

Effect of *Camellia sinensis* and *Euphorbia hirta* extracts on the quality of cobia (*Rachycentron canadum*) fillets during ice storage

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Abstract. The present research evaluated the effects of *Camellia sinensis* and *Euphorbia hirta* on the quality of cobia (*Rachycentron canadum*) fillets over a period of 15 days storage at 4°C. The study included three treatments: soaking cobia fillets in cold tap water as a control treatment, in 0.06% of *C. sinensis* extract solution and in 0.06% of *E. hirta* extract solution. The samples were stored for 15 days and sampling was done at 1, 5, 10 and 15 days. Evaluated parameters included 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. Results showed that cobia fillets treated with *C. sinensis* and *E. hirta* extracts showed reduced lipid oxidation, inhibited bacteria growth, and enhanced sensory properties compared to untreated samples. In addition, treatment of two extracts did not affect the pH, moisture, water holding capacity, total volatile basic nitrogen, and color of fillets during ice storage. Based on the total viable count, shelf-life of cobia fillets untreated or treated with two extracts can be prolonged up to 10 days.

Key Words: cobia, color, sensory properties, shelf-life, total viable count.

Introduction. Cobia (*Rachycentron canadum*) with excellent characteristics, for example good fillet quality, high commercial value, and fast growth, is a noteworthy candidate species for commercial aquaculture. Cobia is a fish with quite high lipid content, lipid in fish muscle mainly polyunsaturated fatty acids. Cobia contains a lot of polyunsaturated fatty acids. Consumption of such fish could reduce the risk of heart disease (Vanschoonbeek et al 2003), increase ability of brain cell formation in children, as well as eye function, and immunity (Innis 1991). Thus, cobia is one of the foods which provide high nutritional value for consumers. However, the lipid content in fish is quite high so they are easily oxidized during storage and processing. This is the main cause of spoilage in food, the formation of odorous compounds and potentially harmful substances in food (Babovic et al 2010). Fat oxidations decrease the quality of food, changes color, taste, muscle structure and forme compounds harmful for health (Kanner 1994). The spoilage of fish during storage is usually caused by the microbial growth and metabolic activities, protein degradation, lipid oxidation and results in short shelf life and the decrease in flesh quality (Arashisar et al 2004).

In recent years, natural antioxidants from plant extracts have been studied to improve fish products preservation. Different natural bioactive compounds have been used in fish preservation for their capacity to retard the lipid oxidation and microbial growth, enhance the quality of fish flesh, thus the shelf-life storage of fish was extended. *Camellia sinensis* leaves show a wide spectrum of pharmacological properties including antioxidant, antimicrobial, anticancer, anti-inflammatory, antiviral and antidiabetic activities (Kris-Etherton & Keen 2002; Yuan 2013; Huang et al 2014). Leaves contain various bioactive compounds e.g., flavonoids, catechols, tannins, alkaloids (caffeine, theophylline, theobromine, xanthin), vitamins C, B1, B2, B3 and enzymes (Chevallier 2000; Yang et al 2011). The preservative effect of tea polyphenols is mainly due to the

inhibition of several enzymes, and thus prevents fat oxidation, which is useful as a preservative and antioxidant in food industry (Fan et al 2008; Song et al 2011). Owing to its strong antibacterial and antioxidant character, tea polyphenol is also commonly used to preserve meat and fish (Yang et al 2009). Analogously, *Euphorbia hirta* shows biological activities such as antioxidant activities from different parts (Basma et al 2011), antibacterial and antifungal activities from leaves, flowers, stems and roots of the plant (Rajeh et al 2010). Perumal et al (2012) reported that *E. hirta* hold potential antimicrobial effects against plenty of pathogenic microorganisms and therefore can be used as a safe, reliable, economical, and natural antimicrobial source for therapeutics. Dao et al (2020) revealed that *E. hirta* extract was the fourth strongest antioxidant extract among 20 Vietnamese plant extracts tested.

In recent years, a lot of studies were conducted about herbals possessing antioxidants and antimicrobials capacity which are used to store fish, improve the quality and to maintain shelf life of fish products (Fan et al 2008; Feng et al 2012; Li et al 2012). However, little work has been conducted on the effects of *C. sinensis* and *E. hirta* extracts to the quality of seafood under storage. Therefore, this study was conducted to evaluate the influence of *C. sinensis* and *E. hirta* extracts on the quality of cobia (*R. canadum*) fillets during ice storage with the aim to provide an improved method for ice preservation.

Material and Method

Preparation of fish and plant extracts. Cobia were reared from sea cage in Nam Du Island, Kien Giang Province and slaughtered in July 2018. At the start of experiment, cobia (24 fish; 18 males and 6 females; 4.4 ± 0.8 kg) was harvested by a sweep net. Fish were killed by ice for 5 minutes in square plastic container (1000 L). Fish were bled for 10 minutes, then scales were removed manually. The length and weight of the fish were recorded, weight of gonads and liver were also recorded, after which each fish was filleted pre-rigor with individually labelled fillets.

The two plants were collected from various areas in the Mekong Delta, Vietnam. They were identified and prepared following the description of Bach et al (2018). All collected parts of plants 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 solvent-containing 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 in the refrigerator until use.

