Food Research 5 (3) : 225 - 235 (June 2021)
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Effect of fish protein hydrolysate on physicochemical properties and oxidative stability of shortfin scad (*Decapterus macrosoma*) emulsion sausage

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Article history:
Received: 13 July 2020
Received in revised form: 11 September 2020
Accepted: 9 November 2020
Available Online: 6 June 2021

Keywords:
Silver catfish protein hydrolysate,
Shortfin scad,
Fish emulsion sausage,
Physicochemical properties,
Oxidative stability

DOI:
https://doi.org/10.26656/fr.2017.5(3).354

Abstract

The present study aimed to investigate the effect of silver catfish (*Pangasius sp.*) protein hydrolysate (SCPH) on physicochemical properties and oxidative stability of emulsion sausage prepared from shortfin scad (*Decapterus macrosoma*). The SCPH with different concentrations (1, 2, and 3%) was added into fish emulsion sausage followed by characterization of its physicochemical properties. The results obtained from this study revealed that the fish emulsion sausages contain high moisture content (63.49-66.85%), protein content (18.04-21.89%), fat content (7.16-8.59%), carbohydrate content (2.28-2.72%) and low ash contents (1.83-2.17%). The addition of SCPH improvised the hardness, cohesiveness, chewiness and resilience of the emulsion sausage ($p<0.05$) compared to the control sample. The finer fat globules were visualized in the sample added with SCPH at higher concentration of 3% (10-20 µm) compared to other concentrations (1 and 2%). Besides, the incorporation of SCPH at all concentrations shows a significant difference on L*, a* and b* values of the emulsion sausages during extended storage of 12 days ($p<0.05$). The SCPH was shown to retard lipid oxidation of sausage after extended storage of 12 days, with lower PV and TBARS values. Therefore, the effectiveness in retarding lipid oxidation achieved with the higher concentration of SCPH and it also capable to retain textural properties of fish emulsion sausage in the refrigerated storage of 12 days. Thus, as an alternative antioxidant in fish emulsion sausage, SCPH showed a potential to be used as it improved the physicochemical properties and oxidative stability of fish emulsion sausage.

1. Introduction

The silver catfish (*Pangasius sp.*) is among the most popular freshwater fish in Malaysia. This *Pangasius sp.* is known with different names at different places such as 'Patin' in Malaysia, 'Plasawai' in Thailand and 'Catra' in Vietnam. It is easily reproduced because these species are fast-growing fish in which it is widely cultured in ponds, floating cages and pens (Normah *et al.,* 2014). Hence, the availability of the *Pangasius sp.* is one of the factors that make it as the potential source of alternative antioxidant agent in fish emulsion sausage (Malaysian Fisheries Department, 2010). The antioxidant activity of the hydrolysate is influenced by the type of proteolytic enzyme used in the protein hydrolysis (Harun *et al.,* 2017). *Pangasius sp.* has a low-fat content with high levels of protein which are 5.5% and 16.6%, respectively. The amount and composition of the fat content will be influenced by the feed used in aquaculture operations. Besides, the amount of *Pangasius sp.* is abundant because it can be cultured (Malaysian Fisheries Department, 2010).

Fish protein hydrolysates (FPH) are products of hydrolysis reaction on peptide bonds in fish proteins and result in shorter peptides or amino acids which are easy for absorption (Shaik and Sarbon, 2020). Generally, the fish protein hydrolysate (FPH), is derived from the three major components of fish which are flesh, skin and discards from the latter which includes, head, frames, trimmings, fins, viscera and roe (Halim *et al.,* 2016; Rasli and Sarbon, 2018). There are several established methods are available to produce hydrolysate from many types of fish which include, autolysis, thermal hydrolysis and enzymatic hydrolysis (Halim *et al.,* 2016; Sarbon *et al.,* 2018). Among these methods, the enzymatic hydrolysis is widely performed in order to improve the functional and nutritional properties of food proteins (Sarbon *et al.,* 2019). Protein hydrolysates possess several beneficial functional properties like solubility,

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eISSN: 2550-2166

emulsifying, foaming, fat and water holding capacity (Amza *et al.,* 2013; Halim *et al.,* 2018). Thus, it provides a variety of application in human or animal nutrition, pharmaceuticals and cosmetics industries (Halim *et al.,* 2016; Nasir and Sarbon, 2019).

Sausage is a product, where meat flesh was mixed with additives and stuffed into suitable casings followed by heat process (Youssef and Barbut, 2010). Emulsified sausages are cooked sausages that have been finely comminute to the consistency of a fine paste. A large amount of emulsion sausage was also prepared commercially by using poultry, beef or pork emulsified with backfat from various sources (Amano, 2012). Recently, the raw materials like fish mince and surimi have been used as a base ingredient in the fish emulsion sausage product (Dincer and Cakli, 2010). Elavarasan *et al.* (2014) reported that fish protein hydrolysates produced from the fish processing food and its waste are having improved surface-active properties like emulsion and foaming. Previously, Intarasirisawat *et al.* (2014) have conducted a study in which FPH was added to the fish emulsion sausage. The addition of skipjack roe protein hydrolysate (SRPH) increased the hardness, cohesiveness and resilience ($p<0.05$) of the fish emulsion sausage.

