

# Sensory Characteristics and Microbiological Quality Changes of Nile Tilapia Fillet Processed by Various Sous-vide Conditions During Chilled Storage

Jaksuma Pongsetkul<sup>1,\*</sup> , Soottawat Benjakul<sup>2</sup> , Surintorn Boonanuntasarn<sup>1</sup> ,  
Jirawat Yongsawatdigul<sup>3</sup> 

<sup>1</sup>Suranaree University of Technology, Institute of Agricultural Technology, School of Animal Technology and Innovation, Nakhon Ratchasima, Thailand, 3000.

<sup>2</sup>Prince of Songkla University, Faculty of Agro-Industry, International Center of Excellence in Seafood Science and Innovation, Hat Yai, Songkhla, Thailand, 90110.

<sup>3</sup>Suranaree University of Technology, Institute of Agricultural Technology, School of Food Technology, Nakhon Ratchasima, Thailand, 30000.

## How to cite

Pongsetkul, J., Benjakul, S., Boonanuntasarn, S., Yongsawatdigul, J. (2022). Sensory Characteristics and Microbiological Quality Changes of Nile Tilapia Fillet Processed by Various Sous-vide Conditions During Chilled Storage. *Turkish Journal of Fisheries and Aquatic Sciences*, 22(9), TRJFAS21079. <https://doi.org/10.4194/TRJFAS21079>

## Article History

Received 01 December 2021

Accepted 18 April 2022

First Online 21 April 2022

## Corresponding Author

Tel.: +6644223641

E-mail: jaksuma@sut.ac.th

## Keywords

Nile tilapia

Sous-vide cooking

Sensory quality

Protein degradation

Storage

## Abstract

Sensory characteristics and microbiological quality of Nile tilapia cooked with various sous-vide (SV) conditions including 50 and 60°C, for 30, 45 and 60 min (S5-30, S5-45, S5-60, S6-30, S6-45 and S6-60) and their changes during storage (4°C) were investigated, compared with control (cooking with boiling water). The result found that increasing temperature and time of SV accelerated protein degradation, both myofibrillar protein and connective tissue. This affected to the lower water-holding capacity (WHC) and shear force of samples treated with severe SV conditions (S6-45 and S6-60) ( $P < 0.05$ ). SV technique may less contribution to the flavor formation, particularly lipid oxidation products, since there were no differences in total volatile base nitrogen (TVB-N) content and thiobarbituric acid reactive substances (TBARS) value among all samples ( $P > 0.05$ ). However, SV cooked samples had higher sensory scores than control, both at day 0 (before storage) or throughout the storage time. This suggested the potential to improve consumer acceptability by this technique. In this study, SV at 60°C, 60 min seem to be the optimal condition for tilapia fillet, which can preserve at 4°C for at least 6 weeks without any spoilage.

## Introduction

A growing trend in consumer demand for “ready-to-eat” (RTE) food has been noted over the last decade since people nowadays have become more pressed for time and the use of convenient, simplified meals has become a way of life. Normally, fish is highly susceptible to spoilage after postmortem due to enzymatic autolysis, oxidation and microbial growth (Velioglu et al., 2015), thus there is a need to preserve or extend its shelf-life. Sous-vide (SV) technique is one of the product alternatives fulfilling consumer demands for RTE foods and increase the food’s shelf-life by means of heat processing (Baldwin, 2012). SV cooking is a process in

which food is cooked in heat stable vacuumed containers under controlled temperature (55–80°C) for a specific time followed by low-temperature storage (Ayub & Ahmad, 2019). With those conditions of heating, juiciness of meat is maintained while the flavor and tenderness are improved (Aguilera, 2018). Normally, cooking not only changes food properties but also make food free from pathogens. There is concern about the safety of sous vide fish as the requirements for a mild heat treatment aimed at preserving a high sensory quality may not ensure a proper bacterial destruction (NACMCF, 1990). In fact, the shelf life and safety of SV products depend on heat treatment as well as the storage temperature (Shakila et al., 2009). Based

on many previous research, couple with the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) standard (NACMCG, 1990), a heat treatment at 60-80°C for 20-40 min is preferable for fish meat to compromise both sensorial characteristics and product's safety. Many previous research exhibited the better sensorial quality of SV cooked fish, compared with traditional cooking. Also, some research focused on the microbiological quality/safety of those products. Gittleston et al. (1992) demonstrated the positive aspects of nutritional composition and sensory perception of SV salmon fillet. Gonzalez-Fandos et al. (2005) stated that SV condition of 90°C, 33 minutes significantly improved the microbiological stability and sensory performance of trout (*Oncorhynchus mykiss*) fillet. The shelf-life of emperorbreem (*Lethrinus lethrinus*) cakes were extended from 4 weeks (conventionally cook-chilled) to 16 weeks with security against pathogenic bacteria after applied with SV cooking (100°C, 20 min). However, after 16 weeks, there was some quality loss were noted (Shakila et al., 2009). Nowadays, there is very little information devoted to assessing both sensorial and microbiological quality of SV cooked fish, especially when compared with those prepared by traditionally. Moreover, there was no information regarding the appropriate heat treatment level of SV cooking, as well as their shelf-life, for tilapia meat. Therefore, the objective of this study was to evaluate the physical, chemical and microbiological characteristics, as well as sensory acceptability of tilapia fillet processed by different SV conditions (time and temperature). The quality changes of all SV cooked samples during refrigerated storage (4°C) for 6 weeks were also monitored, compared with control (regularly cooking with boiling water)

## Materials and Methods

### Sample Preparation

Nile tilapia (*Oreochromis niloticus*) with the average weight of 656.25±82.26 g was procured from a farm at Suranaree University of Technology, Nakhon Ratchasima, Thailand. After capture, the fish were transported in ice with a fish/ice ratio of 1:2 (w/w) in a polystyrene foam box to the laboratory within 30 min. After arrival, 336 tilapia fillets were prepared. Fish were cleaned, beheaded, eviscerated, filleted, deskinning, and then again washed with clean water. Each fillet, with approximately 1.0-1.5 cm thickness, was individually put into a "Three-sided seal bag" retort pouch (PET12 // AL9 // ONy15 // Retort CPP60) (Monotaro, Japan) and vacuum-sealed using a vacuum-packing machine (FVC-II, Furukawa MFG Co., Ltd., Japan) with extent of vacuum 99.6%. Samples were divided to 7 treatment groups (48 pieces/group) including 6 treatment groups for applying various SV cooking conditions and 1 treatment group for control. For SV processing, samples were cooked by immersing in a continuously thermocontrolled water

bath under various temperatures and time as following:

- |                          |                          |
|--------------------------|--------------------------|
| (1) 50°C, 30 min (S5-30) | (2) 50°C, 45 min (S5-45) |
| (3) 50°C, 60 min (S5-60) | (4) 60°C, 30 min (S6-30) |
| (5) 60°C, 45 min (S6-45) | (6) 60°C, 60 min (S6-60) |

Samples directly cooked in boiled water (100°C) until the internal temperature of meat reaches 71°C and holds for 5 min was used as control (C). Temperature data logger series II (ThermaData-K, USA) with an embedded thermocouple probe were inserted into vacuum pouches with the tilapia fillets to monitor the fluctuations of temperature during SV cooking and subsequent chilled storage. After heating process, all 7 samples were rapidly cooled down in water-containing ice for 10 min. Then, fish was stored in refrigerator (4°C) for 6 weeks. During storage, samples were periodically taken every 2 weeks to analyze on their quality. Two replicate experiments were conducted. For each sampling day, 12 random pieces from each group were analyzed as following:

### Microbial Analysis

Microbial population were evaluated as per the method of the International Commission on Microbiological Specifications for Foods (ICMSF, 1978). Fish samples (10 g) were mixed with 90 mL of 0.1% sterile peptone water using a Stomacher 400 Lab Blender (Seward Ltd., Worthing, UK) at high speed for 3 min. Appropriate serial dilutions were prepared with the same diluent. The total mesophilic and psychrophilic aerobic bacterial loads were determined on Plate Count Agar (PCA) (Merck, Darmstadt, Germany) after incubation at 35°C for 24-48 h, and at 7°C for 10 days, respectively. Anaerobes were determined in PCA incubated in anaerobic jar at 35°C for 48 h. Moreover, some pathogen including *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens* as well as *Listeria* spp. were also determined using Baird-Parker agar (Oxoid, Cambridge, UK), mannitol egg yolk polymixin agar (MYP) (Merck, Darmstadt, Germany), Tryptose-sulfite-cycloserine (TSC) agar and Listeria Enrichment Broth (LEB)/Palcam agar (Merck, Darmstadt, Germany) as media respectively, on day 0 and final day of storage to ensure the product's safety. The results were expressed as log CFU/g sample.