Minimum inhibitory concentration (MIC) of *C. sinensis* and *E. hirta* extracts against *Aeromonas hydrophila* were determined following the methods described in Dao et al (2020). The MIC values of both *C. sinensis* and *E. hirta* extracts were $625 \mu\text{g mL}^{-1}$ (corresponding to 0.06%), and they were used as soaking concentrations.

Experimental design. In this study, 48 cobia fillets (950 ± 180 g per fillet) from 6 females and 18 males were randomly assigned into three treatments: soaked in iced tap water (control), soaked in a solution of 0.06% of *C. sinensis* extract and soaked in a solution of 0.06% of *E. hirta* extract. Soaking solutions were maintained below 4°C by adding ice and the soaking time was 30 min. Ratio of fish weight and solution was 1:1 (w:v). Thereafter, fillets were drained for 5 minutes before being packed in polyethylene (PE) bags (1 fillet per bag). The fillets were placed into insulated boxes (100 L) with fish and ice at a ratio of 1:1 (w:w). Fish were transported to College of Aquaculture and Fisheries, Can Tho University. Ice was added and water in the box was removed every day of storage to maintain the temperature below 4°C during the entire storage time. The day of slaughter was considered as day 0.

Sampling was undertaken on days 1st, 5th, 10th, and 15th of ice storage. At each sampling time and for each treatment, four fillets were collected including three male fillets and one female fillet. From each fillet, fish fillets were used for sampling of total

viable counts (TVC), the top part of fish fillets were used for color measurement, the middle of fish fillets were used for sensory analysis and the rest of fish fillets were minced individually for measurement of pH, moisture, water holding capacity (WHC), total volatile base nitrogen (TVB-N), peroxide value (PV), thiobarbituric acid reactive substances (TBARs).

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

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

Total viable counts (TVC). Cobia 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 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 at 105°C until constant weight.

Water holding capacity (WHC). 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 minutes at 300 g using a Mikro 22-R centrifuge (Hettich zentrifugen, Germany). WHC is given as fraction of water bound after centrifugation (% of total water).

Total volatile base nitrogen (TVB-N). 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 minutes, and then the distillate was collected in a flask containing 25 mL boric acid 1% (mixed indicator of methyl red/methylene blue 2:1). Afterward, the boric acid solution was titrated with a 0.1 N sulfuric acid solution.

Peroxide value (PV). PVs 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 hours. After centrifugation at 700 g 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. The sample extract (1 mL) was mixed with 3.9 mL chloroform:methanol (2:1). Then, 50 µL of Fe²⁺ solution (0.018 M) was added and later with 50 µL NH₄SCN 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) per kg fish fat, were calculated based on the concentration of Fe³⁺ determined from regression line ($y = ax + b$) and the fat content of the fish samples.

Thiobarbituric acid reactive substances (TBARs). TBARs were determined according to the spectrophotometric method of Raharjo et al (1992). Fish samples were homogenized and extracted in duplicated in TCA 5%. After centrifugation at 1050 g for 15 minutes at 4°C, the supernatant was collected into 50.0 mL in a volumetric flask,

filled up them by distilled water. In the test tubes, 2.0 mL of each extracted sample and TEP standard solution was added, following an addition of 2.0 mL of TBA reagent 80 mM. The solution was stirred on a vortex mixer for 15 s and placed in a water bath at 94°C for 5 minutes. Samples were cooled in a cold-water bath and the absorbance with the spectrophotometer at 530 nm measured.

Sensory property. The sensory quality of cobia fillets was evaluated by a panel of seven trained members using the quality index method (QIM) (Sveinsdottir et al 2003). 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 cobia 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 cobia fillets). On the day of analysis, the fillets, without skin and bones, were cut in 6-7 cm and then 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 top part of the piece fillet, 4-5 cm from the head of the fish, using a spectrophotometer (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^* indicated the position between red (+) and green (-), and b^* indicated the position between yellow (+) and blue (-). Each treatment was repeated four times (Pathare et al 2013). The values of L^* , a^* , b^* were recorded.

Statistical analysis. All data were expressed as mean \pm 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 $p < 0.05$).

Results and Discussion

Proximate composition. The chemical composition of cobia fillets was characterized by high moisture ($72.7\pm0.02\%$), relatively high protein ($18.7\pm0.76\%$) and lipid ($7.08\pm0.11\%$) and relatively low mineral ($1.25\pm0.02\%$) contents. In this study, the percentage of moisture content was lower than the result of Taheri et al (2012), in which measured moisture of 75.3%, lipid of 5.31%, protein of 16.6% and total ash of 0.97% in cobia fillets were reported.

Temperature. The central temperature of fillets recorded during ice storage for all treatments was below 4°C (from 0.70 ± 0.14 to $1.38\pm0.29^\circ\text{C}$). There was no significant difference in core temperature of the fillets between treatments at each sampling interval ($p > 0.05$). Icing is one of the most prevalent techniques for fresh fish preservation (Roberts et al 2005). Thus, fillet stored in ice in this experiment satisfy the requirements of cryopreservation.