Oxidation of unsaturated fatty acids is the main reaction responsible for lipid degradation, which is related to the final quality of the product (Intarasirisawat *et al.,* 2012). By that, antioxidant compounds in food product play an important role to retard the degradation and changes of product such as fish protein hydrolysate in fish emulsion sausage (Zakaria and Sarbon, 2018). Numbers of ways are available to represent the fish emulsion sausage oxidative stability quantitatively and the most basic methods are peroxide value analysis. Intarasirisawat *et al.* (2014) stated that, the emulsion sausage prepared with the addition of fish protein hydrolysate from skipjack roe used as a natural antioxidant to retard the lipid oxidation during refrigerated storage by the lower peroxide value (PV) obtained. Therefore, the objective of this study is to investigate the effects of FPH from silver catfish on characteristics of fish emulsion sausage from underutilized fish species of shortfin scad.

2. Materials and methods

2.1 Materials

Silver catfish (*Pangasius sp*.) and shortfin scad (*Decapterus macrosoma*) were purchased from the local supplier in Kuala Terengganu, Terengganu and placed in an iced condition during transportation to the laboratory. The other ingredients for sausage making were purchased from the local hypermarket. The enzyme Alcalase® 2.4 L, a bacterial endoproteinase from a strain of *Bacillus licheniformis* (Novozymes, Denmark) was used for the hydrolysis process. Analytical grade chemicals were used for the current study.

2.2 Preparation of silver catfish (Pangasius sp.) protein hydrolysate

Silver catfish (*Pangasius sp.*) protein hydrolysate (SCPH) was prepared according to the method as described by Intarasirisawat *et al.* (2014) with slight modifications. The sample was prepared with 55 g of silver catfish muscle mixed with 37.38 mL of distilled water and homogenized using a food processor. The endogenous enzyme present in the sample has been inactivated prior to enzymatic hydrolysis by incubating at 85^{o}C for 20 mins. The hydrolysis reaction was initiated by addition of Alcalase and the hydrolysis equilibrium took place at 50.11°C and pH at 7.89 for 84.02 mins. The mixture was then placed into a water bath at 85°C for 20 mins to stop or retard the enzymatic reaction. Then the sample was centrifuged (Gyrozen, Korea) at 6000 rpm for 20 mins and supernatant of hydrolysate was filtered and freeze-dried. The freeze-dried SCPH was subjected to fish emulsion sausage preparation.

2.3 Preparation of fish emulsion sausage from shortfin scad (Decapterus macrosoma)

Fish emulsion sausage was prepared based on Intarasirisawat *et al.* (2014) method with slight modifications. Approximately 260 g of shortfin scad flesh was separated and minced in the food processor. Then, about 30 g of canola oil was added and mixed thoroughly about 1 min. The seasoning and 24 mL of chilled water were added to the mixture and mixed in a food processor for 30 s. Then, 9.52 g of isolated soy protein was added and continued to mix for another 30 s. The remaining 30 g of oil was added, followed by 11.08 g of potato starch and continuously homogenized for 30 sec. Silver catfish (*Pangasius sp*.) protein hydrolysate was incorporated at different concentrations (1, 2 and 3%). Finally, 24 mL of chilled water was added and homogenized further 30 s until all the ingredients were mixed well. Then, the mixture was placed into a sausage stuffer and pumped into cellulose casing. The casings were tied at 10-12 cm for each sausage. The sausages were boiled in boiling water (75±4˚C) for 25 mins and the cellulose casing was removed. The sausage samples were packed in polyethylene vacuum bag prior to storage at 4˚C for 12 days. The samples were thawed in room temperature for 30 mins before further analysed.

2.4 Physicochemical properties

2.4.1 Proximate analysis

Proximate composition was determined according to AOAC methods (AOAC, 2000). Carbohydrate is the remained percentage of moisture, fats, protein, ash and fibre. Carbohydrate content was calculated as below:

Carbohydrate (%) = 100 - (% moisture + %crude fats + %crude protein + %ash)

2.4.2 Scanning electron microscopy (SEM)

Microstructure of fish emulsion sausage added with different concentrations (1, 2 and 3) of silver catfish protein hydrolysate (SCPH) and control (without SCPH) were observed with the help of Scanning Electron Microscope (SEM) (JEOL JSM-6360LA, Tokyo, Japan) according to Intarasirisawat *et al.* (2014). The samples with thicknesses of 2-3 mm were fixed with 2.5% of glutaraldehyde in 0.1 M sodium cacodylate buffer for 2-4 hrs. The fixed samples were initially washed with 0.1 M sodium cacodylate buffer (pH 7.2) for 10 mins followed by post-fixation in 0.1 M sodium cacodylate buffer which contains 2% osmium tetroxide for 2-4 hours. The fixed samples were again rinsed with 0.1M sodium cacodylate buffer for 10 mins and then allowed to dehydrate with different concentrations (35%-100%) of ethanol. Then, the dried samples were mounted on a bronze stub and sputter-coated with gold (JEOL JFC-1600, Tokyo, Japan). Finally, the specimens were observed in scanning electron microscope with an acceleration voltage of 5 kV.

2.4.3 Textures profiles

The texture profile analysis (TPA) was carried out with the help of the TA-XT2 texture analyzer (Stable Micro Systems, Godalming, Surrey, UK) with a load cell of 30 kg. The fresh samples on 0 day and thawed samples for 6^{th} and 12^{th} days were used to analysis. About 75 mm diameter cylindrical aluminium probe was used. The sausage samples were placed on the instrument's platform after cutting into cylindrical-shape with 25 mm height X 20 mm diameter. The test was performed with two compression cycles. At room temperature, the TPA was performed with the following testing conditions; crosshead speed 5.0 mm/s, 50% compression of the original sample height, surface sensing force 99 g, threshold 30.0 g. The time interval between first and second compressions fixed as 10 s. The force-time curves generated from each sample used to calculate the hardness, cohesiveness, chewiness, and resilience of the sausage. Textural changes were evaluated at day 0, 6 and 12 (Intarasirisawat *et al.,* 2014).