### Meat Characteristics

The pH of fish meat was determined using pH meter (Sartorius, Gottingen, Germany) according to the method of Nirmal and Benjakul (2009). Total volatile base nitrogen (TVB-N) content was estimated by the Conway micro-diffusion method and expressed as mg N/g sample (Conway, 1950). Thiobarbituric acid reactive substances (TBARS) were determined as described by Nirmal and Benjakul (2009) with some modifications. A standard curve was prepared using 1,1,3,3-

tetramethoxy propane at concentrations ranging from 0 to 2 ppm. TBARS value was calculated and expressed as mg malonaldehyde/ kg sample. TCA-soluble peptide content was determined according to the method of Sriket et al. (2012). Soluble oligopeptide content in the supernatant was measured according to the Lowry method (Lowry et al., 1951) and expressed as  $\mu\text{mol}$  tyrosine equivalent/ g sample. Total collagen content was determined by alkaline hydrolysis as described by Reddy and Enwemeka (1996) with some modifications. Samples were hydrolyzed with 7M NaOH at 120°C for 40 min. The hydrolysate was neutralized with 3.5M H<sub>2</sub>SO<sub>4</sub>, filtered, and reacted with chloramine-T solution and Ehrlich's reagent. The absorbance was measured at 550 nm using hydroxyproline (Sigma-Aldrich Co., St. Louis, MO, USA) as a standard. Total collagen content was calculated using a conversion factor of 7.25 and expressed as mg/g sample. Water holding capacity (WHC) was determined using low-speed centrifugation method as described by Digre et al. (2011). Fifteen grams of minced sample were centrifuged at 1,500×g for 5 min (4°C), then WHC was calculated as percentage of water retained in the mince after centrifugation. For shear force determination, samples were cut into small pieces (1×2×1 cm, width×length×thickness) and measured using the Warner-Bratzler blade equipped with Texture Analyzer (TA.XT.Plus, Stable Micro Systems, Surrey, UK) with the speed test of 4 mm/s and a 50 kg load cell as per the method of Wattanachant et al. (2004).

**Sensory Evaluation**

Sensory evaluation was performed by 50 untrained panelists, who familiar with this fish species, using 9-point hedonic scale (Meilgaard et al., 1999). The samples were served after heating for 1 min at full power in a microwave oven MW 206 of 800 watts and a frequency of 2,450 MHz (EMM20K18GW, Electrolux, Thailand). After cooling for 1-2 min, samples were served on white paper plate. Evaluation was made in individual sensory evaluation booths under fluorescent

white light. Panelists were asked to assess the likeness scores (1=dislike extremely, 9=like extremely) of texture, flavor and overall characteristics.

**Statistical Analysis**

Results were reported as mean values  $\pm$  standard deviation. The effects of different SV treatments and storage time were tested with a two-way analysis of variance using the SPSS package version 16.0 (SPSS for window, SPSS Inc., Chicago, Ill., U.S.A.). All mean separations were carried out by Duncan's multiple range test (DMRT) using the significance level of 95% (P<0.05).

**Results and Discussion**

**Microbiological Changes During Storage**

The changes in microbial population of tilapia fillet during chilled storage are shown in Table 1. SV cooking at all conditions reduced the mesophilic bacteria count of raw tilapia fillet from 4.01 to 1.38-2.35 log CFU/g sample. Among all samples, S6-60 and control had the lower mesophilic bacteria than others (P<0.05). Similar with the level of mesophiles, S6-60 and control had the lowest psychrophilic bacteria count, compared with others (P<0.05). The result was clearly observed that the higher cooking temperature or longer treatment time yielded the lower microorganisms. However, there was no significantly difference in both mesophilic and psychrophilic bacteria count between S6-60 and control (P>0.05), suggesting that SV cooking at 60°C for 60 min can kill microorganisms equally with traditional cooking. The gradually increase in both mesophilic and psychrophilic bacteria of all samples as storage time increased were observed (P<0.05). On the 6<sup>th</sup> week of storage, the mesophilic and psychrophilic bacterial counts of S5-30, S5-45, S5-60 and S6-30 were higher than 6.00 log CFU/g sample, which over the limitation regulated by the International Commission on Microbiological Specifications for Foods (ICMSF, 1986),

**Table 1.** Microbiological analysis of Nile Tilapia processed by various SV conditions during chilled storage

Parameters	Week of storage	S5-30	S5-45	S5-60	S6-30	S6-45	S6-60	Control
Mesophilic aerobic bacteria	0	2.35±0.25 <sup>Da</sup>	2.20±0.10 <sup>Da</sup>	2.02±0.11 <sup>Da</sup>	2.05±0.09 <sup>Da</sup>	2.00±0.15 <sup>Da</sup>	1.45±0.21 <sup>Db</sup>	1.38±0.16 <sup>Db</sup>
	2	3.75±0.23 <sup>Ca</sup>	3.63±0.19 <sup>Ca</sup>	3.40±0.23 <sup>Cab</sup>	3.53±0.09 <sup>Ca</sup>	3.13±0.28 <sup>Cb</sup>	3.02±0.13 <sup>Cb</sup>	2.96±0.09 <sup>Cb</sup>
	4	5.02±0.21 <sup>Ba</sup>	5.11±0.20 <sup>Ba</sup>	4.90±0.25 <sup>Ba</sup>	4.88±0.16 <sup>Ba</sup>	4.36±0.21 <sup>Bb</sup>	4.05±0.22 <sup>Bc</sup>	4.03±0.21 <sup>Bc</sup>
	6	6.34±0.20 <sup>Aa</sup>	6.26±0.17 <sup>Aa</sup>	6.09±0.18 <sup>Aa</sup>	6.12±0.16 <sup>Aa</sup>	6.07±0.11 <sup>Aa</sup>	5.22±0.30 <sup>Ab</sup>	5.55±0.20 <sup>Ab</sup>
Psychrophilic aerobic bacteria	0	2.03±0.11 <sup>Da</sup>	2.10±0.25 <sup>Da</sup>	1.98±0.16 <sup>Dab</sup>	2.05±0.19 <sup>Da</sup>	1.78±0.11 <sup>Dbc</sup>	1.58±0.14 <sup>Dc</sup>	1.60±0.12 <sup>Dc</sup>
	2	3.55±0.20 <sup>Ca</sup>	3.59±0.13 <sup>Ca</sup>	3.43±0.17 <sup>Ca</sup>	3.50±0.09 <sup>Ca</sup>	3.02±0.13 <sup>Cb</sup>	3.03±0.20 <sup>Cb</sup>	3.11±0.10 <sup>Cb</sup>
	4	5.40±0.18 <sup>Ba</sup>	5.26±0.31 <sup>Bab</sup>	5.10±0.16 <sup>Bab</sup>	5.10±0.11 <sup>Bab</sup>	4.92±0.15 <sup>Bb</sup>	4.12±0.16 <sup>Bc</sup>	4.10±0.12 <sup>Bc</sup>
	6	6.29±0.17 <sup>Aa</sup>	6.33±0.15 <sup>Aa</sup>	6.00±0.20 <sup>Aab</sup>	6.15±0.13 <sup>Aa</sup>	6.00±0.20 <sup>Aab</sup>	5.19±0.17 <sup>Ab</sup>	5.60±0.26 <sup>Ab</sup>
Anaerobic bacteria	0	1.01±0.12 <sup>C</sup>	1.00±0.20 <sup>C</sup>	1.15±0.23 <sup>C</sup>	1.06±0.21 <sup>C</sup>	1.03±0.09 <sup>C</sup>	0.93±0.19 <sup>C</sup>	1.02±0.21 <sup>C</sup>
	2	1.23±0.20 <sup>C</sup>	1.09±0.22 <sup>C</sup>	1.15±0.13 <sup>C</sup>	1.26±0.11 <sup>C</sup>	1.30±0.20 <sup>C</sup>	1.09±0.09 <sup>C</sup>	1.18±0.17 <sup>C</sup>
	4	2.22±0.15 <sup>B</sup>	2.29±0.16 <sup>B</sup>	2.15±0.17 <sup>B</sup>	2.07±0.16 <sup>B</sup>	2.06±0.11 <sup>B</sup>	2.04±0.13 <sup>B</sup>	1.99±0.19 <sup>B</sup>
	6	3.95±0.09 <sup>Aa</sup>	3.90±0.16 <sup>Aa</sup>	3.96±0.15 <sup>Aa</sup>	3.76±0.26 <sup>Aa</sup>	3.26±0.14 <sup>Ab</sup>	3.21±0.16 <sup>Ab</sup>	3.21±0.33 <sup>Ab</sup>