Total viable counts (TVC). Changes of TVC of cobia fillets during the refrigerated storage are given in Figure 1. The TVC values of fish fillets in the control treatment were significantly higher than the TVC in the treatment with *E. hirta* extract during storage time ($p < 0.05$), except for day 10. At the day 5 day 15 of storage, the cobia fillets treated with *C. sinensis* extract showed a significant lower ($p < 0.05$) TVC values compared to the control fish. Moreover, the TVC values of fish fillets treated with *E. hirta*

extract were significantly lower than in the treatment with *C. sinensis* extract at day 1 and day 5 of ice storage ($p < 0.05$). The TVC values of three treatments increased gradually over the 15 days of storage. The maximum microbiological acceptable count for fresh fish was $7 \log_{10}\text{cfu g}^{-1}$ as recommended by International Commission on Microbiological Specification for Foods (ICMSF 1986) and Vietnam Ministry of Public Health (2012) proposed that $6 \log_{10}\text{cfu g}^{-1}$ was the microbiological acceptability limit value for human consumption. At day 15 of storage, the TVC values reached above $7 \log_{10}\text{cfu g}^{-1}$ for the all treatments, therefore, a microbiological shelf-life was about 10 days for the cobia fillets during ice storage. In the present study, the results indicated that the use of plant extracts immersion exhibited the capacity in preventing the growth of bacteria, especially soaking with *E. hirta* extract at concentrations of 0.06%. The increasing of TVC during storage were reported by Cakli et al (2007) in sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) refrigerated storage. Feng et al (2012) reported that TVC value of black sea bream (*Sparus macrocephalus*) exceeded $6 \log_{10}\text{cfu g}^{-1}$ at day 9 for a control sample, while fish treated with 0.2% tea polyphenol had a TVC value of more than $6 \log_{10}\text{cfu g}^{-1}$ on day 12 of cold storage. In another study by Bahmani et al (2011), a TVC value of golden gray mullet (*Liza aurata*) at day 12 ($> 6 \log_{10}\text{cfu g}^{-1}$) and on day 16 ($> 7 \log_{10}\text{cfu g}^{-1}$) of stored in ice was noted.

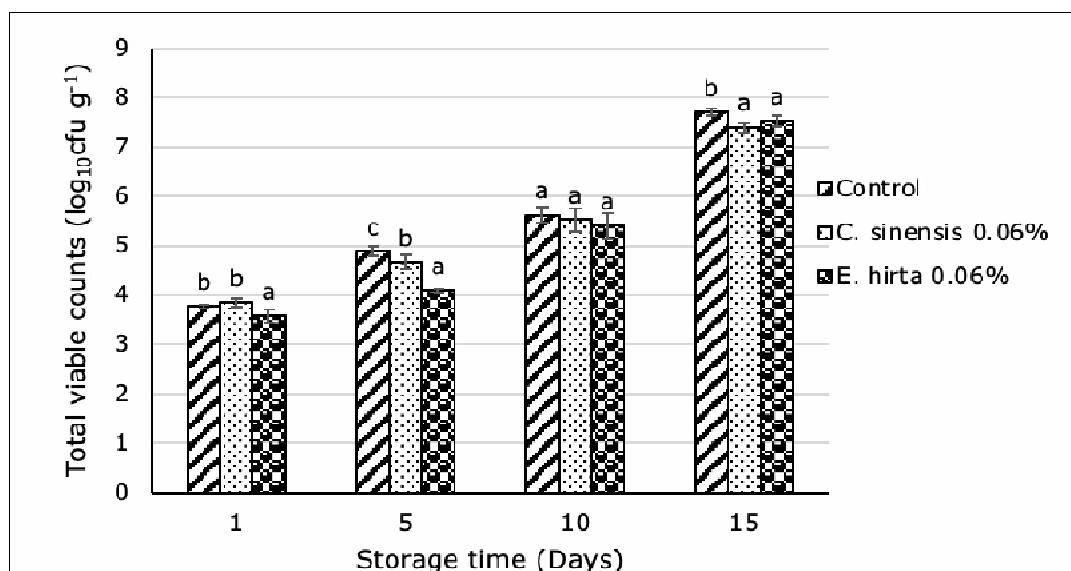


Figure 1. Total viable counts of cobia fillets under treatment of *C. sinensis* 0.06% and *E. hirta* 0.06% extracts during ice storage (values in the same sampling time followed by same letters indicate insignificant differences between treatments ($p > 0.05$, Duncan). Values are mean \pm SD ($n = 4$)).

pH value. pH is an important indicator used to assess fish quality. Changes in pH value of cobia fillets soaked in extract solutions and the control during 15 days of storage are indicated in Table 1.

Table 1
pH of cobia fillets under treatment of *C. sinensis* 0.06% and *E. hirta* 0.06% extracts during ice storage

Samples	pH			
	1	5	10	15
Control	5.93 ± 0.07^a	5.87 ± 0.03^a	5.93 ± 0.01^a	6.07 ± 0.06^a
<i>C. sinensis</i> 0.06%	5.91 ± 0.04^a	5.92 ± 0.06^b	5.97 ± 0.03^b	6.02 ± 0.06^a
<i>E. hirta</i> 0.06%	5.99 ± 0.05^b	5.89 ± 0.04^a	6.01 ± 0.02^c	6.04 ± 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 \pm SD ($n = 4$).