2.4.4 Colour determination

The colour of sausage sample was measured using a colorimeter (Hunter Lab, Model colour Flex, Reston, VIRG, USA) with the port size of 0.50 inches. The colorimeter was calibrated by using a calibration plate (black and white Minolta). The obtained values were placed in the CIE colour profile system as L* value (lightness), a* value (redness/ greenness), and b* value (yellowness/blueness) and recorded. The colour analysis was evaluated at 0, 6^{th} and 12^{th} day. According to Maqsood *et al.* (2012) the total difference value in colour (ΔE*) was calculated by using the following formula.

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Where, ΔL*, Δa* and Δb* are the differences between the corresponding color parameter of the sample and the standard (white colour). (L* = 93.63, a*= 0.92 and b*= 0.42).

2.4.5 Determination of antioxidant activity

2.4.5.1 Peroxide value

The lipid oxidation levels were determined by measuring the peroxide value obtained in the fish sausage produced according to Zakaria and Sarbon (2018). About 5.00 g (±0.05) of homogenized sausage was weighted and put into a 250 mL conical flask. Approximately 30 mL of the acetic acid-chloroform solution with ratio 3:2 was added into the conical flask. The flask was swirled until the sausage is completely dissolved. Then, 1 mL of saturated potassium iodide solution was added to the sample. The flask was swirled for 1 min and then stored in dark place about 5 mins. Approximately 75 mL of distilled water was added immediately, and it was shaken vigorously to liberate the iodine from the chloroform layer. Then, solution in the flask was titrated with 0.01 N sodium thiosulfate, $Na_2S_2O_3$ by constantly and vigorous shaking until the colour changes to light yellow. Next, about 1mL of 1% (w/v) soluble starch as an indicator was added into mixtures which give blue colour and the titration continued until a faint blue colour formed. Finally, 0.01N sodium thiosulfate, $Na_2S_2O_3$ was added drop-wise until the blue colour just disappears. The volume of titrant used was recorded. The Peroxide value was calculated based on the following formula:

$$\text{PV} = \frac{(\text{S} - \text{B}) \times \text{M} \times 1000}{\text{Weight of sample (g)}}$$

Where S = Volume (mL) of $Na_2S_2O_3$ of titration sample, B = Volume of $Na_2S_2O_3$ of titration blank and M = Concentration of the $Na_2S_2O_3$ solution

2.4.5.2 Thiobarbituric acid-reactive substances (TBARS)

The TBA values of the samples were measured

according to Sriket *et al.* (2015). The TBA method was used for evaluating the formation of secondary lipid products like malonaldehyde. This malonaldehyde binds with TBA at low pH and high temperatures (100°C) levels to form red colour complexes that can be measured at 532 nm. The increase in the amount of the red colour complex formed correlates with the oxidative rancidity of the lipid. The 50 µL reaction mixture, was added to distilled water (0.8 mL), 20% acetic acid (pH, 3.5) (1.5 mL), 8.1% sodium dodecyl sulphate (0.2 mL) and 0.8% 2-thiobarbituric acid (TBA) solution (1.5 mL) and the mixture was heated at 100ºC for 60 mins. Then, the mixture was allowed to cool at room temperature and centrifuged at 4300 rpm for 12 mins. The absorbance of the upper layer of mixture solutions was measured at 532 nm. The antioxidant activity levels of the sample were identified as malonaldehyde (MDA) concentrations. A standard curve was plotted from the tetraethoxypropane (TEP) solution to give dilutions containing the equivalent of 1.25, 2.5, 5.0 and 10 µg/mL of malonaldehyde (MDA). TEP solution was used as a standard for quantitative analysis of MDA in products, in relation to the oxidation which are reported as mg of MDA/kg of oil.

2.5 Statistical analysis

All the experiments were carried out with three replications (n=3). Complete random design (CRD) has been used throughout this study. The statistical analysis was performed to determine the difference between the mean of samples using one-way analysis of variance (ANOVA). The significant differences among the means were identified by using Fisher's Least Significant Difference (LSD) at $p<0.05$. The data analysis was performed using Minitab Statistical Software, version 14.0 (Minitab Inc.).

3. Results and discussion

3.1 Proximate composition of fish emulsion sausage

The major components present in fish are protein, fat, water and ash and minor components are carbohydrates, vitamins, nucleotides, non-protein nitrogenous compounds (Ishak and Sarbon, 2018). Fish emulsion sausages contain about 63.49-66.85% of moisture, followed by 18.04-21.89% protein, 7.16-8.59% fat, 1.83-2.17% ash and 2.28-2.72% carbohydrate (Table 1). Generally, the addition of SCPH did not significantly ($p>0.05$) affect the fat, carbohydrate and ash contents in prepared sausage compared to the control sample (without SCPH). However, fish sausages with SCPH addition at different concentrations (1-3%) have the higher protein and lower moisture content compared to the control sample. The decrease in moisture content occurred due to the lyophilization carried out in hydrolysates. The protein contents of fish emulsion sausage increased with SCPH level increased ($p<0.05$).

