0.42[a]	93.0 ± 4.38[a]	93.8 ± 1.20[a]	80.0 ± 0.84[a]	79.4 ± 0.77[a]	80.5 ± 1.05[a]
18	90.4 ± 2.30[a]	92.2 ± 4.48[a]	92.9 ± 1.99[a]	79.0 ± 1.29[a]	80.4 ± 0.87[a]	79.4 ± 0.47[a]

Values in the same sampling time followed by same letters indicate insignificant differences between treatments (P > 0.05, Duncan). Values are mean ± SD (n = 3).

(2017) who reported the inhibition of hydroperoxide during frozen storage when treating products with plant extract before storage. ÖZALP ÖZEN et al. (2011) revealed that pomegranate seed extract could effectively delay the primary oxidation in Chub mackerel (*Scomber japonicus*) and maintain PV below that of the control sample during frozen storage.

Thiobarbituric acid reactive substances

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 oxidized during frozen storage. The formation of secondary oxidation products was significantly impeded in samples treated with *C. sinensis* extract. In particular, TBARs values in fish treated with *C. sinensis* extract at concentrations of 625 µg/mL in this experiment were always significantly lower than those of the control treatment during frozen storage (P < 0.05), apart from months 0 and 9. At months 1, 3, and 18 of storage, the fillets treated with *C. sinensis* extract (7.63 µg/mL) exhibited a significantly lower TBARs values than the control samples (P < 0.05). Moreover, fish fillets treated with *C. sinensis* extract at 625 µg/mL had significantly lower TBARs values than fish fillets treated with *C. sinensis* extract at 7.63 µg/mL at months 1, 6, and 12 of storage (P < 0.05). The TBARs values in all treatments ranged from 0.099 to 0.523 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). Results proved that the treatment of *C. sinensis* extract could inhibit secondary oxidation of the striped catfish fillets during frozen storage, especially in the case of the highest concentration tested of extract (625 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 months of frozen storage. YERLIKAYA & GOKOGLU (2010) demonstrated that green tea and grape seed extracts 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. 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).

Changes in sensory properties of striped catfish fillets during frozen storage

The results of the QI obtained after the application of all treatments to fish over the 18 months

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

Storage time (months)	----------------Peroxide value (PV; meq/kg)----------------			Thiobarbituric acid reactive substances (TBARs; mg MDA/kg)		
	Control	*C. sinensis* 7.63 µg/mL	*C. sinensis* 625 µg/mL	Control	*C. sinensis* 7.63 µg/mL	*C. sinensis* 625 µg/mL
0	4.53 ± 0.74^{b}	3.79 ± 0.65^{a}	3.69 ± 0.24^{a}	0.160 ± 0.03^{a}	0.171 ± 0.01^{a}	0.151 ± 0.01^{a}
1	5.34 ± 0.93^{b}	4.63 ± 0.80^{ab}	4.29 ± 0.27^{a}	0.250 ± 0.01^{c}	0.163 ± 0.003^{b}	0.139 ± 0.01^{a}
3	5.43 ± 0.58^{b}	4.91 ± 0.76^{ab}	4.30 ± 1.02^{a}	0.286 ± 0.03^{b}	0.226 ± 0.02^{a}	0.207 ± 0.01^{a}
6	4.53 ± 0.79^{a}	3.76 ± 0.91^{a}	4.05 ± 0.60^{a}	0.348 ± 0.03^{b}	0.294 ± 0.02^{b}	0.234 ± 0.04^{a}
9	4.16 ± 1.18^{a}	3.69 ± 1.14^{a}	3.63 ± 0.80^{a}	0.173 ± 0.01^{a}	0.201 ± 0.09^{a}	0.176 ± 0.02^{a}
12	3.39 ± 0.90^{a}	3.16 ± 0.88^{a}	3.08 ± 0.63^{a}	0.523 ± 0.06^{b}	0.485 ± 0.08^{b}	0.366 ± 0.06^{a}
18	3.28 ± 0.26^{b}	2.89 ± 0.57^{ab}	2.57 ± 0.27^{a}	0.383 ± 0.13^{b}	0.168 ± 0.06^{a}	0.099 ± 0.03^{a}

Values in the same sampling time followed by same letters indicate insignificant differences between treatments (P > 0.05, Duncan). Values are mean ± SD (n = 3).