Results expressed as log CFU/g sample. Mean  $\pm$  SD from triplicate determinations.

Different uppercase letters in the same column indicate significant differences (P<0.05) as affected by different storage time.

Different lowercase letters in the same row indicate significant differences (P<0.05) as affected by different SV treatment at the same storage time.

indicating the spoilage caused by exceed of microorganisms in these products. Our result was similar with other previous studies. Dogruyol and Mol (2015) stated that mesophilic bacteria of mackerel fillets treated with SV condition of 70°C for 10 min was above 6.00 log CFU/g sample on the 7<sup>th</sup> week of storage. The exceed of mesophilic bacteria of rainbow trout fillet (*Oncorhynchus mykiss*) treated with SV condition of 90°C for 3.3 min, were noted after stored for 45 days at 2°C as reported by Gonzalez-Fandos et al. (2004). In contrast, both mesophilic and psychophilic counts of S6-60 and control samples were still not excess the limitation even stored for 6 weeks, indicating the longer shelf-life of them compared with others.

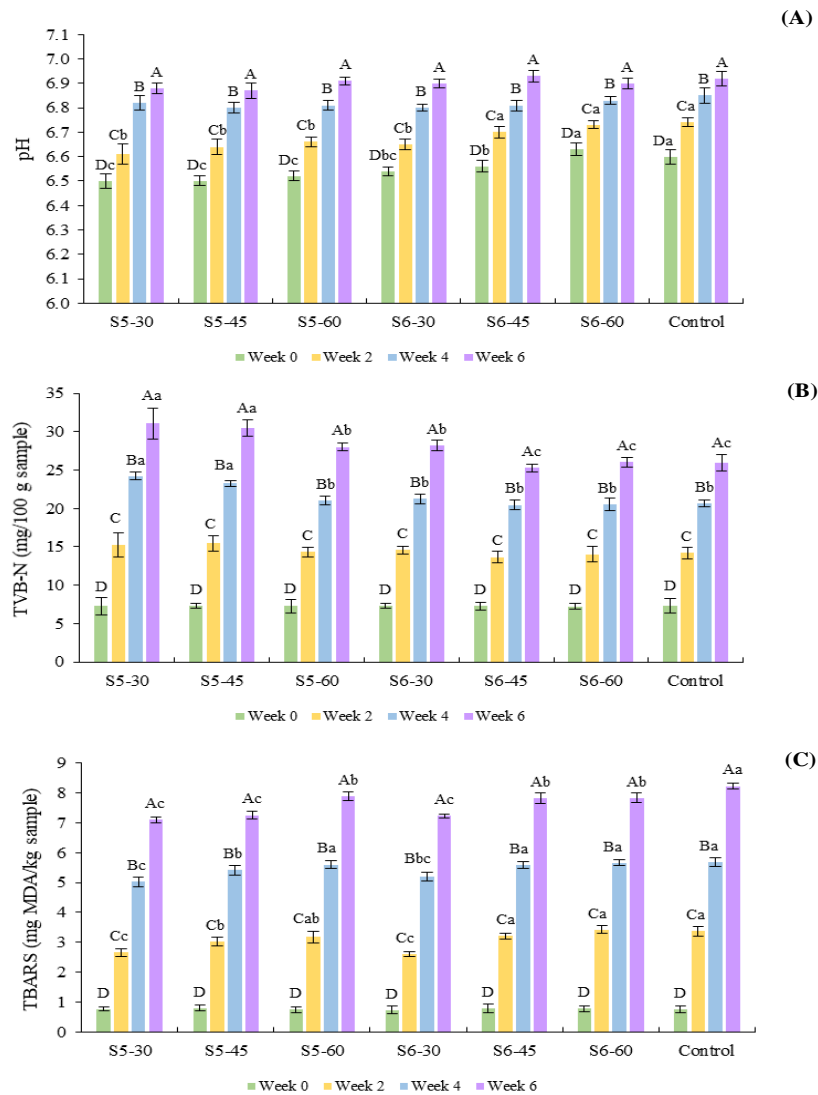
Monitoring the amount of anaerobic bacteria in SV foods is crucial since the foods were packed in the vacuum bag after processing or during storage. At day 0, the total anaerobic bacteria count of all samples was in the range of 0.93-1.15 log CFU/g sample ( $P>0.05$ ). It was also observed that the growth of anaerobic bacteria was retarded for at least 2 weeks during storage at 4°C. Then, the increase in those anaerobes were noticed after storage for 4 weeks. At the 6<sup>th</sup> week of storage, S6-45, S6-60 and control had the lower anaerobic bacteria than others ( $P<0.05$ ). This was correlated well with the changes in mesophilic and psychophilic bacteria, indicating that more severe SV condition (higher temperature or longer time) can eradicate some microorganisms at the beginning and further retard the growth of them during storage. In the aspect of food safety, it was found that the amount of pathogen including *S. aureus*, *B. cereus*, *Cl. perfringens* as well as *Listeria* spp. was not detected in all samples at week 0 (before storage) (data not shown). These pathogens were also not observed in S6-60 and control sample, which were only 2 samples having the microbial population in the limitation of standard, after storage for 6 weeks. This help to indicate the microbial safety of the S6-60 and control product. Our result indicated that having SV condition of 60°C for 60 min can be retard the microbial growth during storage at the same level of traditional cooking (directly heating in boiling water). However, SV cooking at high temperature for long period not only decreased the microbial load, but it can also lead to loss of sensorial acceptability of the product to some extent. Thus, finding the optimal level should be implemented.