As seen from the table, pH values obtained in three treatments did not greatly vary during the storage, ranging from 5.87 to 6.07. The muscle pH remained under 7, showing that the products were not affected by the spoilage. Increases in pH was assumed to be due to an increase in the volatile basic compounds produced by either endogenous or microbial enzymes (Ruiz-Capillas & Moral 2005), and the decomposition of nitrogenous components (Benjakul et al 2002). The pH values in the control treatment were significantly lower than pH values in the treatments with *E. hirta* extract at day 1 and day 10, and *C. sinensis* extract at day 5 and day 10 of ice storage ($p < 0.05$). However, no statistically significant differences were found for the muscle pH of the control and other treatments in day 15 of storage time ($p > 0.05$). The pH value is related to the post-mortem evolution of the flesh and is influenced by the species, diet, seasons, level of activity or stress during the catch as well as category of muscle (Periago et al 2005). This agrees with observations reported by Li et al (2012) for yellow grouper (*Epinephelus awoara*) and in black sea bream (*Sparus macrocephalus*) (Feng et al 2012).

Water holding capacity (WHC). The 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 cobia fillets during a period of 15 days are depicted in Figure 2.

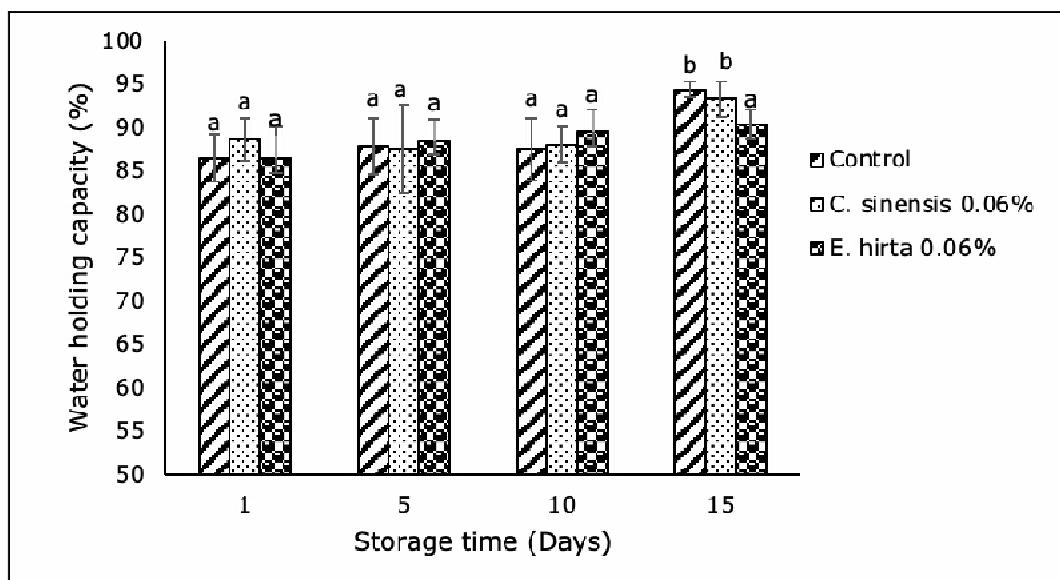


Figure 2. Water holding capacity of cobia fillets under treatment of *C. sinensis* 0.06% and *E. hirta* 0.06% extracts during ice storage (values in the same sampling time followed by same letters indicate insignificant differences between treatments ($p > 0.05$, Duncan)).

Values are mean \pm SD ($n = 4$).

In general, the treated samples showed a gradual increase in WHC over storage time, increasing ranging from 86.5 to 94.8%. The increasing of WHC similar observations during ice storage of fish have been reported (Herland et al 2007; Digre et al 2011). The increase in WHC was probably due to the growth of spoilage bacteria, the increase proteolytic activities leading degradation of protein, which contributes to the increased WHC (Olsson et al 2003). No statistically significant differences were found between the WHC of the control and the other treatments from day 1 to day 10 of storage time ($p > 0.05$). The results showed that soaking *C. sinensis* and *E. hirta* did not significantly affect the WHC of fish fillets during the storage period.

Total volatile base nitrogen (TVB-N). TVB-N value is considered as a poor indicator of fish freshness (Castro et al 2006), it has been commonly used to evaluate fish muscle spoilage (Mazorra-Manzano et al 2000). For several fish species, TVB-N values were reported to increase curvilinearly or linearly with time (Mazorra-Manzano et al 2000).

Changes in the mean TVB-N values of cobia fillets over iced storage are depicted in the Table 2.

Table 2
Total volatile base nitrogen of cobia fillets under treatment of *C. sinensis* 0.06% and *E. hirta* 0.06% extracts during ice storage

Samples	TVB-N ($\text{mgN } 100\text{g}^{-1}$)			
	1	5	10	15
Control	16.3±0.14 ^b	16.5±0.23 ^b	17.3±0.14 ^a	18.3±0.37 ^a
<i>C. sinensis</i> 0.06%	14.1±0.83 ^a	15.0±0.70 ^a	17.1±0.74 ^a	18.1±0.48 ^a
<i>E. hirta</i> 0.06%	15.9±0.80 ^b	16.2±0.62 ^b	16.5±1.07 ^a	17.6±0.62 ^a

Values in the same sampling time followed by same letters indicate insignificant differences between treatments ($p > 0.05$, Duncan). Values are mean±SD ($n = 4$).

