

Effects of packaging method and temperature on the quality and lipid degradation of snakehead fish (*Channa striata*) fillets during frozen storage

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Abstract

Temperature and packaging method are considered the main factors affecting the quality of fishery products during frozen storage. In the present study, snakehead fish fillets were air (AP) and vacuum (VP) packaged and stored at $-20\pm 2^{\circ}\text{C}$ and $-25\pm 2^{\circ}\text{C}$ for six months to investigate the effects of temperature and packaging method on the quality of snakehead fish fillets. Changes in the quality of snakehead fish fillets were assessed by determinations of lipid hydrolysis, lipid oxidation, and their resulting changes in colour, cooking yield (CY), and total volatile basic nitrogen (TVB-N) content. Storage temperature and packaging method showed strong preservative effects on the quality of snakehead fish fillets. Higher cooking yield and lower TVB-N content were observed in the VP samples stored at $-25\pm 2^{\circ}\text{C}$ compared to the AP samples and stored at $-20\pm 2^{\circ}\text{C}$. Vacuum packaging and lower storage temperature (i.e. $-25\pm 2^{\circ}\text{C}$) significantly retarded lipid hydrolysis and lipid oxidation development. This resulted in lower free fatty acid (FFA), lipid hydroperoxides (PV), thiobarbituric acid-reactive substances (TBARS) values, and higher phospholipid content. Significant correlations between lipid oxidation products (PV and TBARS) and yellow discolouration (b^* values) were found in this study. Based on these findings, it could be suggested that frozen snakehead fish fillets should be vacuum-packaged and stored at low temperatures to maintain their quality.

1. Introduction

Fish is an important source of food worldwide. As an affordable product, fish is the primary source of nutritional protein for over two billion people across the world (Garcia and Rosenberg, 2010). There is a growing demand for fishery products especially in countries where due to other factors the cost of livestock meat has risen almost beyond the reach of the low-income people. Fish are not only consumed for their nutritional value but also for their functional properties. Fish contains high-quality proteins, vitamins, minerals, and omega-3 fatty acids that are essential for the prevention of cardiovascular diseases (Rahman *et al.*, 2018).

Striped snakeheads (*Channa striata*), also known as Chevron snakehead, and snakehead murrel are native to Southeast Asia and locally called “Cá lóc” in Vietnam. *Channa striata* is one of the most common and important freshwater food fishes in tropical Asia and the meat is considered delicious. *Channa striata* is a useful source of

proteins ($78.32\pm 0.23\%$ DM), lipids ($2.08\pm 0.08\%$ WM) and vitamin A (0.265 ± 0.013 mg/100 g fish muscle) and it has a high content of arachidonic acid (AA) 20:4 ω 6 and docosahexaenoic acid (DHA) 22:6 ω 3 (Chedoloh *et al.*, 2011). Due to the high content of unsaturated fatty acids, moisture and less connective tissues, snakehead fish fillets are susceptible to spoilage by enzymes, microorganisms and inherent chemical compounds (Ikape and Cheikyula, 2017). Changes in the fish lipid fraction during frozen storage have a detrimental effect on other quality parameters such as colour, cooking yield (CY), water holding capacity and water content (Nguyen *et al.*, 2012). Deterioration in the fish initially begins when the enzyme systems such as lipase and phospholipase hydrolyse lipids and release free fatty acids. A major challenge in the preservation and storage of fish and fishery products is lipid oxidation, a complex process in which unsaturated fatty acids react with oxygen, catalysed by metal ions (e.g., Fe^{+2} , Fe^{+3} , and Cu^{+2}) and UV light to form hydroperoxides. Further

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reaction with unsaturated fatty acids decomposes hydroperoxides to produce thiobarbituric acid reactive substances (TBARS) such as aldehydes, ketones and alcohol. These volatile compounds contribute to unacceptable off-flavour and off-odour in fish products (Vieira *et al.*, 2017). Iron-containing ferrous oxymyoglobin/haemoglobin (Fe^{2+}) may also oxidise to ferric metmyoglobin Fe^{3+} which causes yellow-brownish discoloration in fish fillets (Baron and Andersen, 2002). During frozen storage, ice crystal formation and recrystallisation can disrupt cell organelles and release lipases that cause the formation of free fatty acids via lipolysis. Consequently, free fatty acids interact with hydroperoxide; cross-link, and fragment sarcoplasmic and myofibrillar protein, thus causing protein denaturation. Denatured protein results in a reduced cooking yield and water-holding capacity through drip loss (Nguyen *et al.*, 2011). However, lipid hydrolysis and oxidation in fish muscles can be largely influenced by storage temperature and the method of packaging the fish/fishery products. Enzymatic and microbial activities usually increase at elevated temperatures, but their proliferation and deteriorative impacts are often retarded at low temperatures (Gandotra *et al.*, 2012). The effect of low temperature on bacterial growth can be exerted by decreasing the bacteria's affinity for substrates, reducing its cell fluidity, and extending the lag phase (Nedwell, 1999) which maintains a negligible bacteria load. Packaging creates a barrier to the ingress of gases, light, and water vapor, which would otherwise result in oxidation of lipids, deterioration of colour and denaturation of proteins (Nguyen and Phan, 2018). Packaging also guards against food dehydration and weight loss due to water evaporation to the external environment (Gokoglu, 2020). Many studies have investigated several packaging methods and reported the significant contribution of vacuum packaging in inhibiting the growth of aerobic spoilage microorganisms and the inhibition of deleterious oxidative reactions (Khemir *et al.*, 2020).