As a wet product, the highest moisture content of the fish emulsion sausage was expected. The efficacy of nisin with three different concentrations, 12.5, 25 and 50 ppm were reported by Raju *et al.* (2003) on storage of fish sausage in at ambient temperature of 28±2°C and refrigerated temperature 6±2°C. The fresh fish sausage moisture content was determined as 68.64%. However, the range of moisture contents in shortfin scad emulsion sausage was lower than the nisin fish sausage. Park and Morissey (2000) reported that, the moisture content of a meat-based product will affect the quality of the product especially, the whiteness and gel strength. As expected, the protein content of the fish emulsion sausage was increased with an increase in the proportion of silver catfish protein hydrolysate (SCPH). The protein content of shortfin scad emulsion sausage was higher than the protein content of fish sausage (16.76%) reported by Raju *et al.* (2003). The percentages of fish protein hydrolysate used during preparation portray the highest protein contents of the samples due to high content of proteins in SCPH the protein content in sausage was increased.

Similarly, the fat contents of shortfin scad emulsion sausage were higher (5.64%) than the fish sausage produced by Raju *et al.* (2003). This might due to the use of shortfin scad flesh in the production of fish sausage which has higher fat content. The fat content level depends on the type of fish species where, the higher value of fat content found in oily fish (>8%) and lower value fat content observed with lean fish (<4%) (King, 2002). In addition, high ash contents in the fish sausage

Table 1. Proximate composition of Shortfin Scad emulsion sausage added with and without Silver Catfish (*Pangasius sp.)* protein hydrolysate (SCPH)

Sample	Moisture	Protein	Fat	Ash	Carbohydrate
Control	66.85±0.09^{a}	18.04±0.74^{c}	8.15±0.18^{a}	1.83±0.10^{b}	2.28±0.17^{a}
1% SCPH	63.85±0.15^{b}	19.61±0.50^{b}	8.30±0.82^{a}	2.17±0.04^{a}	2.63±0.09^{a}
2% SCPH	63.67±0.14^{bc}	21.71±0.50^{a}	7.16±1.08^{a}	2.13±0.23^{ab}	2.72±0.08^{a}
3% SCPH	63.49±0.19^{c}	21.89±0.25^{a}	8.59±1.21^{a}	2.12±0.22^{ab}	2.64±0.04^{a}

Values are expressed as mean±standard deviation, n = 3. Values with different superscript within the same column indicate significant differences ($p<0.05$).

added with SCPH might be due to the high contents of minerals in SCPH compared to control samples. Besides, the addition of salt during the processing also contributed to increase ash content as 2.5% salt was added during fish sausage preparation by Venugopal (2006). However, the ash contents in the shortfin scad emulsion sausages (1.83-2.17%) were lower than the fish sausage (2.67%) prepared by Raju *et al.* (2003).

As shown in Table 1, the carbohydrate content was reduced with the increasing concentration of the fish protein hydrolysate. The reduced carbohydrate content was balanced with the addition of potato starch during the preparation of fish emulsion sausage. Venugopal (2006) reported in his study that, around 1.5% of potato starch was added during fish sausage preparation. The nutritional value of the food product also depends on the level of carbohydrates content, either low or high, thus the reasons for the addition of starch with fish emulsion sausage. This result suggested that, the SCPH is possible to use for improving the nutritional value of shortfin scad emulsion sausages.

3.2 Microstructure of fish emulsion sausage

The different microstructures of fish emulsion sausage depicted in Figure 1 and the variation between SCPH added sausage and control sausage microstructures was analysed with scanning electron microscopy. The size of the fat globules present in the fish emulsion sausage was as the concentration of SCPH increased, and the same was shown in descending order, control (34-65 µm)> 1% SCPH (24-42 µm)> 2% SCPH (16-26 µm) and 3% SCPH (14-20 µm). Fish emulsion sausage containing SCPH had the slightly compact structure with fewer cavities, especially samples added with 3% SCPH. The control sample shown the widen structure with larger cavities compared to the SCPH added sausage samples.

Increasing SCPH concentration more likely resulted in smaller size fat globule formation with uniform distribution due to emulsifying properties of the SCPH and formation of an interfacial thin film around the fat globules (Intarasirisawat *et al.,* 2014). After emulsification, the SCPH with myofibrillar proteins can migrate to the interface rapidly and stabilize an emulsion formed (Cáceres *et al.,* 2008). The SCPH with small size peptides probably exhibit greater emulsifying activity to larger peptides or proteins. The high protein matrix density is governed by the higher degree of protein aggregation and unfavourable textural properties.

The uniform oil droplets distribution and emulsion formation in the sausages with 3% of SCPH was noticed, which were smaller in size, compared to others (Figure 1). The hardness and greater consistency of the product promotes due to compact matrix (Cáceres *et al.,* 2008). The highest concentrated (3%) SCPH might inhibit the combination of the emulsion due to its protective role towards the retardation of the oxidative damage in protein, which acts as an emulsifier.