Overall, TVB-N values of all fillet samples had a slightly growing trend from the beginning to the end of process storage (from 14.1 to 18.3 $\text{mgN } 100\text{g}^{-1}$). However, no significant differences in TVB-N levels were observed between the control and other treatments from day 10 to day 15 of storage time ($p > 0.05$). Therefore, the result showed that *C. sinensis* and *E. hirta* extracts did not significantly affect the TVB-N of fillets during the storage period. This observation was in accordance with the result reported by Fogaça et al (2017) on cobia, and Lu et al (2010) on snakehead fillets. After 15 days of storage, TVB-N values of cobia fillets in all treatments were acceptable for human consumption. It is because they were lower than 35 $\text{mgN } 100\text{g}^{-1}$, the maximum acceptable limit proposed by Huss (1995) and smaller than the limit of 35-40 $\text{mgN } 100\text{g}^{-1}$ suggested by Lakshmanan (2000).

Peroxide value (PV). Fish lipids contain polyunsaturated fatty acids which are highly sensitive to oxidation. During storage time, cobia underwent lipid oxidation resulting in the formation of hydroperoxides. The PV was employed for determining the formation of fatty acid hydroperoxides, which were primary oxidation products during the iced storage of fish (Olafsdottir et al 1997). Changes in PV of cobia fillets soaked in *C. sinensis* and *E. hirta* extracts solutions and the control during ice storage are depicted in Figure 3.

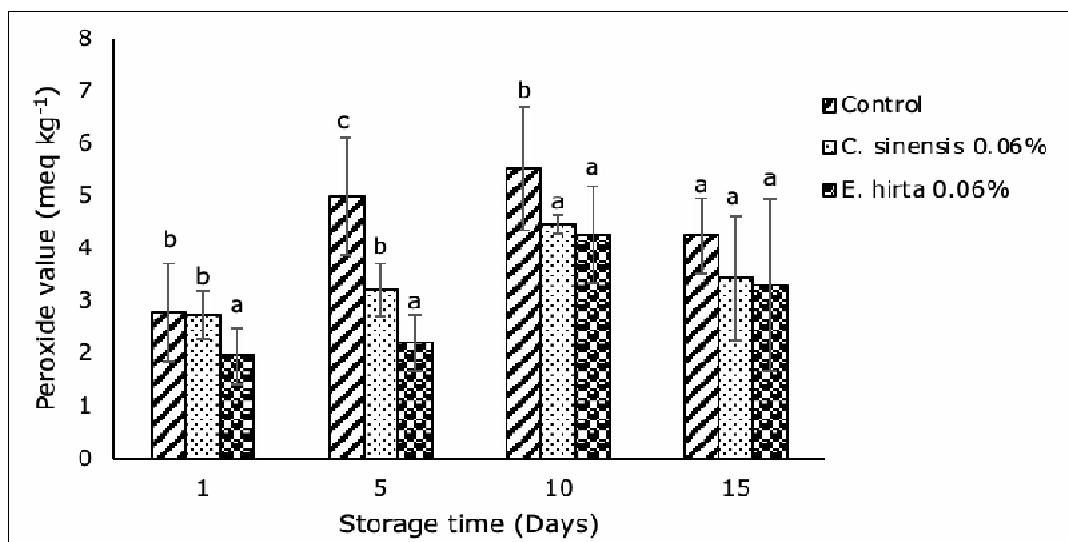


Figure 3. Peroxide value of cobia fillets under treatment of *C. sinensis* 0.06% and *E. hirta* 0.06% extracts during ice storage (values in the same sampling time followed by same letters indicate insignificant differences between treatments ($p > 0.05$, Duncan). Values are mean±SD ($n = 4$)).

Generally, PV increased in the three treatments until day 10 of storage, then declined at day 15 of the storage experiment. This is in accordance with the result reported by

Chaijan et al (2006) on sardine (*Sardinella gibbosa*) muscle during ice storage, where PV increased until day 9 and then gradually decreased until the end of storage period. The reduction of PV observed with extended storage time is due to the decomposition of hydroperoxide and formation of secondary oxidation products (Underland 2001). Hydroperoxides break down in several steps, yielding a wide variety of decomposition products, including aldehydes (Nawar 1996). The PV of the control treatment was significantly higher than those of fish treated with *E. hirta* extract at the first ten days, and *C. sinensis* extract at day 5 and day 10 of storage ($p < 0.05$). Moreover, *E. hirta* extract treatment seemed more effective to delay the fatty acid oxidation than *C. sinensis*, as PV were significantly lower in fillets soaked in *E. hirta* extract solution than in *C. sinensis* in day 1 and day 5 of iced storage ($p < 0.05$). 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 per 100 mg plant extract, and 10.3 mg gallic acid equivalent per 100 mg *E. hirta* extract (Dao et al 2020). Generally, these PV are below the acceptable threshold of PV content for fat oxidation of 8-10 meq kg⁻¹ (Linhartová et al 2019) and 10-20 meq kg⁻¹ (Huss 1995; Lakshmanan 2000). The present study indicated that the used plant extracts were effective at delaying lipid peroxidation in cobia fillets during chilled storage, especially soaking with 0.06% of *E. hirta* extract. This finding agrees with the results obtained by Bensid et al (2014) who treated fish with natural extracts before ice storage. Haghparast et al (2011) revealed that green tea extract and onion juice could effectively delay the primary oxidation in Persian sturgeon (*Acipenser persicus*) fillets and maintained PV below that of the control sample during ice storage.