The aim of the present study was to investigate the effect of vacuum and air packaging on the quality of snakehead fish fillets stored at $-20\pm 2^\circ\text{C}$ and $-25\pm 2^\circ\text{C}$ for six months. Changes in the quality of snakehead fish fillets were assessed by determinations of colour, cooking yield (CY) and TVB-N. Lipid hydrolysis was carried out by measurements of free fatty acid (FFA) and phospholipid (PL) contents. Lipid oxidation was determined by the measurements of primary and secondary lipid oxidation products.

2. Materials and methods

2.1 Materials

All snakehead fish samples used for this research were bought from the local fish farm in Nha Trang city. The average weight of the snakehead fish was 700-800 g. The fish were transported to the labs alive in water containers.

2.2 Chemicals

1,1,3,3-tetraethoxypropane, cumene hydroperoxide, thiobarbituric acid, trichloroacetic acid, oleic acid, and phosphatidylcholine were purchased from Sigma-Aldrich Company (USA). All other chemicals used in this study were of analytical grade.

2.3 Sample preparation and storage

At the laboratory, snakehead fish were rested for 2 hrs before bleeding and filleting following the procedure described by Nguyen *et al.* (2021). The fillets were frozen in an IQ freezer (Tempura IQF, ARICO, Vietnam) at -40°C for 30 mins when the core temperature of the fillets reached -18°C . After freezing, frozen fillets were divided into four groups (35 fillets in each group) for different packaging methods and storage temperatures. The frozen fillets of the four groups were packaged and stored for 6 months as described in Table 1. Samples were evaluated after freezing (month 0) and after 1, 2, 3, 4, 5, and 6 months of storage. At each sampling point, five fillets of each group were collected randomly and thoroughly thawed at $2\pm 1^\circ\text{C}$. The thawed fillets were used for physicochemical determinations.

Table 1. Packaging method and storage temperature of frozen snakehead fish fillets.

Sample code	Packaging method	Storage temperature
AP-20	Air packaging	$-20\pm 2^\circ\text{C}$
VP-20	Vacuum packaging	$-20\pm 2^\circ\text{C}$
AP-25	Air packaging	$-25\pm 2^\circ\text{C}$
VP-25	Vacuum packaging	$-25\pm 2^\circ\text{C}$

2.4 Colour measurement

The intensity of the flesh colour was measured by using the Minolta CR-400 chromameter (Minolta Camera Co., Ltd; Osaka, Japan) in Lab* system (CIE, 1976) with CIE IlluminantC as described in Nguyen *et al.* (2012). Three positions of the fillets ($n = 3$) were measured. The average L^* , a^* , and b^* value of three measurements for each fillet was used to calculate the mean and standard deviation for each group.

2.5 Cooking yield determination

Three slices (25 ± 5 g) in the middle part of each fillet ($n = 3$) were individually weighed in aluminium boxes

and steam-cooked in a steamed pot for 10 mins. All cooked samples were drained from the excess liquid in a plastic grid for 10 mins and weighed again. The cooking yield was expressed as the percentage of retaining weight compared to the weight of the sample before cooking. The average cooking yield value of three samples for each fillet was used to calculate the mean and standard deviation.

2.6 Lipid extraction

The total lipids (TL) of the fish samples were extracted according to the Bligh and Dyer (1959) method. The extract was used for the determination of free fatty acid and phospholipid contents.

2.7 Lipid oxidation

2.7.1 Lipid hydroperoxides value

Lipid hydroperoxide (PV) was determined by the ferric thiocyanate method of Shantha and Decker (1994). Briefly, 500 μ L of lipid extract was added to 500 μ L ice-cold chloroform: methanol solution (1:1, v/v). A total amount of 5 μ L of ammonium thiocyanate (4M) and ferrous chloride (80 mM) mixture (1:1, v/v) was added. The samples were brought to room temperature for 10 mins and the absorbance was read at 500 nm (Libra S50 UV/VIS spectrophotometer, Biochrom, UK). A standard curve was prepared using cumene hydroperoxide. The results were expressed as μ mol lipid hydroperoxides per g of sample.