Thus, the microstructure of the emulsion sausage was decreased with increasing in the concentration of SCPH and the size of fat globules in sausage was reported as: control (24-65 µm)> 1% SCPH (24-42 µm)> 2% SCPH (15-25 µm)> 3% SCPH (10-20 µm). Similar

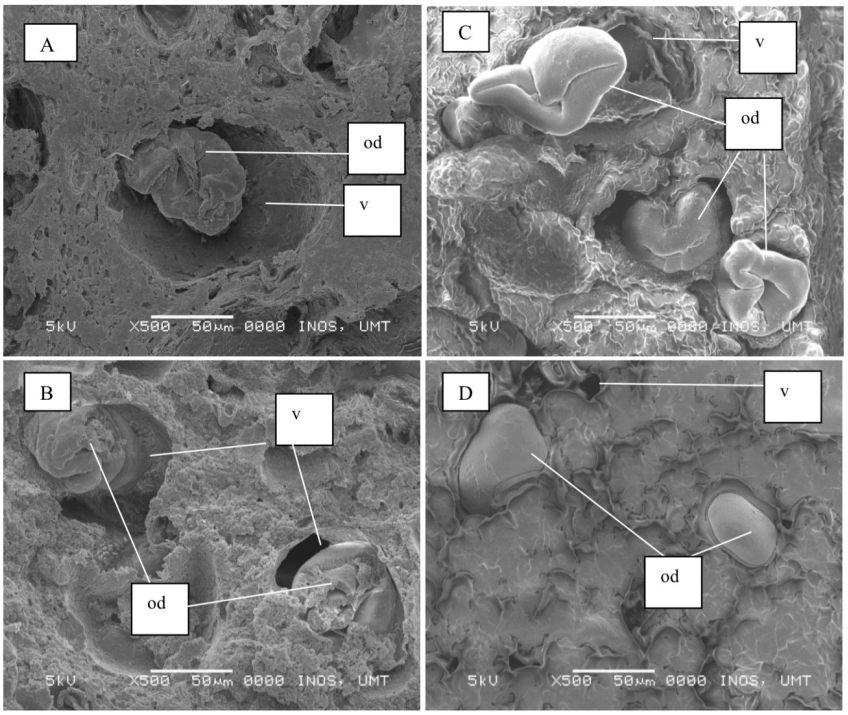

Figure 1. Magnification of 500 x. (A); control (without SCPH), (B); sample added with 1% SCPH, (C); sample added with 2% SCPH and (D); sample added with 3% SCPH. v; Void and od: oil droplet

study reported by Intarasirisawat *et al.* (2014) revealed that, the size of fat globules in the sausage was in a descending order with increasing of SRPH: control (24-65 µm)> 0.5 g SRPH (24-42 µm)> 1 g SRPH (15-25 µm) and 3 g SRPH (10-20 µm). The skipjack roe protein hydrolysate (SRPH) with smaller size peptides probably exhibited greater emulsifying activity compared to larger peptides or proteins. Therefore, SCPH have higher stabilizing property on the emulsion sausage.

3.3 Changes in oxidative stability and properties of shortfin scad emulsion sausage during 12 days of storage

3.3.1 Colour properties of fish emulsion sausage

The colour analysis values are expressed as L*, a*, b* and Hue angle of fish emulsion sausages with SCPH (1-3%) and without SCPH during 12 days storage at refrigerated temperature were shown in Table 2. At day 0, the results were not showing any significant difference for all colour parameters between all the samples ($p>0.05$). As storage time increased, the lightness L* and b* values of the products increased ($p<0.05$), while a* value decreased. However, all colour parameters of all samples increased with increasing level of SCPH in the fish emulsion sausage ($p<0.05$) except the yellowness parameter that decreased with increasing SCPH level.

The lightness value (L*) of fish emulsion sausage was increased with the increasing of SCPH concentration (1-3%) ($p<0.05$). However, the L* value was not changed with the increasing storage time (0-6 days) due to the same formulation used in the production process. The L* value of fish emulsion sausage was ranging from (60.91-66.75), (64.76-67.00) and (65.07-67.37) at 0, 6 and 12 days of storage. As shown in Table 2, the fish sausage with SCPH addition had higher whiteness value than the control fish sausage at all the storage times, it is due to delay in the oxidation process in SCPH sausage and this activity was conforming with similar studies conducted by Intarasirisawat *et al.* (2014). The lightness value of the fish emulsion sausage was similar to the Malaysian fish sausage (Salman and Tuna with other ingredients) lightness value which is ranged between 58.73 to 79.56 (Huda *et al.,* 2012).

The emulsion sausage redness value (a*) calculation revealed that, it is significantly affected by the storage time ($p<0.05$) and the concentration of SCPH (1-3%) ($p<0.05$). Due to the oxidation, levels of redness parameter (a*) of Hunter Lab colour space consequently decreased, which indicates the changes in some colour constituents like myoglobin. The myoglobin is the predominant pigment which is the responsible for 80% of meat colour. Myoglobin was unstable compound, and it is easily converted into the oxidized brown metmyoglobin when exposed to oxygen (Dolatowski and Olszak, 2007). The effectiveness of the SCPH can be observed with the increasing levels of redness value and decreasing levels of yellowness value in sausage. The study of Thomas *et al.* (2008) reported the similar results, in which 3% of textured soy protein usage significantly increased ($p<0.01$) the redness in pork sausage.