Thiobarbituric acid reactive substances (TBARs). The development of lipid oxidation depends on several factors, such as storage period, temperature, presence of inhibitors or catalysts, availability of oxygen, and degree of unsaturated fatty acids (Aubourg & Medina 1999; Erickson 2002). TBARs values are widely used to describe the degree of lipid oxidation (Sallam 2007). The presence of TBARs is due to second stage autoxidation, during which peroxides are oxidized to aldehydes and ketones, alcohols, small carboxylic acids, and alkanes (Lindsay 1991). Figure 4 depicts the TBARs values measured expressed as mg of malondialdehyde (MDA) per kg of cobia fillets during 15 days of iced storage.

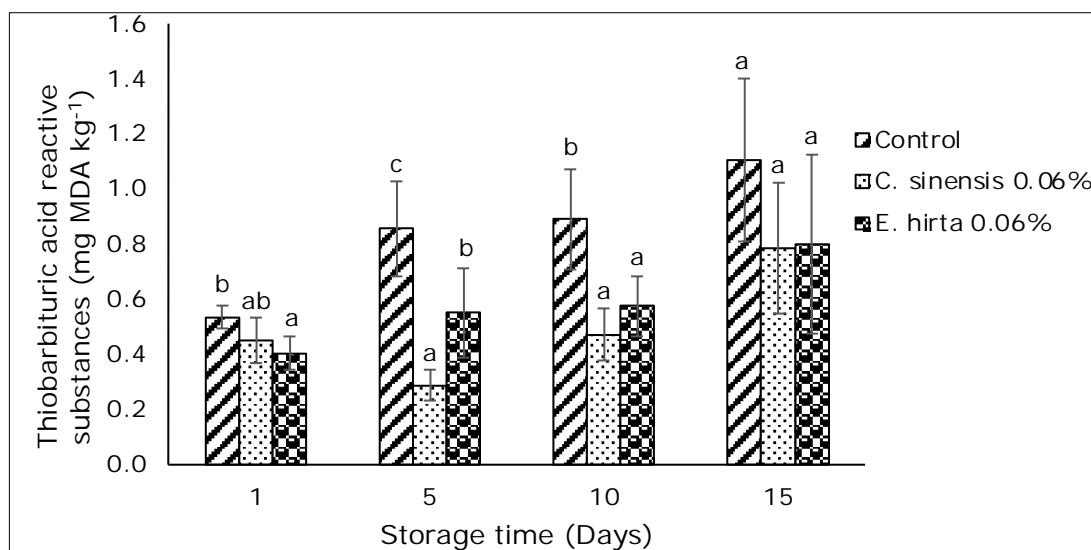


Figure 4. Thiobarbituric acid reactive substances of cobia fillets under treatment of *C. sinensis* 0.06% and *E. hirta* 0.06% extracts during ice storage (values in the same sampling time followed by same letters indicate insignificant differences between treatments ($p > 0.05$, Duncan). Values are mean \pm SD ($n = 4$)).

TBARS values in fish treated with *E. hirta* extract in this experiment were always significantly lower than those of the control treatment from day 1 to day 10 of storage ($p < 0.05$). Besides, at day 5 and day 10, the fillets treated with *C. sinensis* extract exhibited a significant lower TBARS values than the control samples ($p < 0.05$). The results proved that the treatment of *C. sinensis* and *E. hirta* extracts could inhibit secondary oxidation of the cobia fillets during ice storage. Additionally, TBARS values in the range 5-8 mg MDA kg⁻¹ in all the samples were within the acceptable limit throughout the period of storage (Sallam 2007). Thus, TBARS values in all the samples were within the acceptable limit throughout the storage period and lower than research of Rawdkuen et al (2008) and Bensid et al (2014). Feng et al (2012) found that the lipid oxidation of black sea bream treated with tea polyphenol (TP and TP + O₃ treatment groups) showed better results than fish treated with O₃ and control sample, suggesting tea polyphenol could reduce lipid oxidation in fish. Moreover, an investigation by Tang et al (2001), found that tea catechins reduced lipid oxidation in mackerel patties with a significant decrease ($p < 0.05$) in TBARS content. Haghparast et al (2011) demonstrated that green tea extract and onion juice could effectively delay the peroxidation in Persian sturgeon fillets and maintain the TBARs below those of the control sample.

Moisture. Moisture content influences the quality of the products. Changes in the moisture values of cobia fillets during a period of 15 days are depicted in Table 3. Overall, no significant difference in moisture was observed between the control and the plant extracts treated fish fillets during storage time ($p > 0.05$). The moisture content of all samples was seen to slightly decrease over the 15 days of iced storage (from 73.4 to 71.5%). This decrease in moisture content over storage time was associated with the increase of WHC which could be explained by the increase in drip loss during storage. It was reported that the denaturation of muscle protein in combination with the increase of degraded enzyme activities leads free water being released out of fish muscle tissue (Tsuchiya et al 1992). The present study indicates that treating cobia fillets with *C. sinensis* and *E. hirta* extracts did not affect the moisture of the fillet during the storage period. This observation was in accordance with the result reported by Duan et al (2010), moisture content decreased with storage time of cold-stored lingcod (*Ophiodon elongatus*).