#### Quality Characteristic Changes During Storage

The initial pH value of raw tilapia samples was  $6.35\pm 0.03$ . A slight increase in pH after SV cooking was observed, accounting for 6.50-6.63 as shown in Figure 1A. The result was in agreement with other previous studies that the pH value of fish meat increases after heating, particularly with increase in the temperature treated. Crobotova et al. (2019) reported that the pH of raw mackerel increased from 6.31 to 6.43-6.75 after treated with SV cooking in the range of 60-90°C, 10-20

min. Also, the pH of the SV cooked trout increases to 6.63-7.00 as compared to raw trout with pH 6.56 (Ayub & Ahmad, 2019). Among all samples, S6-60 and control had the higher pH, compared with others ( $P<0.05$ ). The higher in pH of these 2 samples may because of the bond cleavage involving different sulfhydryl and hydroxyl group which is more at high-temperature processing (Oz & Seyyar, 2016). The increase in pH as storage time increased was noticed in all samples ( $P<0.05$ ). However, there was no difference in pH value of all samples when stored for 4 and 6 weeks ( $P>0.05$ ). Normally, the acceptable upper limit for pH value of fish is 6.8-7.0 (Ludorff & Meyer, 1973). In our study, the pH values of all samples did not exceed this limit, although continuously increased and almost reached the limit. This indicated that freshness of fillet gradually reduced as storage time increased, which may affect to consumer's acceptability of the product to some extent. The increase in pH values of SV salmon and trout fillets during cold storage was also reported by Garcia-Linares et al. (2004) and Gonzalez-Fandos et al. (2004).

Figure 1B exhibited the TVB-N content of tilapia fillet and its changes during storage. At week 0, samples treated with different level of SV cooking had no difference in TVB-N content, which was in the range of 7.24-7.34 mg N/100 g sample ( $P>0.05$ ). TVB-N represents the quantity of non-protein nitrogen such as nucleotides, sulfur-containing amino acids, and trimethylamine oxide, converted to volatile basic nitrogenous substances such as trimethylamine, methyl-mercaptan, and ammonia. Therefore, the accumulation of TVB-N is usually used as a reliable freshness index for fishery products (Chang & Rx, 2012). TVB-N of all samples increased as storage time was prolonged ( $P<0.05$ ). There was no difference in TVB-N of all samples when stored for 2 weeks ( $P>0.05$ ). However, at the 4<sup>th</sup> and 6<sup>th</sup> week of storage, the higher rate of the increase in TVB-N content were observed in S5-30 and S5-45, compared with others ( $P<0.05$ ). This may relate with the higher microorganisms found in S5-30 and S5-45 samples (Table 1), which revealed that more severe of SV condition, both higher temperature or longer treatment time, can better delay the formation of basic compounds caused by both endogenous and microbial enzymes. The increase in TVB-N content of SV-cooked fish during chilled storage were reported by Mol et al. (2011), which revealed that the TVB-N content of Bonito (*Sarda Sarda*) applied SV cooking at 70°C for 10 min increased from 9.83 to 49.04 mg/100 g sample when stored for 27 days. Normally, the higher amount was directly correlated with the undesirable odor/flavor of fish meat, in which the highest acceptable level of TVB-N content for fish product is 30 mg/100 g as reported by Sikorski et al. (1990). Therefore, S5-30 and S5-45, which contained TVB-N content of 31.04 and 30.45 mg/100 g sample at 6<sup>th</sup> week of storage, can be announced as "not acceptable". This result was related well with the lower flavor-liking scores of samples treated with mild SV



**Figure 1.** Change in pH (A), TVB-N (B), and TBARS value (C) of Nile tilapia processed by various SV conditions during chilled storage. Different uppercase letters indicate significant differences ( $P < 0.05$ ) as affected by different storage time. Different lowercase letters indicate significant differences ( $P < 0.05$ ) as affected by different SV conditions at the same storage time.

condition, especially when prolonged the storage time for more than 4 weeks (Table 2).

There was no significantly difference in TBARS value among all samples at week 0, which was in the range of 0.74-0.80 mg MDA/kg sample ( $P > 0.05$ ) as shown in Figure 1C. This indicated that SV condition of 50-60°C, for up to 60 min were not significantly governed lipid oxidation of the fish meat. Normally, TBARS value indicated the formation of secondary lipid oxidation products, which notably related with the bad odor/flavor of fishery products (Kolakowska, 2002), thus it can be use as index to determine fish freshness. A quiet low of the initial TBARS value of all sample before storage (week 0) can be explained that lipid oxidation product occurred during SV cooking may mostly stuck in the intermediate process, in which intermediate product likes hydroperoxides still not fully changes into aldehyde or ketone, which are the secondary products determined by TBARS value. During storage, TBARS value of all samples sharply increased as storage time increased ( $P < 0.05$ ). This was coincidental well with the

decrease in flavor-liking score of all samples when extended the storage time (Table 2). Among all samples, the highest TBARS value was found in control at all the period test, followed by S6-60 an S6-45, respectively ( $P < 0.05$ ). The increase in TBARS value of SV-cooked fish during storage has been reported in bonito (*Sarda sarda*) (Mol et al., 2011) and Atlantic mackerel (*Scomber scombrus*) (Croptova et al., 2019). Normally, fish meat containing more than 8 mg MDA/kg sample are considered as unacceptable (Kolakowska, 2002). It was noted that control sample contained TBARS value of 8.22 mg MDA/kg sample, which over the limitation, after storage for 6 weeks. Whilst, other samples were not exceeded. This may suggest that hard condition of cooking can accelerate lipid oxidation throughout the storage. However, the results were also noticed that the level of SV cooking did not affect or governed lipid oxidation as much as the prolonged storage time since all samples exhibited the sharply increase in TBARS value as storage time increased.

The initial TCA-soluble peptides after SV cooking (before storage) were 25.22-110.13  $\mu\text{mol}$  tyrosine/ g sample (Figure 2A). Among all samples, control sample had the highest TCA-soluble peptides, followed by S6-60 and S6-45, respectively ( $P < 0.05$ ). Normally, TCA-soluble peptide has been used as the index for the protein degradation of fish muscle, particularly myofibrillar and sarcoplasmic proteins (Benjakul et al., 1997). It was clearly observed that the level of SV cooking condition directly affected to the formation of these oligopeptides. The higher temperature can accelerate the degradation of myofibrillar protein since the TCA-soluble peptides of samples using SV at 60°C (S6-30, S6-45 and S6-60) was significantly higher than samples treated at 50°C (S5-30, S5-45 and S5-60) ( $P < 0.05$ ). Higher TCA-soluble peptides indicate higher hydrolysis rates of muscle proteins during SV cooking, which possibly affect to the meat characteristics, particularly texture. During storage, the continuously increase in TCA-soluble peptides were observed in all samples ( $P < 0.05$ ). Also, control had the highest TCA-soluble peptides at all period test, followed by the samples treated with severe SV condition (S6-60, S6-45, respectively) ( $P < 0.05$ ). During chilled storage, endogenous or microbial proteases represent a potential source of proteolytic muscle degradation, in which resulting in protein breakdown and the formation of peptides, amino acids, and their metabolites causing the deterioration of fish quality (Rodrigues et al., 2016). The increase in TCA-soluble peptides of fish meat during storage has been noted for rainbow trout (*Oncorhynchus mykiss*) (Rodrigues et al., 2016) and grass carp (*Ctenopharyngodon idellus*) (Yu et al., 2016) as well. In this study, the resulted revealed that different SV conditions displayed different muscle protein degradation occurring in sample. Higher temperature or longer treatment time resulted in more protein degradation of the product. Moreover, this protein degradation continuously occurred during chilled storage, which affected to meat quality. The greater muscle protein degradation may associate with the lower water-holding capacity (WHC) of the samples treated with severe SV cooking conditions (Table 2). Moreover, Roseiro et al. (2008) stated that the taste of fish and fish products can be developed through the formation of several low-molecular weight compounds, such as peptides, amino acids, aldehydes, organic acids and amines, thus TCA-soluble peptides can be use as taste indicator of fish meat. Higher degree of muscle proteins degradation could contribute to the development of taste. This was corresponding well with the higher flavor-liking scores observed in S6-45, S6-60 or control samples (Table 2).