The yellowness value of the fish emulsion sausage was decreased with the increasing of SCPH level. This might be due to the oxidation of fat content in fish emulsion sausage and can be reduced by using an antioxidant agent (Suhaj, 2006). SCPH acts as the antioxidant agent that breaks the oxidation chain by stabilizing free radicals (Galla *et al.,* 2012). Besides, the addition of SCPH could prevent the oxidation of haem

Table 2. Changes in L*, a* and b* value of fish emulsion sausage during 12 days storage

Storage time (days)	SCPH levels (%)	Colour analysis			
		L*	a*	b*	Hue angle
0	0 (Control)	65.05 ± 1.57^{Aa}	5.96 ± 0.84^{Aa}	9.54 ± 0.75^{Ba}	58.01
	1	60.91 ± 5.11^{Aa}	6.02 ± 1.10^{Aa}	8.28 ± 1.02^{Ba}	53.98
	2	66.62 ± 2.66^{Aa}	6.58 ± 0.41^{Aa}	9.21 ± 0.44^{Ba}	54.47
	3	66.75 ± 5.77^{Aa}	6.22 ± 0.99^{Aa}	9.10 ± 1.38^{Ba}	55.65
6	0 (Control)	64.76 ± 0.50^{Abc}	2.28 ± 0.17^{Bb}	13.81 ± 0.39^{Aa}	80.63
	1	65.10 ± 0.70^{Ab}	2.63 ± 0.09^{Ba}	13.19 ± 0.35^{Ab}	78.72
	2	66.39 ± 1.41^{Aab}	2.72 ± 0.08^{Ba}	13.24 ± 0.10^{Ab}	78.39
	3	67.00 ± 0.32^{Aa}	2.64 ± 0.04^{Ba}	13.62 ± 0.23^{Aab}	79.03
12	0 (Control)	65.07 ± 0.20^{Abc}	2.14 ± 0.04^{Bb}	14.41 ± 0.13^{Aa}	81.55
	1	65.60 ± 0.81^{Ab}	2.80 ± 0.05^{Ba}	13.21 ± 0.55^{Ab}	78.03
	2	66.84 ± 0.51^{Aab}	2.85 ± 0.15^{Ba}	13.10 ± 0.31^{Ab}	77.73
	3	67.37 ± 1.52^{Aa}	2.37 ± 0.35^{Bb}	13.12 ± 0.39^{Ab}	79.76

Values are expressed as mean±standard deviation, $n = 3$. Values with different uppercase superscript within the same column of the SCPH levels (%) while values with different lowercase superscript within the same column of the same storage time indicate significant differences ($p<0.05$).

pigments, thus the red colour of the muscle particles did not turn into yellowish brown hue. Marianski and Marianski (2008) reported that, soy protein also prevents the melting of fat. Similarly, 3% of textured soy protein in pork sausage significantly decreased the yellowness value ($p<0.01$) (Thomas *et al.,* 2008). This is the reasons why the samples with the addition of SCPH had higher redness value and lower yellowness value compared to the control sample. According to the results, it is clear that, the addition of SCPH has a remarkable impact on the changes of colour parameters. Therefore, the result proved that the addition of 3% SCPH is able to stabilize the colour of the fish emulsion sausage during the storage time.

3.3.2 Texture properties of fish emulsion sausage

The textural properties change of the fish emulsion sausages during the storage were tabulated in the Table 3. There was a significant difference in all the textural parameters among all the samples tested ($p<0.05$) on day 0. After 12 days storage, all samples showed decreases in hardness and chewiness ($p<0.05$), while an increase in springiness, cohesiveness, and also resilience ($p<0.05$). In general, storage time did affect textural properties of sausage samples. The results indicated that the formation of softening texture was correlated with the storage duration. It occurred probably due to the proteolytic action which promoted by muscle endopeptidases and microbial (bacteria and yeasts) proteinases when prolonged storage time was applied (Toldra, 2006). A study of Zapata and Pava (2018) reported that, antioxidants must add to the formulations of sausages made with tilapia in order to reduce lipid oxidation to maintain quality of sausage. Some of the textural parameter value might be decreasing due to the oxidation occurred in samples. The oxidation of protein could be induced by radicals being generated from lipid oxidation (Shacter, 2000). Two mechanisms have been proposed for oxidation-induced changes in texture through formation of protein cross-links and less tenderization through reduced proteolysis (Yulong and Ertbjerg, 2019). In addition, the lipid and protein oxidation are also associated with deteriorating processes occurring in the meat products (Mercier *et al.,* 2004). Protein oxidation can severely affect the sensory qualities of the fresh meat as well as the meat products in terms of its colour, texture and tenderness (Rowe *et al.,* 2004).

It was also reported that, the oxidation process increased with the prolonged storage time because, degradation of the protein film presents around the fat globules in the emulsion system (Chaiyasit *et al.,* 2005). The slowdown of the lipid oxidation process in the sausage with SCPH might prevent the adverse effects at some degrees. These results were in agreement with the study conducted by Intarasirisawat *et al.* (2014). From the study, the results indicated that hardening texture, decreased over the storage time, which was probably due to increase in moisture content in the sausage samples. Though, all the textural parameters of all samples increased with increasing concentration of SCPH in the fish emulsion sausage ($p<0.05$). The addition of SCPH to emulsion sausage resulted in increased hardness, chewiness, springiness, cohesiveness, and resilience ($p<0.05$) as compared to the control sample. In general, the concentration of SCPH directly affects the textural properties of the sausage samples. It implied that SCPH with the concentration from 1-3% used in the present study might exhibit the effective emulsifying activity, in which emulsion can be stabilized in the matrix. Samples added with SCPH showed higher values of all the

Table 3. Texture properties of Shortfin Scad emulsion sausage during extended 12 days of storage.

