Production of Protein Hydrolysate from Yellowfin (*Thunnus albacares*) and Skipjack Tuna (*Katsuwonous pelamis*) Viscera

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ABSTRACT

Tuna viscera consist of high levels of protein and enzymes which have the potential to be used for protein hydrolysate production. The objective of this study was to produce protein hydrolysates from yellowfin and skipjack tuna viscera. Tuna viscera were autolyzed at various temperatures (33, 35, 55°C) for 10 days. Protein hydrolysate produced from tuna viscera contained 15.09-22.65% protein, 0.48-0.59% fat, and 1.88-1.95% salt. The levels of TVB-N (9.52-45.39 mg·100 g^{-1}) and histamine (276.87-289.95 mg·kg^{-1}) were below the maximum levels for human consumption. Protein hydrolysate from skipjack tuna viscera contained a greater amount of protein and a lower level of fat than yellowfin tuna viscera. Protein hydrolysate from yellowfin and skipjack tuna viscera showed antioxidant activity as determined by DPPH (2,2-diphenyl-l-picrylhydrazyl) and ABTS (2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)) radical scavenging activities with acceptable qualities. The protein hydrolysate from skipjack tuna viscera could be a new source of protein for humans.

Keywords: protein hydrolysate, yellowfin, skipjack, tuna, viscera

INTRODUCTION

Thailand is the world's largest producer and exporter of canned tuna. Large amounts of wastes from tuna processing are generated each year, causing environmental, health and economic problems that affect many communities in Thailand. The companies involved in the industry have to pay a lot of money for the treatment and management of discarded items such as heads, bones and viscera. However, these wastes still contain useful compounds such as protein and amino acids. Therefore, these discarded materials can be used as protein sources in other forms such as fish meal and protein concentrate (Klomklao *et al.*, 2004). Unfortunately, they are usually transformed into low value products, for instance, animal feed, fish meal and fertilizer. Many researchers have tried to increase their market value (Herpandi *et al.*, 2011; Chalamaiah *et al.*, 2012).

Enzymatic hydrolysis is one of the approaches to recover protein effectively from wastes obtained from tuna industry and can be used to improve the nutritional properties of proteins. The autolysis or

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self-digestion is a part of the process of enzymatic hydrolysis. The end product of autolysis process is called hydrolysates, and it is generally a fairly viscous liquid rich in free amino acids and small peptides (Hsu, 2010; Kim, 2014). Endogenous enzymes are commonly used in the autolysis for production of fish sauce and silage (Kristinsson and Resco, 2000). However, the autolysis processes depend on time for the completion of the processes, pH, salt content, temperature, fish species, seasonality, the type and amount of enzymes therefore the control of autolysis by the endogenous enzymes (Hsu, 2010; Kim, 2014). Tuna viscera contain important proteolytic enzymes, such as pepsin, trypsin and chymotrypsin (Simpson, 2000). These proteinases can be used for protein hydrolysate production in the fishery industries (Herpandi *et al*., 2011). The optimal temperature of these proteolytic enzymes was found in a range of 40°C - 55°C (Prasertsan and Prachumratana, 2008). The objective of this study was to produce protein hydrolysate using the autolysis process from yellowfin and skipjack tuna viscera.

MATERIALS AND METHODS

Raw materials

Viscera of yellowfin (YF, *Thunnus albacares*) and skipjack (SJ, *Katsuwonous pelamis*) tuna were obtained from Thai Unicord Public Co., Samutsakorn, Thailand. The viscera were packed in polyethylene bags, kept on ice and transported to the Department of Fishery Products, Kasetsart University, Bangkok within 2 hours. The samples were stored at -20°C until used.

Sample preparation

Tuna viscera of each species were thawed at 4°C and then blended thoroughly, using a MK-5080M Model food processor (Panasonic, Malaysia).

Determination of raw material composition

The moisture, fat and ash contents of raw materials were determined according to the AOAC method (AOAC, 2006). The crude protein and salt contents were determined according to the Nielsen method (Nielsen, 2010). Crude protein was estimated from the total amount of nitrogen using the conversion factor of 6.25.

The histamine content was determined according to the AOAC method (AOAC, 2012). The pH was measured as described by Miwa and Ji (1992) using a Metrohm 744 pH meter (Metrohm, Switzerland).