Total collagen content of tilapia fillet after SV cooking at various conditions varied from 2.01-6.12 mg/g sample (Figure 2A). During SV cooking, collagen content was markedly reduced by 49.79-83.51% in comparison to initial collagen content in raw tilapia (12.19 $\pm$ 0.24 mg/g sample). Among all samples, control

had the lowest total collagen content, followed by S6-60 and S6-45, respectively, while the highest collagen content was found in S5-30 ( $P < 0.05$ ). The result clearly indicated that increasing temperature and time for SV treatment can lowering total collagen content, suggesting more intensive collagen degradation of them. Total collagen of all samples decreased as storage time increased ( $P < 0.05$ ). The intensive collagen content reduction was observed at the first 2 weeks of chilled storage, accounting for more than 50%, compared with week 0 ( $P < 0.05$ ). Then, the rate of reduction was slower when prolonged the storage time for 4 and 6 weeks. However, control and S5-30 had the highest and lowest total collagen content, respectively, at all period tests ( $P < 0.05$ ). This result revealed the impact of cooking condition on protein degradation, which reflected to the meat quality. In this study, control, S6-60 and S6-45 had the greater protein degradation, both myofibrillar protein and connective tissue. The result was corresponding well with the changes in WHC (Table 2). The more denaturation of myofibril and collagen proteins with the increasing temperature and time of SV cooking may let the increase in aggregation, then resulting in the decrease in water holding capacity of the fish muscles. This phenomenon caused changes in texture to some extent. Hatae et al. (1996) stated that the decrease in total collagen content, which can be explained by the partial solubilization of collagen and the shrinkage of muscle fiber, allowing the juice dripping out from the fish flesh.

WHC of tilapia fillet during chilled storage are shown in Table 2. After applied with various SV cooking conditions, the initial WHC of raw tilapia fillet, accounting for 93.05% (data not shown), decreased into 70.05-82.26%. Among all samples, S5-30 had the highest WHC (82.26%), while S6-60 and control had the lowest WHC (70.05 and 70.16%, respectively) ( $P < 0.05$ ). The result revealed that more severe SV conditions, both temperature and time, resulted in lower WHC of sample. This was in agreement with the higher TCA-soluble peptides and lower total collagen content when applied more severe SV cooking condition (Figure 2). Actin and myosin hold 80% of the total water in muscles so with denaturation of these filaments the water holding capacity is reduced. The decrease in WHC of the fish flesh may lead to increased cooking loss and detrimental changes in texture. During cooking, particularly when increasing the temperature or time of cooking, extracellular spaces of the fish flesh can expand, and the breakage of pericellular layers along with the shrinkage of myofibrils and collagen can induce. This phenomenon results in the emergence of intracellular gaps in the flesh, leading to impaired muscle integrity and reduction in texture parameters of the fish (Cropotova et al., 2018). WHC of all samples tended to decrease as storage time increase, but with the slow rate. After storage for 6 weeks, WHC of all samples was still higher than 70%. However, the decrease in WHC may directly associated with the

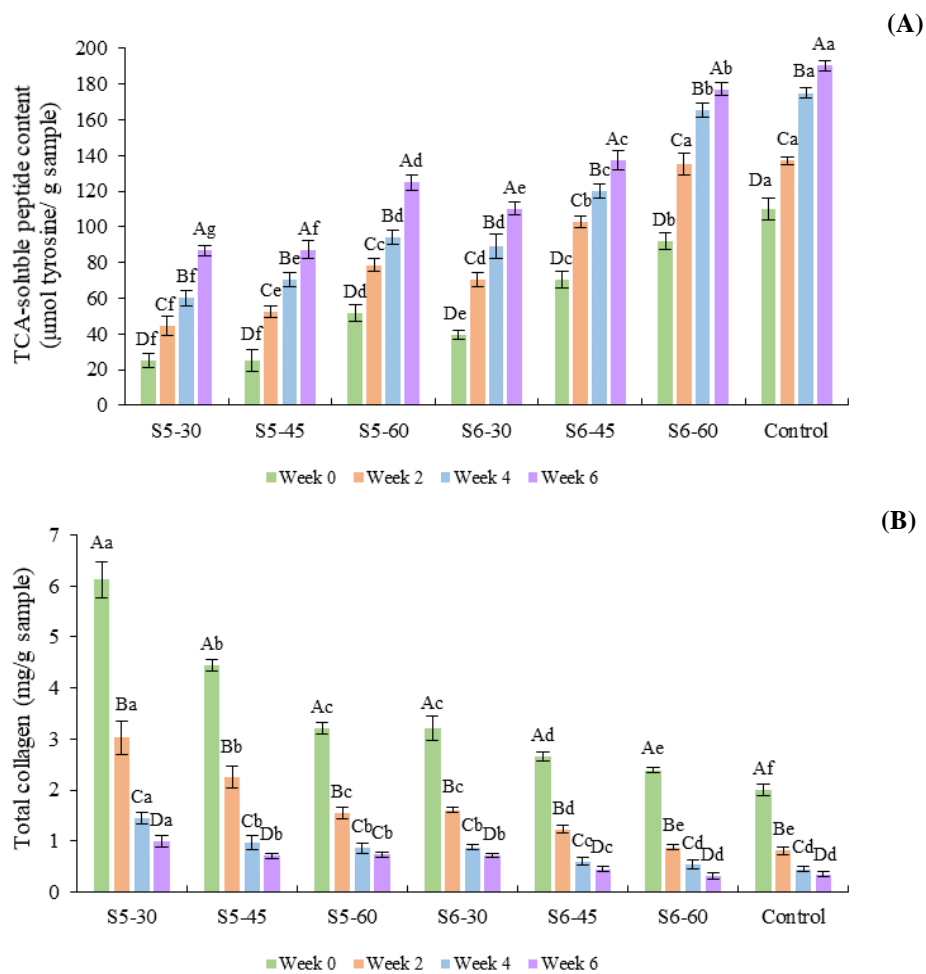
**Table 2.** Changes in WHC, shear force and sensory scores of Nile Tilapia processed by various SV conditions during chilled storage