2.7.2 Thiobarbituric acid-reactive substances value

Thiobarbituric acid-reactive substances were measured by the method of Lemon (1975) with modifications. A 5.0 g sample was homogenized with 10 mL of 7.5% trichloroacetic acid (TCA) using an Ultra-Turrax homogenizer (Kika Labortechnik, T25 basic, Staufen, Germany) at 2400 rpm for 10 s. The homogenate was centrifuged at $1500\times g$ for 15 mins at 4°C (Heraeus Biofuge Stratos Reconditioned 75005289R, Rotor 3335, DJB Labcare Limited, England). A mixture of 0.5 mL of supernatant and 0.5 mL of 0.02 M thiobarbituric acid solution was heated in a water bath at 95°C for 40 mins. The heated samples were cooled down on ice and absorbance was read at 530 nm (Sunrise Microplate Reader, Tecan Austria GmbH, A-5082 Grödig, Austria). The results were expressed as μ mol malondialdehyde per kg of the sample (μ mol MDA/kg) and calculated using a standard curve prepared from 1,1,3,3-tetraethoxypropane (TEP).

2.8 Lipid hydrolysis

2.8.1 Free fatty acid content

Free fatty acid content was determined on the TL extract by the method of Bernardez *et al.* (2005) based

on complex formation with cupric acetate-pyridine. Briefly, 1 mL of lipid extract was deposited into Pyrex tubes and the chloroform was removed by using liquid nitrogen. After that, 3 mL of cyclohexane and 1 mL of cupric acetate-pyridine reagent was added. Tubes were vortexed for 2 mins and centrifuged for 20 mins. The upper layer was read at 710 nm (Sunrise Microplate Reader, Tecan Austria GmbH, A-5082 Grödig, Austria). The FFA content was calculated as micromolar oleic acid (OL) based on a standard curve spanning a 2-22 μ mol range. Results were expressed as g oleic acid equivalents per 100 g of total lipids extract (g oleic acid Eq/100 g TL).

2.8.2 Phospholipid content

Phospholipid content (PL) of the fish muscle was determined on the TL extract by using a spectrophotometric method (Stewart, 1980), based on the complex formation of phospholipid with ammonium ferriothiocyanate, followed by absorbance reading at 488 nm (Sunrise Microplate Reader, Tecan Austria GmbH, A-5082 Grödig, Austria). The PL content was calculated using a standard curve prepared from phosphatidylcholine (PC). The results were expressed as g phosphatidylcholine equivalents per 100 g of total lipids extract (g PC Eq/100 g TL).

2.9 Total volatile basic nitrogen determination

The method of Malle and Poumeyrol (1989) was used for measuring total volatile basic nitrogen (TVB-N). TVB-N was measured by steam distillation (Struer TVN distillatory, STRUERS, Copenhagen) and titration, after extracting the fish muscle with 7.5% aqueous trichloroacetic acid solution. The distilled TVB-N was collected in boric acid solution and then titrated with sulphuric acid solution.

2.10 Statistical analysis

The data sets obtained were analysed by General Linear Modelling (GLM) to investigate the main effects of the packaging method and storage temperature on the indicators of quality and lipid oxidation. Means were compared by using ANOVA and Duncan's Multiple-Comparison Test using NCSS 2000 software (NCSS, Kaysville, Utah, USA). The significance of differences was defined at the 5% level ($p < 0.05$).

3. Results and discussion

3.1 Lipid degradation during storage

3.1.1 Lipid hydrolysis

Lipid hydrolysis in the snakehead fish fillets was indicated by an increase in free fatty acid (FFA) content

(Figure 1a) and the decomposition of phospholipid (PL) content (Figure 1b) in the fish muscles. After the freezing process, the FFA content of the fish muscle increased slightly, whereas the PL content slightly decreased. The increased FFA is believed to result from the enzymatic hydrolysis caused by lipase and phospholipase. The same results have been reported by Dang *et al.* (2018) for Tra catfish fillets. The FFA content of all samples increased throughout the storage period. During the first two months of storage, the FFA content of all samples increased slightly but increased significantly ($P < 0.05$) during the subsequent storage time. The FFA value of vacuum-packaged fillets was significantly lower ($P < 0.05$) than air-packaged fillets at the same storage temperatures (i.e. $-20 \pm 2^\circ\text{C}$ and $-25 \pm 2^\circ\text{C}$). The FFA content of the samples stored at $-25 \pm 2^\circ\text{C}$ remained significantly lower than that of the samples stored at $-20 \pm 2^\circ\text{C}$ throughout the storage time. It has been documented that the formation of free fatty acids in fish muscles derives from the hydrolysis of phospholipids in the fish muscle during storage (Nguyen and Phan, 2018). The FFA content in the frozen snakehead fish fillets in this work was in negative correlation ($r^2 = -0.9014$) with the phospholipid content (Figure 1b). Generally, the phospholipid content (PL) of all samples decreased with increasing storage time. Higher PL values were recorded for fillet samples stored at $-25 \pm 2^\circ\text{C}$ (e.g., AP-25 and VP-25) compared to their counterparts stored at $-20 \pm 2^\circ\text{C}$ (e.g., AP-20 and VP-20). No significant difference in PL content was observed between the air-packaged and vacuum-packaged fish samples stored at $-25 \pm 2^\circ\text{C}$. The PL value of the air-packaged snakehead fish samples stored at $-20 \pm 2^\circ\text{C}$ was significantly lower compared to other group samples throughout the storage period. The results were in agreement with the previous publication by Nguyen and Phan (2018) who investigated lipid hydrolysis in the cobia fillets during superchilled storage for 16 days and similarly found an increasing FFA and decreasing PL values along storage time. The lower values of FFA and higher PL contents in vacuum-packaged samples are due to vacuum packaging's inhibitory effect on the growth of bacteria, suppressing the production of enzymes (lipase and phospholipase) responsible for lipid hydrolysis. Findings from previous studies confirmed that lipid hydrolysis in frozen fish muscles can be induced by lipase and phospholipase from the fish muscles as well as from bacteria (Zhang *et al.*, 2019). During frozen storage, bacteria acclimatise to the low-temperature environment and produce lipase and phospholipase (Ghaly *et al.*, 2010). These enzymes are thought to have exhibited optimum hydrolytic activities at the freezing temperature (-20 to -25°C) of the snakehead fish fillets investigated in this research. The accumulation of free fatty acids can lower the pH and reduce the water