Table 3
Moisture of cobia fillets under treatment of *C. sinensis* 0.06% and *E. hirta* 0.06% extracts during ice storage

Samples	Moisture (%)			
	1	5	10	15
Control	73.1±1.14 ^a	73.0±0.94 ^a	73.3±1.76 ^a	71.8±1.59 ^a
<i>C. sinensis</i> 0.06%	72.5±0.71 ^a	72.4±1.85 ^a	71.5±1.72 ^a	72.6±0.66 ^a
<i>E. hirta</i> 0.06%	73.4±2.27 ^a	72.8±1.64 ^a	71.5±1.39 ^a	71.7±1.00 ^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 = 4).

Color measurements. Quality parameters, including flesh coloration, determine acceptability and the price of fish (Skrede & Storebakken 1986). Changes in the instrumental color values of cobia fillet during chilled storage are depicted in Table 4. Overall, no significant difference of lightness (L*) value was observed between control and both plant extracts treated fish fillets during storage time ($p > 0.05$), apart day 10. Lightness (L*) values of all fillet samples showed a slightly decreasing trend from the beginning to the end of storage period (from 60.8 to 66.9), whilst the redness (a*) and yellowness (b*) values gradually increased with storage period. The cause of these color changes is due to the changes in the components of fish muscle, such as lipid oxidation, enzyme, and microbial activity. Lipid oxidation and the breakdown of proteins form dark brown complexes, leading to a light color of fish fillets decreased while yellow and red increased. Similar result was also found by Lu et al (2010), were L* values of snakehead

fillets declined and a^* values rose for 15 days storage at 4°C. In addition, the decreasing trend in L^* value was found in the study by Mohan et al (2012) in the chilled storage of filleted Indian oil sardines (*Sardinella longiceps*).

Table 4
Color of cobia fillets under treatment of *C. sinensis* 0.06% and *E. hirta* 0.06% extracts during ice storage

Storage time (days)	Samples	Color		
		L^*	a^*	b^*
1	Control	66.9±1.06 ^a	-3.96±0.41 ^a	3.98±0.90 ^{ab}
	<i>C. sinensis</i> 0.06%	65.6±1.23 ^a	-3.47±0.25 ^b	4.29±0.63 ^b
	<i>E. hirta</i> 0.06%	66.5±1.65 ^a	-3.33±0.37 ^b	3.14±1.29 ^a
5	Control	62.7±1.65 ^a	-3.05±0.60 ^a	4.16±1.68 ^a
	<i>C. sinensis</i> 0.06%	63.0±0.74 ^a	-3.11±0.22 ^a	5.24±0.63 ^a
	<i>E. hirta</i> 0.06%	63.3±0.53 ^a	-2.56±0.42 ^b	6.98±1.43 ^b
10	Control	63.2±0.81 ^b	-2.62±0.20 ^a	5.89±0.83 ^a
	<i>C. sinensis</i> 0.06%	62.2±0.63 ^a	-2.02±0.34 ^b	5.71±0.74 ^a
	<i>E. hirta</i> 0.06%	62.5±1.15 ^{ab}	-2.07±0.31 ^b	6.16±0.42 ^a
15	Control	61.5±1.55 ^a	-2.48±0.32 ^a	6.30±0.31 ^a
	<i>C. sinensis</i> 0.06%	60.8±0.84 ^a	-2.31±0.45 ^a	6.24±0.96 ^a
	<i>E. hirta</i> 0.06%	61.7±0.84 ^a	-1.54±0.24 ^b	6.36±0.73 ^a

Values in the same sampling time followed by same letters indicate insignificant differences between treatments ($p > 0.05$, Duncan). Values are mean±SD ($n = 4$).

Sensory properties. Sensory properties of food products are the key factors in consumer attraction. In this research, the sensory properties of raw cobia fillets (color, odor, gapping, and texture, surface) were evaluated by seven members trained panelists. Results of the total quality index (QI) difference testing for all treatments fish over the 15 days of ice storage are presented in Figure 5a. On the scale of QI used, zero represented fresh fish and 14 was defined as a completely deteriorated fish. Generally, the sensory quality of fish fillets declined in all treatments over the 15 days. However, the fillets treated with *C. sinensis* and *E. hirta* extracts exhibited a significant lower QI than the control samples from day 5 onwards ($p < 0.05$). Furthermore, cobia fish treated with *E. hirta* extract had significantly higher sensory quality than those of *C. sinensis* extract at day 10 of iced storage ($p < 0.05$). The results show that fish fillets were treated with *C. sinensis* and *E. hirta* extracts of fish had less fishy smells and the fish with plant extracts treatment were preferred by the panelists.