Storage time (days)	SCPH levels (%)	Texture profile analysis (TPA)				
		Hardness (N)	Springiness	Cohesiveness	Chewiness (N)	Resilience
0	0 (Control)	8.87 ± 0.65^{Ab}	0.45 ± 0.06^{Bc}	0.34 ± 0.04^{Bc}	1.39 ± 0.45^{Bd}	0.11 ± 0.01^{Bc}
	1	10.18 ± 1.12^{Ab}	0.71 ± 0.06^{Ab}	0.40 ± 0.02^{Ab}	2.89 ± 0.37^{Ac}	0.14 ± 0.01^{Ab}
	2	16.02 ± 0.29^{Aa}	0.76 ± 0.02^{Bab}	0.45 ± 0.01^{Bb}	5.41 ± 0.09^{Bb}	0.15 ± 0.00^{Bb}
	3	16.47 ± 0.80^{Aa}	0.80 ± 0.01^{Aa}	0.50 ± 0.01^{Aa}	6.56 ± 0.37^{Aa}	0.18 ± 0.00^{Aa}
6	0 (Control)	8.52 ± 1.54^{Ab}	0.71 ± 0.13^{Ab}	0.49 ± 0.07^{Aa}	2.93 ± 0.67^{Ac}	0.18 ± 0.04^{Aa}
	1	8.59 ± 0.52^{Bb}	0.58 ± 0.02^{Bc}	0.38 ± 0.02^{Ab}	1.88 ± 0.04^{Bd}	0.13 ± 0.01^{Ab}
	2	14.80 ± 0.21^{Ba}	0.84 ± 0.01^{Aa}	0.51 ± 0.01^{Aa}	6.26 ± 0.21^{Ab}	0.19 ± 0.01^{Aa}
	3	15.91 ± 1.20^{Aa}	0.86 ± 0.01^{Aa}	0.53 ± 0.00^{Aa}	7.18 ± 0.62^{Aa}	0.21 ± 0.01^{Aa}
12	0 (Control)	7.60 ± 1.08^{Bc}	0.60 ± 0.06^{Bb}	0.42 ± 0.03^{Bb}	1.91 ± 0.37^{Bc}	0.14 ± 0.02^{Ba}
	1	9.67 ± 0.47^{Ab}	0.76 ± 0.12^{Aa}	0.32 ± 0.01^{Bc}	2.36 ± 0.48^{ABc}	0.10 ± 0.01^{Bb}
	2	10.22 ± 0.43^{Cb}	0.74 ± 0.03^{Aab}	0.41 ± 0.01^{Ab}	3.11 ± 0.26^{Ab}	0.15 ± 0.01^{Ba}
	3	14.84 ± 0.57^{Ba}	0.85 ± 0.02^{Aa}	0.46 ± 0.02^{Ba}	5.80 ± 0.30^{Ba}	0.17 ± 0.00^{Ba}

Values are expressed as mean±standard deviation, $n = 3$. Values with different uppercase superscript within the same column of the SCPH levels (%) while values with different lowercase superscript within the same column of the same storage time indicate significant differences ($p<0.05$).

textural parameters when compared to the control samples ($p<0.05$), especially SCPH at 3%.

Based on the result obtained in the current study, the texture of sausage could be improved, and shelf life of sausage can be extended with SCPH in fish emulsion sausages. This finding suggested that the textural properties of fish emulsion sausage could be stabilized during storage with the addition of SCPH at 3%.

3.3.3 Lipid oxidation of shortfin scad emulsion sausage

3.3.3.1 Peroxide value of fish emulsion sausage

The effect of SCPH (1, 2 and 3%) on lipid oxidation of shortfin scad emulsion sausages during 12 days storage at refrigerated temperature was depicted in Figure 2. There are no significant changes in peroxide value (PV) in emulsion sausage were observed in all the samples during the 0 day of storage ($p>0.05$). The sharp increase in PV was noticed in all the samples up to 6 days storage ($p<0.05$). From the 6th day of storage, the reduced PV was observed in all the samples up to the end of the stipulated storage time i.e. 12 days ($p<0.05$). However, the control samples have a higher rate increase of PV compared to the sample with SCPH added.

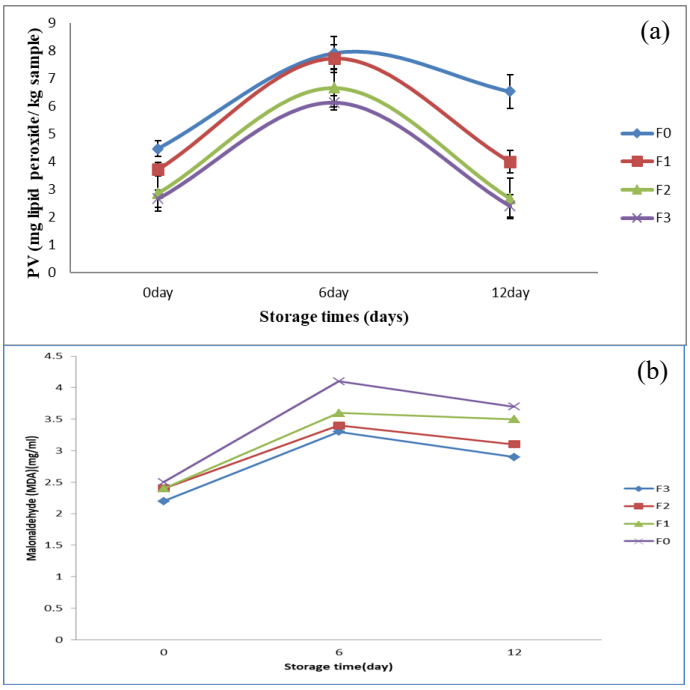

Figure 2. Effect of SCPH at various levels (1-3%) on (a) peroxide value and (b) TBARS of shortfin scad emulsion sausage during refrigerated storage.