holding capacity of fish muscles. This is proven by the negative correlation ($r^2 = -0.9096$) seen between the FFA and the cooking yield of the snakehead fish fillet samples. The disintegration of the phospholipid content to form FFA may have damaged the fish muscle structure and released water upon steam cooking. Hematyar *et al.* (2019) proved that the decomposition of lipids can lead to the disintegration of fish muscle structure. A higher concentration of free fatty acids released in fish muscle is quite susceptible to react with atmospheric oxygen which leads to the formation of lipid hydroperoxide (PV). The increase in FFA content of the frozen snakehead fish fillets strongly correlated with the hydroperoxide values ($r^2 = 0.8972$) and TBARS values ($r^2 = 0.9668$). It has been demonstrated that an increase in FFA content could trigger the formation of TBA reactive substances (Taheri *et al.*, 2012).

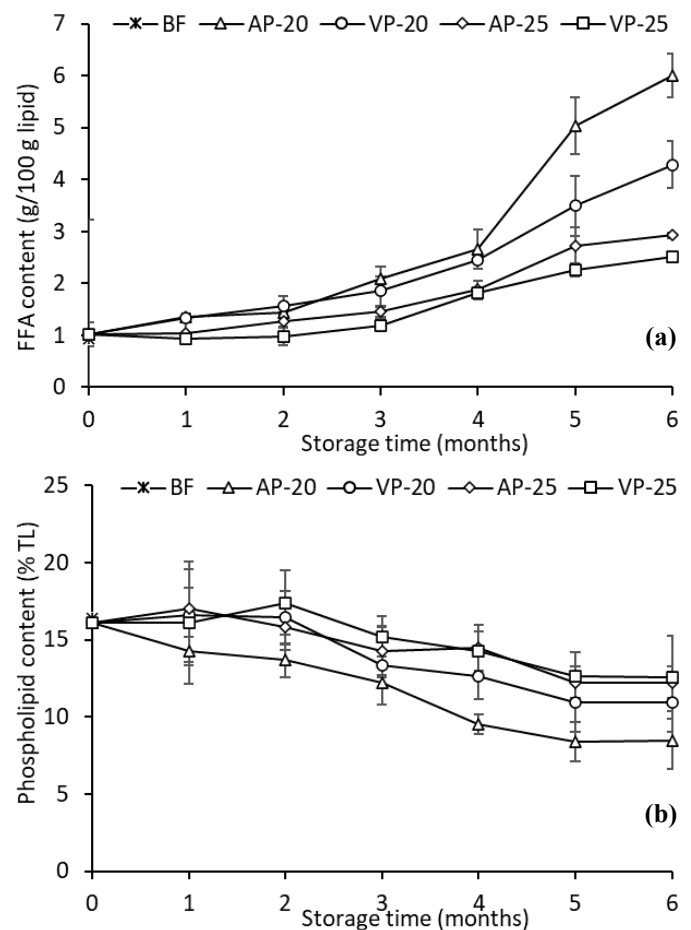


Figure 1. Changes in FFA (a) and PL (b) of snakehead fish fillets during frozen storage as affected by packaging method and temperature [BF: Before freezing; AP-20: air-packaged and stored at $-20 \pm 2^\circ\text{C}$; VP-20: vacuum-packaged and stored at $-20 \pm 2^\circ\text{C}$; AP-25: air-packaged and stored at $-25 \pm 2^\circ\text{C}$; VP-25: vacuum-packaged and stored at $-25 \pm 2^\circ\text{C}$].