Production of protein hydrolysate

Fifty-five grams of blended tuna viscera were placed in separate glass bottles. The samples were subjected to autolysis at 33°C (room temperature), 35°C and 55°C for 10 days. Liquid samples were collected every day and used in analysis. At the designated time, the samples were centrifuged at 20,000 ×g (4°C) for 30 min, using a Suprema 21 Model centrifuge (Tomy, Japan). The supernatant was filtered with Whatman filter paper No.1 and collected for further analysis.

Determination of optimal production

Protein hydrolysates obtained from each treatment were analyzed for yield, pH, color and total soluble protein content (Lowry *et al*., 1951; Peterson, 1977). The optimal condition for the production of protein hydrolysates was selected based on high levels of total soluble protein content.

Determination of degree of hydrolysis

The degree of hydrolysis (%DH) is defined as the percentage of peptide bonds cleaved during the hydrolysis reaction relative to total peptide bonds (Rutherfurd, 2010). The DH of these protein hydrolysates was estimated according to Ovissipour *et al*. (2010). Twenty ml of 20 % trichloroacetic acid (TCA) were added to precipitate the protein, followed by centrifugation at 10,000 ×g (4°C) for 20 min to obtain the soluble fractions in 10 % TCA, using a Suprema 21 Model centrifuge (Tomy, Japan). The total nitrogen content of the supernatant was determined by Kjeldahl method (AOAC, 2006). The degree of hydrolysis was calculated as: % DH = (10 % TCA soluble nitrogen in tuna viscera protein hydrolysates / Total nitrogen in fresh tuna viscera) × 100.

Determination of total nitrogen, formaldehyde nitrogen, ammonia nitrogen and amino nitrogen of protein hydrolysate

Total nitrogen content

Total nitrogen content of the samples was measured using Kjeldahl method (AOAC, 2006). Total nitrogen content was expressed as $g \cdot l^{-1}$.

Formaldehyde nitrogen content

Formaldehyde nitrogen content was determined by the titration method (Thai Industrial Standard, 1983). The 10-fold diluted sample (10 ml) was titrated to pH 7.0 with 0.1 M NaOH before addition of 10 ml of formaldehyde solution (37% v/v). Titration was continued to pH 9.0 with 0.1 M NaOH. Formaldehyde nitrogen content was calculated as follows: Formaldehyde nitrogen content ($g \cdot l^{-1}$) = X×0.1×14; where X was the volume of NaOH (pH 7 – pH 9).

Ammonia nitrogen content

Ammonia nitrogen content was determined by the titration method (Thai Industrial Standard, 1983). The 10-fold diluted sample (30 ml) was transported to Kjeldahl flask containing 60 ml of distilled water and 3 g of MgO. The mixture was distilled to release volatile nitrogen into 30 ml of 4% boric acid containing methyl red bromocresol green. The distillate was finally titrated with 0.05 M H_2SO_4 until the end-point was obtained. Ammonia nitrogen content was calculated as follows: Ammonia nitrogen content ($g \cdot l^{-1}$) = 5.6×0.05×Y; where Y was the volume of H_2SO_4 (ml).

Amino nitrogen content

Amino nitrogen content was calculated using the following formula: Amino nitrogen content ($g \cdot l^{-1}$) = Formaldehyde nitrogen content ($g \cdot l^{-1}$) – Ammonia nitrogen content ($g \cdot l^{-1}$)

Determination of chemical composition and antioxidative activities of protein hydrolysates

Protein hydrolysates were analyzed for total nitrogen, salt (Nielsen, 2010), fat (Iverson *et al*., 2001), TVB-N (Hasegawa, 1987), histamine (AOAC, 2012), and amino acid composition (Bo *et al*., 2007). DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)) radical scavenging activity were also determined (Yu *et al*., 2014; Binsan *et al*., 2008). pH, and color were measured (Miwa and Ji, 1992).

Microbiological analysis

Total bacterial count, *Salmonella* spp. *Staphylococcus aureus*, *Vibrio cholera*, *Escherichia coli*, yeast and mold were obtained from the protein hydrolysates according to AOAC (2012).