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 increasing for the flesh samples of all treatments with increasing storage time. The fillets of the control group exhibited an observable dull color and loss of fresh odor, compared to the fish samples treated with *C. sinensis* extracts leading to a higher QI for the control samples than for the treated samples. However, there was no significant difference between the two treatments tested during frozen storage except at months 9 and 18 of storage (P < 0.05). It can be concluded that soaking fillets with *C. sinensis* extract solutions (7.63 and 625 µg/mL) before storage enhanced sensory properties compared to the control treatment at months 9 and 18 of frozen storage.

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. At months 6 and 9 of storage, the sensory score of striped catfish fillets treated with *C. sinensis* extract (625 µg/mL) was significantly higher than those of the control sample (P < 0.05). Moreover, no significant difference in sensory scores was observed between the control samples and the fillets treated with *C. sinensis* (7.63 µg/mL) extract during the 18 months of storage, except for the values measured after 6 months of storage. The treatment of *C. sinensis* extract improved the sensory properties through the presence of herbal flavor and taste at month 6 and month 9 of frozen storage.

Color

The color of fish and fish products is one of the most important criteria for consumers to determine acceptability and the price of catfish (SKREDE & STOREBAKKEN, 1986). Changes in the instrumental color values of striped catfish fillets during frozen storage are given in table 4. It can be seen that there was no significant difference of lightness (L^*) value among control samples and *C. sinensis* (7.63 and 625 µg/mL) extract treated fish fillets during the 18 months of storage. L^* values of all fillet samples showed a gradually decreasing trend from the beginning to the end of storage period (from 64.8 to 44.9). Additionally, no significant difference was observed between treatments of a^* value, apart from month 1. The redness (a^*) values showed an increasing trend over storage time, whilst the yellowness (b^*) was reduced with storage period. The cause of these color changes is due to the changes in the components of fish muscle, such as lipid oxidation, enzymatic, and microbial activity. Lipid oxidation and the breakdown of proteins form dark brown complexes, leading to a light color of fish fillets decreasing while the red increased. Similar results were also found by SRIKET & 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 a study by RODEZNO et al. (2013) in a 6 month frozen storage of catfish fillets.