3.1.2 Lipid oxidation

The extent of lipid degradation in the air-packaged and vacuum-packaged snakehead fish fillets stored at $-20 \pm 2^\circ\text{C}$ and $-25 \pm 2^\circ\text{C}$ for six months was determined and the results are presented as peroxide value (PV) and

thiobarbituric acid-reactive substances (TBARS) value in Figure 2a and 2b, respectively. The results from GLM analyses show that both storage temperature and packaging method significantly ($P < 0.05$) affected the PV and TBARS values of snakehead fish fillets. Both PV and TBARS contents of the snakehead fish muscle increased significantly after the freezing process. The increase in PV and TBARS is believed to result from the oxidation PL (Nguyen *et al.*, 2012), which is further supported by the significant negative correlation ($r = -0.9626$) between the PV and PL content observed in this study. Similar trends have been observed by Dang *et al.* (2018) for Tra catfish after freezing. Peroxide value (PV) in all the snakehead fish fillet samples showed no noticeable change during the first two months of storage. However, a significant ($P < 0.05$) increase in PV values of all samples was observed from the 2nd month to the 5th month and tended to be stable during further storage (Figure 2a). The samples stored at $-25\pm 2^\circ\text{C}$ (i.e., AP-25 and VP-25) had significantly lower PV values compared to their counterparts stored at $-20\pm 2^\circ\text{C}$ (i.e., AP-20 and VP-20) during the frozen storage. On the basis of packaging methods, VP samples showed lower peroxide values compared to AP samples. This means that storage temperature and packaging method exerted a significant influence on lipid hydrolysis and oxidation in the snakehead fish fillets. The relative invariable PV in the snakehead fish fillets during the first two months of storage is thought to be due to the low storage temperature temporary effect of slowing down biochemical reaction, resulting in less degree of lipid hydrolysis (Sampels, 2015). This can also be explained by the fact that the rate of hydroperoxide formation was equal to the rate of hydroperoxide decomposition into secondary oxidation products (Nguyen *et al.*, 2012). The subsequent increase in PV values of the snakehead fish fillets is the result of lipid oxidation induced by enzymes (lipase, phospholipase and lipoxygenase) which are believed to have been released from microorganisms that adjusted to the frozen environment. The lower PV values in vacuum-packaged fillets compared to air-packaged fillets derived from a low concentration of oxygen (O_2) in the vacuum package. The removal of oxygen or barrier to the ingress of light, and water vapour from the vacuum packaging is said to have obstructed lipid hydrolysis and oxidation in the snakehead fish muscle tissues. Hydroperoxide (PV) is an unstable primary oxidation product; its concentration in frozen fish muscles depends on the rate of production and decomposition to form thiobarbituric acid-reactive substances (TBARS). The PV of the snakehead fish fillets in the present study was in strong positive correlation ($r^2 = 0.9269$) with the TBARS values.

The TBARS values (Figure 2b) of all the samples

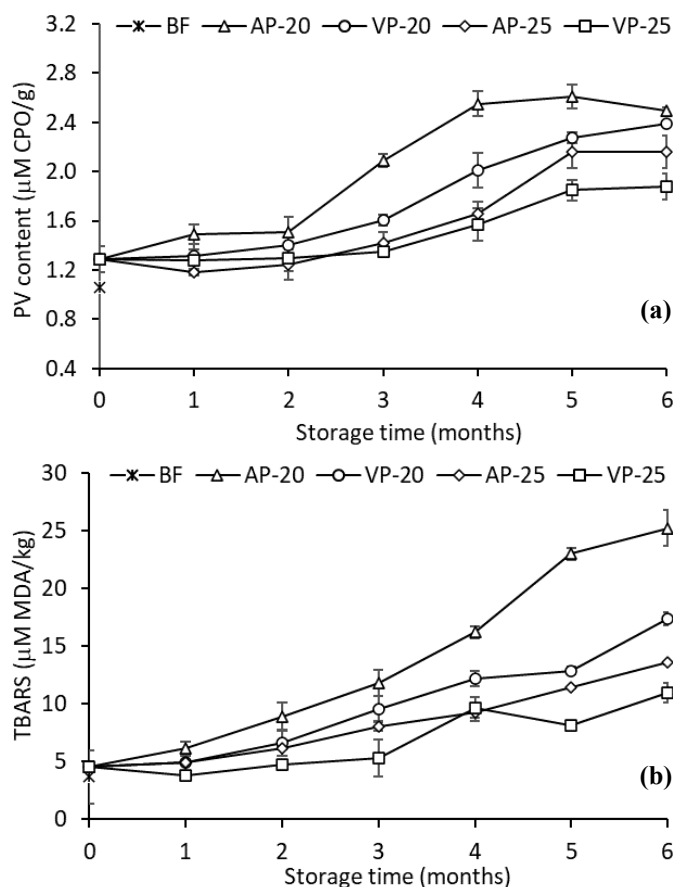


Figure 2. Changes in PV (a) and TBARS (b) of snakehead fish fillets during frozen storage as influenced by packaging method and temperature [BF: Before freezing; AP-20: air-packaged and stored at $-20\pm 2^\circ\text{C}$; VP-20: vacuum-packaged and stored at $-20\pm 2^\circ\text{C}$; AP-25: air-packaged and stored at $-25\pm 2^\circ\text{C}$; VP-25: vacuum-packaged and stored at $-25\pm 2^\circ\text{C}$].