Parameters	Week of storage	S5-30	S5-45	S5-60	S6-30	S6-45	S6-60	Control
WHC (%)	0	82.26±1.11 <sup>Aa</sup>	79.23±1.44 <sup>Ab</sup>	79.02±0.78 <sup>Ab</sup>	78.02±2.01 <sup>b</sup>	75.23±2.05 <sup>Ac</sup>	70.05±2.04 <sup>d</sup>	70.16±2.02 <sup>Ad</sup>
	2	82.05±0.96 <sup>Aa</sup>	80.01±2.02 <sup>Ab</sup>	77.88±1.44 <sup>Bc</sup>	78.16±1.66 <sup>c</sup>	72.12±1.99 <sup>Bd</sup>	71.63±2.61 <sup>d</sup>	69.13±2.60 <sup>Ad</sup>
	4	80.43±1.32 <sup>Ba</sup>	76.98±2.14 <sup>Bb</sup>	79.15±1.23 <sup>Aa</sup>	77.24±1.98 <sup>b</sup>	74.44±2.41 <sup>Ac</sup>	70.15±1.33 <sup>d</sup>	67.78±1.05 <sup>Be</sup>
	6	80.99±1.05 <sup>Ba</sup>	78.24±1.97 <sup>ABb</sup>	77.26±1.09 <sup>Bb</sup>	77.02±1.53 <sup>b</sup>	72.09±2.08 <sup>Bc</sup>	70.28±1.29 <sup>d</sup>	70.12±1.37 <sup>Ac</sup>
Shear force (raw) (N)	0	9.23±0.08 <sup>Aa</sup>	9.01±0.06 <sup>Ab</sup>	8.44±0.07 <sup>Ac</sup>	8.50±0.07 <sup>Ac</sup>	8.02±0.12 <sup>Ad</sup>	7.83±0.07 <sup>Ae</sup>	7.99±0.05 <sup>Ad</sup>
	2	8.88±0.09 <sup>Ba</sup>	8.43±0.04 <sup>Bb</sup>	8.20±0.10 <sup>Bc</sup>	8.13±0.05 <sup>Bc</sup>	7.76±0.06 <sup>Bd</sup>	7.55±0.05 <sup>Be</sup>	7.59±0.05 <sup>Be</sup>
	4	8.75±0.15 <sup>Ba</sup>	8.26±0.08 <sup>Cb</sup>	8.04±0.05 <sup>Cc</sup>	7.88±0.06 <sup>Cd</sup>	7.50±0.08 <sup>Ce</sup>	7.12±0.13 <sup>Cf</sup>	7.22±0.09 <sup>Cf</sup>
	6	7.92±0.08 <sup>Ca</sup>	8.05±0.07 <sup>Da</sup>	7.81±0.05 <sup>Da</sup>	7.38±0.06 <sup>Db</sup>	7.21±0.03 <sup>Dc</sup>	6.93±0.08 <sup>Dd</sup>	6.98±0.06 <sup>Dd</sup>
Sensory score (Flavor likeness)	0	6.25±0.15 <sup>Ab</sup>	6.28±0.20 <sup>Ab</sup>	6.20±0.19 <sup>Ab</sup>	6.44±0.22 <sup>Aa</sup>	6.46±0.28 <sup>Aa</sup>	6.45±0.11 <sup>Aa</sup>	6.50±0.20 <sup>Aa</sup>
	2	6.02±0.12 <sup>Bb</sup>	6.05±0.22 <sup>Bb</sup>	6.01±0.11 <sup>Bb</sup>	6.08±0.20 <sup>Bb</sup>	6.20±0.28 <sup>Aa</sup>	6.26±0.24 <sup>Aa</sup>	6.21±0.12 <sup>Ba</sup>
	4	4.99±0.26 <sup>Cc</sup>	5.30±0.21 <sup>Cb</sup>	5.49±0.24 <sup>Cb</sup>	5.78±0.19 <sup>Ca</sup>	5.60±0.30 <sup>Bab</sup>	5.80±0.19 <sup>Ba</sup>	5.80±0.26 <sup>Ca</sup>
	6	-	-	-	-	-	5.62±0.25 <sup>C</sup>	5.33±0.31 <sup>D</sup>
Sensory score (Texture likeness)	0	6.88±0.24 <sup>Ab</sup>	7.02±0.30 <sup>Ab</sup>	6.96±0.24 <sup>Ab</sup>	6.89±0.20 <sup>Ab</sup>	7.26±0.17 <sup>Aa</sup>	7.32±0.20 <sup>Aa</sup>	6.06±0.19 <sup>Ac</sup>
	2	6.70±0.22 <sup>Aab</sup>	6.65±0.25 <sup>Bb</sup>	6.50±0.26 <sup>Bb</sup>	6.63±0.21 <sup>Bb</sup>	6.95±0.20 <sup>Ba</sup>	6.88±0.19 <sup>Ba</sup>	6.10±0.22 <sup>Ac</sup>
	4	6.13±0.19 <sup>Bab</sup>	6.09±0.22 <sup>Cb</sup>	5.91±0.18 <sup>Cb</sup>	6.03±0.20 <sup>Cb</sup>	6.26±0.25 <sup>Ca</sup>	6.27±0.21 <sup>Ca</sup>	6.01±0.25 <sup>Ab</sup>
	6	-	-	-	-	-	6.33±0.22 <sup>Ca</sup>	5.78±0.29 <sup>Bb</sup>
Sensory score (Overall likeness)	0	7.02±0.20 <sup>Aa</sup>	7.06±0.25 <sup>Aa</sup>	7.11±0.22 <sup>Aa</sup>	7.08±0.30 <sup>Aa</sup>	7.26±0.29 <sup>Aa</sup>	7.09±0.22 <sup>Aa</sup>	6.78±0.23 <sup>Ab</sup>
	2	6.66±0.22 <sup>Bb</sup>	6.75±0.20 <sup>Bab</sup>	6.59±0.26 <sup>Bb</sup>	6.70±0.21 <sup>Bb</sup>	6.93±0.24 <sup>Ba</sup>	6.97±0.23 <sup>Aa</sup>	6.66±0.23 <sup>Ab</sup>
	4	5.98±0.19 <sup>Cb</sup>	6.15±0.25 <sup>Cb</sup>	6.03±0.19 <sup>Cb</sup>	6.32±0.27 <sup>Cab</sup>	6.56±0.17 <sup>Ca</sup>	6.67±0.22 <sup>Ba</sup>	6.14±0.26 <sup>Bb</sup>
	6	-	-	-	-	-	6.02±0.31 <sup>Ca</sup>	5.10±0.29 <sup>Cb</sup>

Mean ± SD from triplicate determinations.

Different uppercase letters in the same column indicate significant differences (P<0.05) as affected by different storage time.

Different lowercase letters in the same row indicate significant differences (P<0.05) as affected by different SV treatment at the same storage time.



**Figure 2.** Change in TCA-soluble peptides (A) and total collagen (B) of Nile tilapia processed by various SV conditions during chilled storage.

Different uppercase letters indicate significant differences (P<0.05) as affected by different storage time. Different lowercase letters indicate significant differences (P<0.05) as affected by different SV conditions at the same storage time.

slightly decrease in texture-liking score when extended the storage time (Table 2).

Shear force of raw tilapia fillet was  $14.17 \pm 0.35$  N (data not shown), while shear force of SV-cooked fish was in the ranges of 7.83-9.23 N (Table 2). Similar with the amount of WHC, the highest shear force was observed in S5-30, which is the mildest SV condition of this experiment, while the lowest shear force was found in control, followed by S6-60 and S6-45, respectively ( $P < 0.05$ ). The decrease in shear force after SV-cooking may associate with the degradation of connective tissue surrounding the fish muscle fiber. Therefore, the result was in accordance well with the amount of total collagen (Figure 2B). It revealed that more severe SV cooking condition, more protein degradation, which further directly affected to the meat characteristics, particularly texture. Shear force of all samples decreased when prolonged the storage time ( $P < 0.05$ ). However, this value did not change much, compared with the impact of cooking condition at the beginning of storage. Overall, the results clearly exhibited that temperature used, and the duration of both SV cooking and subsequent chilled storage are the main parameters influencing tenderization of the fish flesh. However, the optimal SV cooking condition for this fish species (the appropriate level to improve sensorial characteristics, particularly texture or flavor) need to be interpreted with the sensory evaluation by directly testing with consumer.