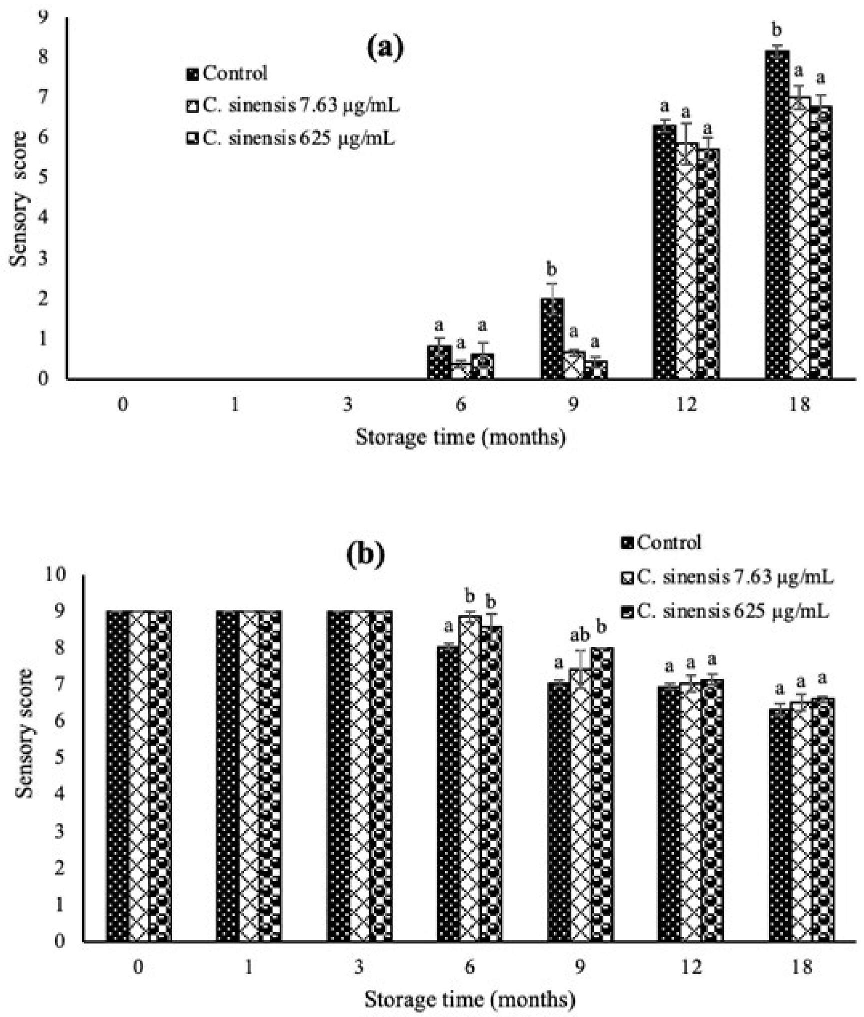

Figure 2 - Sensory analysis of striped catfish fillets after no treatment (control) or with treatment with *C. sinensis* extracts during frozen storage: quality index (QI) (a) and tasting of cooked fillets (b). Values in the same sampling time followed by same letters indicate insignificant differences between treatments (P > 0.05, Duncan). Values are mean ± SD (n = 3).

CONCLUSION

Results indicated that pre-soaking striped catfish fillets with *C. sinensis* extract slightly reduced the total viable count, inhibited the formation of primary and secondary lipid oxidation, and improved the sensory properties during frozen storage. In addition, using *C. sinensis* extract did not affect the pH, moisture, TVB-N, WHC, and color compared to the control group during frozen storage. Based on

Table 4 - Color values of striped catfish fillets under treatment of *C. sinensis* 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]
	C. sinensis 7.63 µg/mL	64.5 ± 0.66[a]	-0.66 ± 0.39[a]	9.11 ± 0.03[ab]
	C. sinensis 625 µg/mL	64.2 ± 0.73[a]	-1.33 ± 0.48[a]	9.54 ± 0.70[b]
1	Control	64.5 ± 0.66[a]	-1.12 ± 0.08[b]	8.77 ± 0.12[a]
	C. sinensis 7.63 µg/mL	64.3 ± 0.95[a]	-0.82 ± 0.18[c]	10.55 ± 0.37[b]
	C. sinensis 625 µg/mL	64.5 ± 0.12[a]	-1.44 ± 0.10[a]	9.91 ± 0.53[b]
3	Control	63.6 ± 0.38[a]	-1.67 ± 0.13[a]	9.41 ± 0.99[a]
	C. sinensis 7.63 µg/mL	64.0 ± 0.70[a]	-1.35 ± 0.08[a]	9.81 ± 1.05[a]
	C. sinensis 625 µg/mL	63.7 ± 0.56[a]	-1.72 ± 0.29[a]	8.72 ± 0.09[a]
6	Control	62.7 ± 0.11[a]	-1.83 ± 0.19[a]	8.52 ± 0.74[a]
	C. sinensis 7.63 µg/mL	62.5 ± 0.41[a]	-1.60 ± 0.08[a]	9.88 ± 0.64[b]
	C. sinensis 625 µg/mL	62.7 ± 0.24[a]	-1.76 ± 0.08[a]	9.72 ± 0.45[ab]
9	Control	56.7 ± 1.74[a]	-1.65 ± 0.31[a]	8.71 ± 0.50[a]
	C. sinensis 7.63 µg/mL	55.9 ± 1.46[a]	-1.65 ± 0.37[a]	8.72 ± 0.60[a]
	C. sinensis 625 µg/mL	54.6 ± 1.68[a]	-1.72 ± 0.15[a]	8.80 ± 0.67[a]
12	Control	55.5 ± 1.64[a]	-1.91 ± 0.32[a]	8.77 ± 0.84[a]
	C. sinensis 7.63 µg/mL	54.1 ± 1.88[a]	-2.03 ± 0.13[a]	8.90 ± 1.18[a]
	C. sinensis 625 µg/mL	54.6 ± 0.97[a]	-1.77 ± 0.18[a]	8.99 ± 0.75[a]
18	Control	45.5 ± 0.69[a]	1.25 ± 0.05[a]	2.23 ± 0.60[a]
	C. sinensis 7.63 µg/mL	46.3 ± 1.00[a]	1.53 ± 0.17[a]	2.52 ± 0.43[a]
	C. sinensis 625 µg/mL	44.9 ± 1.61[a]	1.33 ± 0.61[a]	3.11 ± 0.50[a]

Values in the same sampling time followed by same letters indicate insignificant differences between treatments ($P > 0.05$, Duncan). Values are mean ± SD ($n = 3$).

the total viable count, physicochemical parameters and sensory quality, it can be concluded that striped catfish fillets untreated or treated with *C. sinensis* (7.63 and 625 µg/mL) extract can be stored for up to 18 months before consumption.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest for this article. The founding sponsors had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the manuscript. All authors critically revised the manuscript and approved of the final version.

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