increased throughout the frozen storage time. However, the rates of increased TBARS values during the first two months of storage were less than those during the subsequent three months of storage. Snakehead fish fillets stored at $-25\pm 2^\circ\text{C}$ revealed significantly ($P < 0.05$) lower TBARS values than those stored at $-20\pm 2^\circ\text{C}$. At each storage regime, the air-packaged samples (AP-20 and AP-25) produced higher TBARS values compared to the VP samples (VP-20 and VP-25). TBARS are stable secondary lipid oxidation products whose formation depends on the free fatty acids, hydroperoxide, oxygen, temperature, and the rate of oxidation (Nguyen *et al.*, 2012; Huang and Ahn, 2019). The TBARS value of the snakehead fish fillets treated in this work was in correlation with values of FFA ($r^2 = 0.9668$) and PV ($r^2 = 0.9269$). The low rates of increased TBARS values during the first two months are attributed to the low rate of oxidation induced by low storage temperature. However the unprecedented increase in TBARS content during the subsequent storage time is due to the higher content of hydroperoxide whose decomposition leads to the formation of aldehydes and TBA-reactive substances (Nguyen *et al.*, 2012; Huang and Ahn, 2019). The accumulation of aldehydes in fish muscle can further

damage protein and release rancid flavours. It has also been demonstrated that TBARS interaction with proteins through the Maillard reaction leads to undesirable yellow-brown discolouration in fish muscles (Vieira *et al.*, 2017). This rationalises the strong positive correlations between TBARS ($r^2 = 0.8970$) and b^* values (yellowness) and between PV ($r^2 = 0.9191$) and b^* values of the snakehead fish fillets investigated in this study. Irrespective of packaging methods, fillet samples stored at $-25\pm 2^\circ\text{C}$ showed significantly lower TBARS values than their counterparts stored at $-20\pm 2^\circ\text{C}$. This implies that storage temperature plays a significant role in inhibiting lipid oxidation (Aubourg *et al.*, 2004; Cyprian *et al.*, 2017). The values of TBARS and PV in the present study were in harmony with findings from Basa fish (*Pangasius bocourti*) fillets during frozen storage at $-20\pm 2^\circ\text{C}$ (Sriket and La-ongnual, 2018). However, at different storage temperatures ($-20\pm 2^\circ\text{C}$ and $-25\pm 2^\circ\text{C}$) the lower values of TBARS observed in vacuum-packaged samples are due to the barrier property of the packaging material in synergism with low storage temperature (Cyprian *et al.*, 2017; Tsogas *et al.*, 2019). This suggests that the quality of snakehead fish fillets can be better preserved by vacuum packaging and storage at a temperature lower than -25°C which will synergistically double the shelf life of the fish fillets.

3.2 Changes in colour

The variations in fillet colour expressed as lightness (L^* value), redness (a^* value) and yellowness (b^* value) during the frozen storage of air-packaged and vacuum packaged snakehead fish fillets are presented in Figure 3. After freezing, the L^* value (Figure 3a), a^* value (Figure 3b) and b^* value (Figure 3c) of the fish flesh slightly increased. The increase in L^* values may be due to the formation of ice crystals in the fish muscle, leading to a change the light refraction. Whereas, the increase in a^* and b^* values is thought to be resulted from lipid oxidation occurring during the freezing process (Nguyen *et al.*, 2012; Dang *et al.*, 2018). The L^* value of all the samples followed a decreasing pattern toward the end of storage time (Figure 3a). Unlike the L^* value, a^* value (redness) and b^* value (yellowness) of all the fillet samples followed an increasing order as a function of storage time (Figure 3b and c, respectively). These results were in accordance with colour variation reported for frozen grass carp (*Ctenopharyngodon idellus*) fillets (Yin *et al.*, 2014). The authors attributed the colour change in the grass carp fillets to the accumulation of secondary lipid oxidation products such as aldehyde, ketone, carbonyl, etc. These reactive substances are known for oxidizing haemoglobin (Hb) and myoglobin (Mb) to form a yellow/brown MetHb or MetMb in the fish muscles (Richards and Hultin, 2002). In this current

study, the strong positive correlation of b^* value with FFA ($r^2 = 0.8393$), PV ($r^2 = 0.9191$), and TBARS ($r^2 = 0.8980$) indicated that the change in the snakehead fish fillets colour was driven from lipid oxidation that produced darker and yellow pigments; thus, decreasing the lightness and increasing the yellowness of the snakehead fish fillets during frozen storage. The fillet samples stored at $-25\pm 2^\circ\text{C}$ revealed higher L^* values than those stored at $-20\pm 2^\circ\text{C}$ with the VP samples appearing lighter than the AP samples at all storage levels. This could be due to the inhibitory effect of low storage temperature and the absence of pro-oxidants in the vacuum package. A similar increase in a^* and b^* values was found in Basa fish (*Pangasius bocourti*) stored for 20 weeks at -20°C (Sriket and La-ongnual,