Acceptability score for taste of cooked cobia fillets was evaluated by using nine-point scale. The cooked fish samples were 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 5b. The results indicate that sensory scores showed a similar pattern of decreasing acceptability for the flesh samples of the control, *C. sinensis* and *E. hirta* extracts treated with increasing storage period. The same result of fresh samples, the treated with both plant extracts were observed significantly higher than the control samples ($p < 0.05$), apart day 1. Moreover, at day 15 of storage, the sensory score of cobia fillets treated with *E. hirta* extract was significantly higher than those of *C. sinensis* extract ($p < 0.05$). It was noted that the control samples displayed neutral taste, off-flavors, and loss of sweetness, but the treatment of *C. sinensis* and *E. hirta* improved the sensory properties through the presence of herbal flavor and taste. Therefore, using plant extracts soak treatments on cobia fillets could remain their good quality characteristics in terms of sensory assessment.

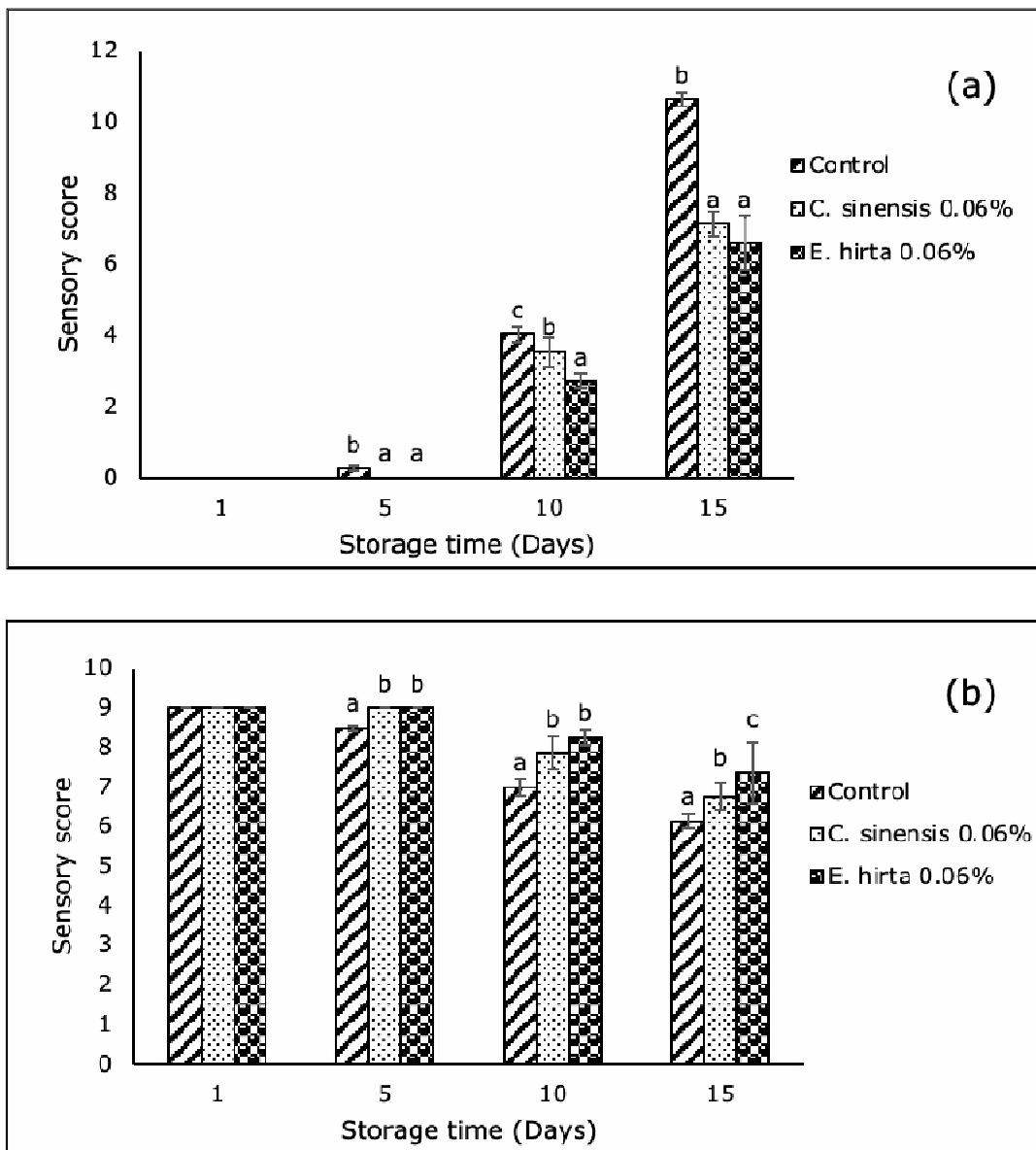


Figure 5. Sensory properties of cobia fillets under treatment of *C. sinensis* 0.06% and *E. hirta* 0.06% extracts during ice storage: quality index (QI) (a) and tasting of cooked fillets (b) (values in the same sampling time followed by same letters indicate insignificant differences between treatments ($p > 0.05$, Duncan). Values are mean \pm SD ($n = 4$)).

Conclusions. The results indicate that pre-soaking cobia fillets with *C. sinensis* and *E. hirta* extracts significantly reduced the total viable count, inhibited the formation of primary and secondary lipid oxidation, and improved the sensory properties during ice storage. Based on the total viable count, it can be concluded that cobia fillets untreated or treated with *C. sinensis* (0.06%) and *E. hirta* (0.06%) extracts can be used for up to 10 days.

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