### Sensory Acceptability During Storage

Table 2 showed the flavor-liking, texture-liking, and overall-liking scores of samples treated by various SV conditions during storage every 2 weeks (samples having the compositions or microorganism population out of standard were except). Overall, scores of all characteristics decreased as storage time increased ( $P < 0.05$ ). For flavor characteristic, samples treated with SV cooking of  $60^\circ\text{C}$  had the higher flavor-liking score than samples having SV cooking at  $50^\circ\text{C}$  ( $P < 0.05$ ), in which the scores were comparable with the control. The results indicated that the level of temperature used for SV cooking had more impact on flavor development than duration time. The greater protein or lipid degradation when applied higher temperature of SV may influence taste development through the formation of several low-molecular weight compounds, i.e., peptides, amino acids, aldehydes, organic acids, amines, etc. (Roseiro et al., 2008). During storage, flavor-liking score of all samples markedly decreased when prolonged the storage time ( $P < 0.05$ ). S5-30 had the flavor-liking score lower than 5.00 when stored for 4 weeks, indicating unacceptable quality in the aspect of undesirable flavor occurred as evaluated by consumer. While, S6-60 and control, which still having microbial standard in the range regulated by ICMSF (1978), still had the flavor-liking score more than 5.00 when stored for 6 weeks. As for the texture characteristic, texture

liking scores of S6-30, S6-45 and S6-60 were higher than other 3 samples treated at  $50^\circ\text{C}$  ( $P < 0.05$ ). While, control sample had the lowest texture-liking scores among all samples ( $P < 0.05$ ). This result indicated that SV cooking had more impact on texture improvement of cooked fish meat, when compared with traditional/general cooking (directly cooking with boiling water). Texture-liking scores of all samples decreased when extended the storage time ( $P < 0.05$ ). It was observed that S6-60 and S6-45 had the highest texture-liking scores, while the lowest was still found in control sample, at all period tests ( $P < 0.05$ ). This help to confirm that the SV cooking which can improve better texture of cooked fish can further keep the good texture throughout the storage, compared with control. This may be explained by the fact that the temperature of  $70\text{--}80^\circ\text{C}$  allows these textural changes to reach their maximum (Llave et al., 2018). In this experiment, the temperature using for SV cooking was  $50\text{--}60^\circ\text{C}$ , which lower than  $70\text{--}80^\circ\text{C}$ , particularly lower than control sample (boiling water,  $100^\circ\text{C}$ ), the textural changes during cooking, therefore, were difference. Control sample confronted with the intensive protein degradation, resulting in more aggregation and coarse in texture characteristic. Crobotova et al. (2018) reported that fish meat softens with cooking and the hardness of mackerel meat is reduced with 14-37% on SV cooking, compared with cooking with boiling water. Llave et al. (2018) also stated that shrinkage during SV cooking is smaller as compared to the conventional techniques like boiling, frying and barbecuing.

Overall-liking score can be used for the index to identify the optimal condition for SV cooking used in this experiment. At week 0 (before storage), overall-liking score of SV-cooked fish was in the range of 7.02-7.26, which higher than 7.00 ( $P < 0.05$ ). While, the overall-liking score lower than 7.00 was only found in control sample, accounting for 6.78. The lowest score of control sample was associated with the lower in texture-liking score of this sample. The result can prove that texture is a crucial characteristic, which highly impacted to the consumer's acceptability of cooked fish fillet. The result suggested the potential of using SV technique to improve the sensory acceptability of this product. Gluchowski et al. (2019) revealed that cooked Atlantic salmon (*Salmo salar*) processed by SV technique at  $57^\circ\text{C}$  for 20 min and  $63^\circ\text{C}$  for 80 min had higher sensory scores, particularly in the aspects of texture-liking and overall-liking, compared with the fish processed by roasting and steaming. Due to the quality loss during storage as indicated by biochemical and microbiological changes, overall-liking score of all samples also decreased gradually as storage time increased ( $P < 0.05$ ). Moreover, among all samples, S6-60 contained better acceptability of all aspects throughout the storage and still got acceptability at high level even stored for 6 weeks. Thus, this SV condition ( $60^\circ\text{C}$ , 60 min) seems to be the greatest condition since it can provide the product shelf-life for at least 6 weeks at chilled



temperature, in which the quality can be improved and further preserved thorough the storage, compared with traditional cooked.

## Conclusion

SV cooking has an impact to improve sensorial characteristics of tilapia fillet, compared with traditional boiling. In this experiment, SV cooked tilapia exhibited the higher sensory scores, particularly texture-liking score, than control since the beginning of storage and during stored at 4°C for 6 weeks. Thus, the quality of the product before storage, governed by cooking method plays the most crucial role to produce the product with prime quality. Among all SV conditions, the SV-cooked tilapia fillet prepared at 60°C for 60 min exhibited the best results for consumer's acceptability. This condition can preserve the shelf-life for at least 6 weeks, in which chemical parameters as well as microbial population were still in the standard regulation. Further studies focused on the insight composition changes related with meat characteristics and consumer's acceptability should be evaluated to ensure this optimal SV conditions for cooked tilapia products. Further suggestion, this experiment used a quiet low temperature for SV cooking (40-60°C), which have a positive effect on flavor, increased juiciness, reduced thermal shrinkage of the product, and it has its enthusiasts. However, it may pose a microbiological hazard, thus, it was suggested to consume freshly after cooking. In our aspect, therefore, the further study focused on the growth/survival of pathogens during storage, should be evaluated to ensure that the optimal SV condition can eradicate those pathogens since those pathogenic bacteria may contaminate in the raw fish or there was a subsequent contamination in the final product. This can ensure the microbial safety of the product throughout the storage

## Ethical Statement

The experiment was reviewed and approved by the ethical committee (ethical number U1-02631-2559) of the Suranaree University of Technology, Nakhon Ratchasima, Thailand.

## Funding Information

This research was supported by Suranaree University of Technology (Research Brotherhood Scholarship, Grant No. BRO3-303-64-12-07).

## Author Contribution

**J.P.:** Responsible for the entire process from idea, design, experiment to final draft, review and modification, etc. **S.B.:** Methodology, Supervision, Visualization, Resources. **S.B.:** Supervision, Resources. **J.Y.:** Methodology, Supervision, Resources.

## Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

## References

- Aguilera, J. M. (2018). Relating food engineering to cooking and gastronomy. *Comprehensive Reviews in Food Science and Food Safety*, 17, 1021-1039. <https://doi.org/10.1111/1541-4337.12361>
- Ayub, H., & Ahmad, A. (2019). Physiochemical changes in sous-vide and conventionally cooked meat. *International Journal of Gastronomy and Food Science*, 17, 100145. <https://doi.org/10.1016/j.ijgfs.2019.100145>
- Baldwin, D. E. (2012). Sous vide cooking: a review. *International Journal of Gastronomy and Food Science*, 1, 15-30. <https://doi.org/10.1016/j.ijgfs.2011.11.002>
- Chang, H., & Rx, W. (2012). Textural and biochemical properties of Cobia (*Rachycentron canadum*) sashimi tenderised with the ultrasonic water bath. *Food Chemistry*, 132, 1340-1345. <https://doi.org/10.1016/j.foodchem.2011.11.116>
- Conway, E. J. & Nyrne, A. (1936). An absorption apparatus for the micro-determination of certain volatile substances I: The micro-determination of ammonia. *Biochemical Journal*, 27, 419-429. <https://doi.org/10.1042/bj0292221>
- Cropotova, J., Mozuraityte, R., Stadal, I. B., & Rustad, T. (2018). A non-invasive approach to assess texture changes in sous-vide cooked Atlantic mackerel during chilled storage by fluorescence imaging. *Food Control*, 92, 216-224. <https://doi.org/10.1016/j.foodcont.2018.04.060>
- Cropotova, J., Mozuraityte, R., Standal, I. B., & Rustad, T. (2019). The influence of cooking parameters and Cilled storage time on quality of sous-vide atlantic mackerel (*Scomber scombrus*). *Journal of Aquatic Food Product and Technology*, 28, 505-518. <https://doi.org/10.1080/10498850.2019.1604595>
- Digre, H., Erikson, U., Skaret, J., Lea, P., Gallart-Jornet, L., Misimi, E. (2011). Biochemical, physical and sensory quality of ice-stored Atlantic cod (*Gadus morhua*) as affected by pre-slaughter stress, percussive stunning and AQUI-S™ anaesthesia. *European Food Research and Technology*, 233, 447-456. <https://doi.org/10.1007/s00217-011-1531-8>
- Dogruyol, H., & Mol, S. (2015). Effect of irradiation on shelf life and microbial quality of cold-stored sous-vide mackerel fillets. *Journal of Food Processing and Preservation*, 41, e12804. <https://doi.org/10.1111/jfpp.12804>
- Garcia-Linares, M. C., Gonzalez-Fandos, E., Garcia-Arias, M. T., & Garcia-Fernandez, M. C. (2004). Microbiological and nutritional quality of sous vide or traditionally processed fish: Influence of fat content. *Journal of Food Quality*, 27, 371-387. <https://doi.org/10.1111/j.1745-4557.2004.00676.x>
- Gittleson B., Saltmarch M., Cocotas P., & Proud L. (1992). Quantification of the physical, chemical and sensory