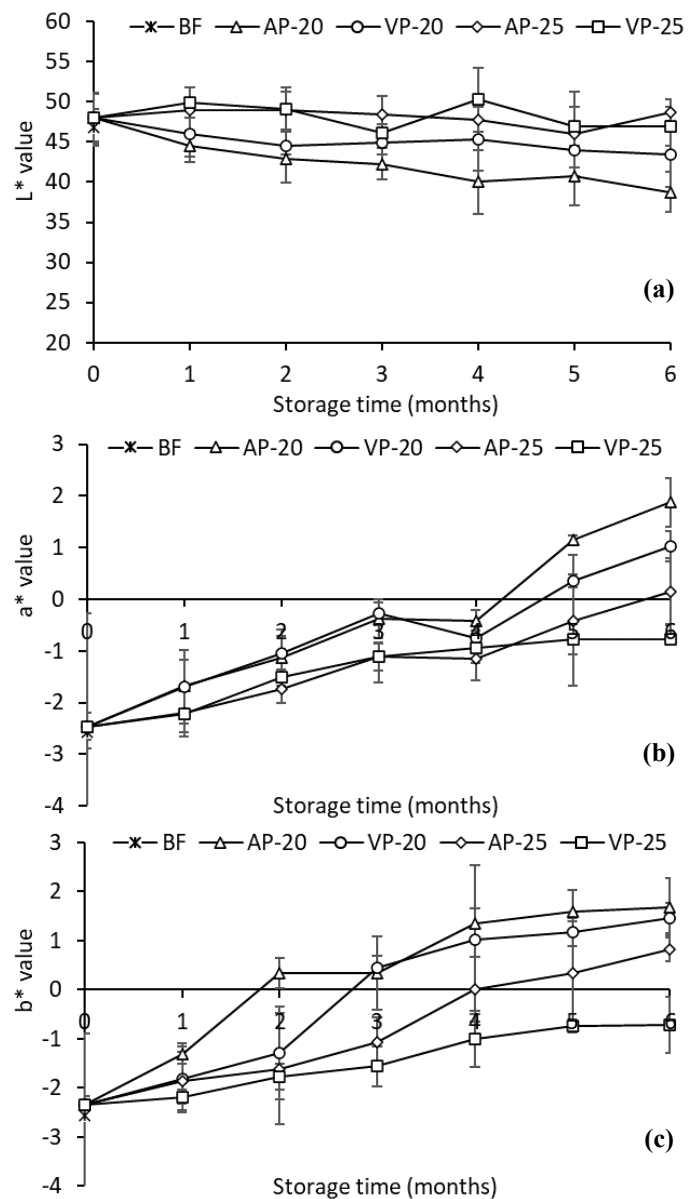


Figure 3. Changes in L^* value (a), a^* value (b) and b^* value (c) of snakehead fish fillets during frozen storage as influenced by packaging method and temperature [BF: Before freezing; AP-20: air-packaged and stored at $-20\pm 2^\circ\text{C}$; VP-20: vacuum-packaged and stored at $-20\pm 2^\circ\text{C}$; AP-25: air-packaged and stored at $-25\pm 2^\circ\text{C}$; VP-25: vacuum-packaged and stored at $-25\pm 2^\circ\text{C}$].

2018), and in cobia fillets during 24 weeks of frozen storage at -20°C (Nguyen and Phan, 2018). Such increasing discoloration could stem from the oxidation of oxymyoglobin in the fish muscle tissues which led to the formation of yellow-brown pigmented metmyoglobin (Richards and Hultin, 2002; Vieira et al., 2017).

3.3 Changes in cooking yield and total volatile basic nitrogen

Cooking yield (CY) is an imperative quality attribute that indicates structural changes due to protein denaturation and lipid degradation in muscle food. It can be seen from Figure 4a that the CY of snakehead fish fillets slightly decreased after the freezing process. This is thought to be due to protein denaturation caused by ice crystal formation in the fish muscle during the freezing process. During the storage, the CY of all the snakehead fish fillet samples generally decreased though in varying magnitudes as a function of storage time. It is evidenced that vacuum packaging and low storage temperature exerted a significant influence on the cooking yield of the snakehead fish muscles. The snakehead fish fillets stored at $-25\pm 2^{\circ}\text{C}$ (i.e., AP-25 and VP-25) remained at higher CY values compared to their counterparts stored at $-20\pm 2^{\circ}\text{C}$ (i.e., AP-20 and VP-20) throughout the storage period. At each storage temperature, vacuum packaging has a higher percentage of cooking yield relative to air-packaged fillets. The order of cooking yield follows $\text{VP-25} > \text{AP-25} > \text{VP-20} > \text{AP-20}$. The reduction in the CY of the snakehead fish fillets during frozen storage can be attributed to damage to the fish muscle structure as a result of protein denaturation and lipid oxidation that negatively impacted the water holding capacity of the fish muscle during heating (Nguyen et al., 2011; Hematyar et al., 2019; Zhang et al., 2019). Due to proximity to protein, aldehydes from lipid oxidation may have initiated denaturation of the fish muscle protein by oxidizing the amino acid side chains and protein backbones which usually leads to protein fragmentation or protein-protein cross-linkages (Zhang et al., 2013). The denatured protein forfeited its water holding capacity through drip loss upon heating and cooling. The slow pace of reduction of CY content in the vacuum-packaged fillets and fillets stored at lower temperatures could be ascribed to low temperature and vacuum packaging effects on prohibiting microbial activity biochemical reactions that would otherwise damage the fish muscle structure. However, as storage time increased, the loss of CY increased as a result of lipolytic enzymes produced by adapted psychrotrophic bacteria (Zhang et al., 2013). The changes in CY content of the snakehead fish muscle during frozen storage exhibited a strong negative correlation ($r^2 = -0.9472$) with the changes in TVB-N content. Generally, the TVB-