The increase in PV of the samples with SCPH throughout the storage time indicated that, the samples were in the propagation stage of lipid oxidation and poor rates of decomposition of hydroperoxide formed (Maqsood, Benjakul and Balange, 2012). In general, the control sample exhibited the highest PV up to 6 days of storage compared to other the samples ($p<0.05$). After extended storage time, the reduced PV was observed and it was presumed due to the decomposition of the hydroperoxides formed into secondary oxidation products (Boselli *et al.,* 2005). The secondary oxidation products such as aldehydes, ketone, acid, etc., were produced from the decomposition of hydroperoxides (Chaijan *et al.,* 2006).

However, the samples with SCPH, showed the poor rate of increase in PV when compared with the control sample, especially with higher concentration of SCPH ($p<0.05$). It was noticed that the efficacy of silver catfish protein hydrolysate (SCPH) in preventing lipid oxidation was performed by dose-independent manner. However, the PV of the samples with SCPH (2 and 3%) were not significantly different throughout the storage time ($p>0.05$). These results also indicate that, the SCPH samples were more effective than compared to the control sample in retarding the formation of hydroperoxide. The SCPH exhibit potential radical scavenging activity through hydrogen donating and reducing power, thereby terminating the propagation (Maqsood and Benjakul, 2010). The *in vitro* antioxidative activity of SCPH was similar to the study conducted by Intarasirisawat *et al.* (2012). Besides, this finding also agreed with the similar study reported by Maqsood *et al.* (2012) in which the treated sample has a lower rate of PV values than untreated sample.

3.3.3.2 Thiobarbituric acid-reactive substances (TBARS)

The TBARS is one of the most commonly used methods to determine the secondary products formed during the oxidation process. Based on the colour product resulting from the condensation of TBA with aldehydes including malonaldehyde (MDA) this is generated in the oxidized fats (Halim *et al.,* 2018). This test was widely used as a marker for oxidative stress and an index of lipid peroxidation (Alghazeer *et al.,* 2008).

The effect of the various contents of SCPH (1, 2 and 3%) on TBARS values of shortfin scad emulsion sausages was displayed in Figure 2. There were no significant differences ($p>0.05$) in TBARS value of emulsion sausages were observed in all samples on the 0 -day storage. However, it increased sharply up to day 6 for the control ($p<0.05$). After day 6, the TBARS values continuously decreased until the end of the storage and for the samples with SCPH, the continuous increase was found up to 6 days of storage, followed by the decrease from day 6 to day 12 ($p<0.05$). The control sample showed the highest formation of TBARS during the storage of 12 days, when compared to the other samples ($p<0.05$). There is a significant change ($p<0.05$) in TBARS formation found between the sample with 3%

SCPH and other samples which indicates lowest TBARS formation in 3% SCPH sample.

The TBARS values of all samples ranged from the 2 to 2.5 mg MDA/mL, indicating the occurrence of lipid oxidation during the processing and cooking of the emulsion sausages on day 0. The decrease in the TBARS values during 6-12 days of storage was occurring due to the loss of volatile secondary oxidation products. Furthermore, these products may react with the free amino acids, proteins and peptides present in the emulsion sausages to form the Schiff bases (Nordvi *et al.,* 2007). The TBARS formation was increased in control sample might be due to the higher loss of volatile secondary products on day 12 that leading to the lower retained amount compared with the amounts found in samples with SCPH. The TBARS value obtained from the study conducted by Intarasirisawat *et al.* (2014) reported that the increase in TBARS of all fish emulsion sausage was noticeable throughout the refrigerated storage and the same agreed with the present study. Besides, Cavalheiro *et al.* (2014) reported that the mortadella-type sausages added with mechanically deboned chicken meat protein hydrolysate showed good oxidative stability during 60 days of storage (maximum TBARS value = 0.688 mg MDA/kg).

This study suggested that the addition of SCPH at different concentrations (1-3%) caused the TBARS formation in shortfin scad emulsion sausage retarded effectively. It was noticed that SCPH has efficacy in the retardation of TBARS in a dose-dependent manner and this result was well correlated with a PV of SCPH samples. Thus, SCPH at different concentrations (1-3%) was effective in retarding lipid oxidation in shortfin scad emulsion sausage during the 12 days storage at refrigerated temperature.

4. Conclusion

The fish emulsion sausages from shortfin scad (*Decapterus macrosoma*) were successfully produced without and with the incorporation of silver catfish (*Pangasius sp*.) protein hydrolysate, SCPH at three concentrations (1-3%). The proximate compositions of fish emulsion sausage with the addition of SCPH improved the protein content, higher in L* value and a* value but lower in b* value compared to the control. Additionally, 3% of SCPH added fish emulsion could improve the textural properties during storage for 12 days of refrigerated storage. Finer fat globules were visualised in the emulsion sausage sample with SCPH added, contained continuously and homogeneously dispersed oil droplets, which were smaller in size compared to others. The lower TBARS formation as well as lower PV value indicating the effectiveness of SCPH at different concentrations (1-3%) in the reduction of the lipid oxidation in fish emulsion sausage during 12 days storage. SCPH, especially with the higher level (3%), could be used as a natural antioxidative emulsifier in the preparation of fish emulsion sausage.

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