- modes of deterioration in sous-vide processed salmon. *Journal of Food Service System*, 6, 209-232. <https://doi.org/10.1111/j.1745-4506.1992.tb00295.x>
- Gonzalez-Fandos, E., Garcia-Linares, M. C., Rodriguez, A. V., Garcia-Arias, M. T., & Garcia-Fernandes, M. C. (2004). Evaluation of the microbiological safety and sensory quality of rainbow trout (*Oncorhynchus mykiss*) processed by the sous-vide method. *Food Microbiology*, 21, 193-201. [https://doi.org/10.1016/S0740-0020\(03\)00053-4](https://doi.org/10.1016/S0740-0020(03)00053-4)
- Gonzalez-Fandos, E., Villarino-Rodríguez A., & García-Linares M.C. (2005). Microbiological safety and sensory characteristics of salmon slices processed by sous vide method. *Food Control*, 16, 75-85. <https://doi.org/10.1016/j.foodcont.2003.11.011>
- Głuchowski, A., Czarniecka-Skubina, E., Wasiak-Zys, G., & Nowak, D. (2019). Effect of various cooking methods on technological and sensory quality of Atlantic salmon (*Salmo salar*). *Foods*, 8, 1-15. <https://doi.org/10.3390/foods8080323>
- Hatae, K., Nakai, H., Tanaka, C., Shimada, A., & Watabe, S. (1996). Taste and texture of abalone meat after extended cooking. *Fisheries Science*, 62, 643-647. <https://doi.org/10.2331/fishsci.62.643>
- ICMSF. (1978). Microorganisms in Foods. 1. Their significance and methods of enumeration. International Commission on Microbiological Specifications for Foods (ICMSF), 2nd Edition. University of Toronto Press, Toronto.
- Kolakowska, A., Olley, J., & Dunstan, G. A. (2002). Fish lipids. In Z. E. Sikorski & A. Kolakowska (Eds.), Chemical and functional properties of food lipids (pp. 221-264). FL, USA: CRC Press. <https://doi.org/10.1201/9781420031997>
- Llave, Y., Shibata-Ishiwatari, N., Watanabe, M., Fukuoka, M., Hamada-Sato, N., & Sakai, N. (2018). Analysis of the effects of thermal protein denaturation on the quality attributes of sous-vide cooked tuna. *Journal of Food Processing and Preservation*, 42, e13347. <https://doi.org/10.1111/jfpp.13347>
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R.J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, 193, 265-275. [https://doi.org/10.1016/S0021-9258\(19\)52451-6](https://doi.org/10.1016/S0021-9258(19)52451-6)
- Meilgaard, M., Civille, G. V., & Carr, B.T. (1999). Sensory Evaluation Techniques. 3<sup>rd</sup> ed. Boca Raton, Fla: CRC Press. pp. 387. <https://doi.org/10.1201/9781439832271>
- Mol, S., Ozturan, S., & Cosansu, S. (2011). Determination of the quality and shelf life of sous vide packaged bonito (*Sarda sarda*, Bloch, 1793) stored at 4 and 12°C. *Journal of Food Quality*, 36, 137-143. <https://doi.org/10.1111/j.1745-4557.2011.00430.x>
- NACMCF. (1990). Recommendations for refrigerated foods containing cooked, uncured meat or poultry products that are packaged for extended, refrigerated shelf-life and that are ready-to-eat, or prepared with little or no additional heat treatment. National Advisory Committee on Microbiological Criteria for Foods (NACMCF). Washington, DC.
- Nirmal, N. P., & Benjakul, S. (2009). Effect of ferulic acid on inhibition of polyphenoloxidase and quality changes of Pacific white shrimp (*Litopenaeus vannamei*) during iced storage. *Food Chemistry*, 116, 323-331. <https://doi.org/10.1016/j.foodchem.2009.02.054>
- Oz, F., & Seyyar, E. (2016). Formation of heterocyclic aromatic amines and migration level of bisphenol-A in sous-vide cooked trout fillets at different cooking temperatures and cooking levels. *Journal of Agricultural and Food Chemistry*, 64, 3070-3082. <https://doi.org/10.1021/acs.jafc.5b05716>
- Reddy, G. K. & Enwemeka, C. S. A. (1996). Simplified method for the analysis of in biological tissues hydroxyproline. *Clinical Biochemistry*, 29, 225-229. [https://doi.org/10.1016/0009-9120\(96\)00003-6](https://doi.org/10.1016/0009-9120(96)00003-6)
- Rodrigues, B. L., Costa, M. P., Frasso, B. S., Silva, F. A., Mársico, E. T., Alvares, T. S., & Conte-Junior, C. A. (2017). Instrumental texture parameters as freshness indicators in five farmed Brazilian freshwater fish species. *Food Analysis Methods*, 10, 3589-3599. <https://doi.org/10.1007/s12161-017-0926-y>
- Roseiro, L. C., Santos, C., Sol, M., Borges, M. J., Anjos, M., Goncalves, H., & Carvalho, A.S. (2008). Proteolysis in *Painho de Portalegre* dry fermented sausage in relation to ripening time and salt content. *Meat Science*, 79, 784-794. <https://doi.org/10.1016/j.meatsci.2007.11.012>
- Shakila, R. J., Jeyasekaran, G., Vijayakumar, A., & Sukumar, D. (2009). Microbiological quality of sous-vide cook chill fish cakes during chilled storage (3°C). *International Journal of Food Science and Technology*, 44, 2021-2026. <https://doi.org/10.1111/j.1365-2621.2009.02047.x>
- Sriket, C., Benjakul, S., Visessanguan, W., Hara, K., Yoshida, A., & Liang, X. (2012). Low molecular weight trypsin from hepatopancreas of freshwater prawn (*Macrobrachium rosenbergii*): Characteristics and biochemical properties. *Food Chemistry*, 134, 351-358. <https://doi.org/10.1016/j.foodchem.2012.02.173>
- Velioglu, H. M., Temiz, H. T., & Boyaci, I. H. (2015). Differentiation of fresh and frozen-thawed fish samples using Raman spectroscopy coupled with chemometric analysis. *Food Chemistry*, 172, 283-290. <https://doi.org/10.1016/j.foodchem.2014.09.073>
- Wattanachant, S., Benjakul, S., & Ledward, D. A. (2004). Composition, color, and texture of Thai indigenous and broiler chicken muscles. *Poultry Science*, 83, 123-128. <https://doi.org/10.1093/ps/83.1.123>
- Yu, D., Xu, Y., Jiang, Q., Yang, F., & Xia, W. (2016). Freshness assessment of grass carp (*Ctenopharyngodon idellus*) fillets during storage at 4°C by physicochemical, microbiological and sensorial evaluations. *Journal of Food Safety*, 37, e12305. <https://doi.org/10.1111/jfs.12305>