N content of all samples was rather stable during the first two months of storage and no significant ($P > 0.05$) differences between the groups were observed (Figure 4b). During the subsequent storage time, the TVB-N content of the snakehead fish fillets stored at $-20\pm 2^{\circ}\text{C}$ (i.e., AP-20 and VP-20) increased significantly ($P < 0.05$) and a significant difference in TVB-N content between the AP-20 and VP-20 groups was noted. Whereas, the TVB-N content of the fillet samples stored at $-25\pm 2^{\circ}\text{C}$ (AP-25 and VP-25) increased slightly and no significant ($P > 0.05$) difference was found. At the end of the storage period, the TVB-N values of all groups were much lower than the limitation for human consumption of TVB-N value (i.e., 30-35 mg N/100 g).

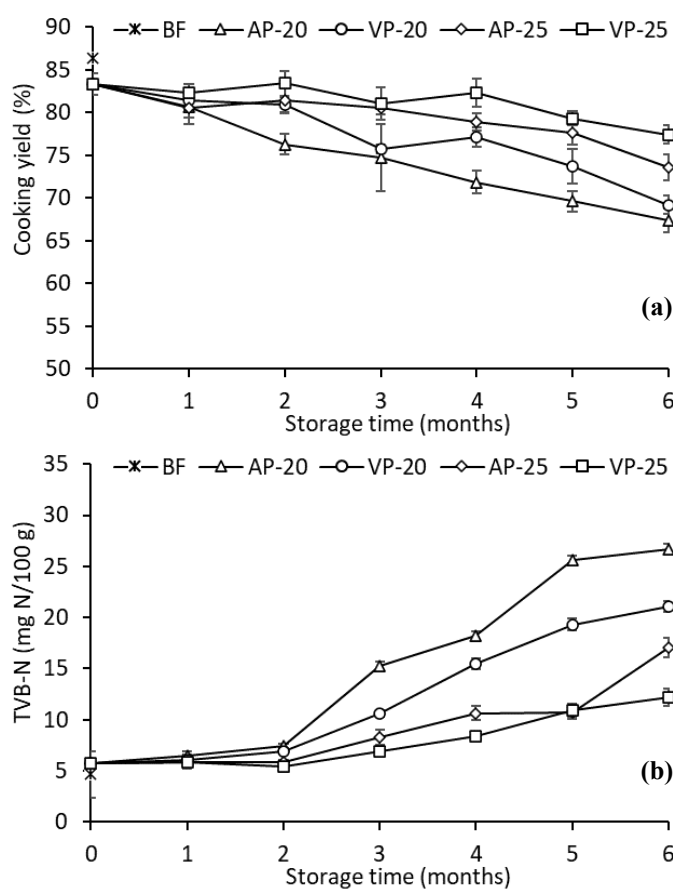


Figure 4. Changes in CY (a) and TVB-N (b) of snakehead fish fillets during frozen storage as functions of packaging method and temperature [BF: Before freezing; AP-20: air-packaged and stored at $-20\pm 2^{\circ}\text{C}$; VP-20: vacuum-packaged and stored at $-20\pm 2^{\circ}\text{C}$; AP-25: air-packaged and stored at $-25\pm 2^{\circ}\text{C}$; VP-25: vacuum-packaged and stored at $-25\pm 2^{\circ}\text{C}$].

4. Conclusion

Results from the study indicate that storage temperature and packaging method had a strong positive influence on the quality of the snakehead fish fillets. Higher cooking yield value and lower TVB-N content were observed in the VP samples stored at $-25\pm 2^{\circ}\text{C}$ compared to the AP samples and stored at $-20\pm 2^{\circ}\text{C}$. Vacuum packaging and lower storage temperature (i.e., $-25\pm 2^{\circ}\text{C}$) significantly retarded lipid hydrolysis and lipid

oxidation progress. This resulted in lower free fatty acid (FFA), lipid hydroperoxides (PV), thiobarbituric acid-reactive substances (TBARS) values and higher phospholipid content. Significant correlations between lipid oxidation products (PV and TBARS) and yellow discolouration (b^* values) were found in this study. Based on these findings, it could be suggested that frozen snakehead fish fillets should be vacuum packaged and stored at low temperatures to maintain their quality.

Conflict of interest

The authors declare no conflict of interest.

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