Sensory evaluation

Protein hydrolysates were evaluated for acceptance by a panel of 50 untrained people with ages ranging from 19 to 33 years old. Protein hydrolysates were coded with random three-digit numbers and served to the panelists in a randomized complete block design. Each sample of protein hydrolysates (about 10 ml) was poured into a plastic cup and directly consumed by the panelists. After evaluation of one sample, the panelists were served with water before testing on the next sample. The panelists were then asked to give scores based on how they like the samples in terms of color, odor, taste (saltiness, sweetness, and bitterness) and overall satisfaction using a nine-point hedonic scale. The categories were on a 1-9 scale where 9 means 'extremely like', 8 as 'like very much' , 7 as 'moderately like', 6 as 'slightly like', 5 as 'neither like or dislike', 4 as 'slightly dislike' , 3 as 'moderately dislike' , 2 as 'dislike very much' and 1 as 'extremely dislike'.

Statistical analysis

Completely Randomized Design (CRD) with a 3 × 2 factorial arrangement (3 temperatures and 2 tuna species) was used in statistical analysis for determination of optimal production. T-test was used in statistical analysis to search for mean differences between different samples. The observable values are presented as means ± standard deviation (SD). The parallels, the number of replications = 3, were used for all analyses. Statistical analysis was performed using the Statistical Package for Social Science (SPSS 15.0 for windows, SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Composition of raw materials

SJ and YF tuna viscera contained about 17.51 and 10.91% protein, respectively (Table 1), which was higher than that of catla viscera (8.52 % protein) (Bhaskar and Mahendrakar, 2008). Therefore, such high levels of protein from viscera of both species made them a suitable source of protein produced from protein hydrolysate production. There was an increased amount of protein and a decreased amount of fat after production in the protein hydrolysate compared to raw material (Tables 1 and 5). The changes in

Table 1. Chemical composition of yellowfin and skipjack tuna viscera

Chemical analysis (%)	Yellowfin	Skipjack
Protien	10.91 ± 0.04	17.51 ± 0.30
Fat	4.42 ± 1.10	2.60 ± 0.37
Ash	1.88 ± 0.02	1.90 ± 0.20
Moisture	73.17 ± 0.22	74.51 ± 0.13
Salt	2.18 ± 0.12	2.14 ± 0.07

The data are presented as mean ± standard deviation. They were statistically analyzed for mean differences between the species of each composition using T-test (T - values, $P<0.05$).

protein and fat contents were caused by the removal of fat and impurities during production (Salwanee *et al*., 2013). The fat in samples was removed using a centrifuge at low temperature (4°C). The reduced fat content in samples may make them a good source of protein for producing protein hydrolysate since the presence of the protein-lipid complex causes resistance to protein hydrolysis (Kristinsson *et al*., 2000). The salt content of SJ and YF tuna viscera was 2.14 % and 2.18 %, respectively (Table 1). The pH of SJ and YF tuna viscera was 5.12 and 5.22, respectively. The histamine content of SJ and YF tuna viscera was about 28.03 and 28.78 in $mg \cdot kg^{-1}$, respectively, and was claimed to be lower than the maximum histamine levels (50 $mg \cdot kg^{-1}$) declared by the Fish Inspection and Quality Control Division, Department of Fisheries (2013); therefore, SJ and YF tuna viscera would not result in histamine poisoning.

The optimal production

Percent of yield

The yields of protein hydrolysates from SJ and YF tuna viscera increased when autolysis time increased. The yield of protein hydrolysates at 55°C (> 60%) was higher than those at 35 and 33°C (room temperature) in both species ($P < 0.05$) (Figure 1). It was suggested that the endogenous enzymes and high temperature could stimulate the breaking down of biomolecules into smaller peptides through autolysis process (Pastoriza *et al*., 2004). Therefore, amounts of small peptides would be produced to a higher extent at high than at low temperature.

The yield of protein hydrolysate from SJ tuna viscera was higher than that from YF tuna viscera ($P < 0.05$) (Figure 1). The differences in yield are usually caused by many factors such as fish species, seasonality, or the type and amount of enzymes that affect the digestion of protein by autolysis processes (Hsu, 2010; Kim, 2014)

pH

The pH of protein hydrolysates showed a similar pattern at different autolysis times and at three temperatures. The pH ranged between 5.06 and 6.0 (Figure 2). This result was not different from Thai commercial fish sauce which has a pH range of 5.0 to 6.0 (Funatsu *et al*., 2004).

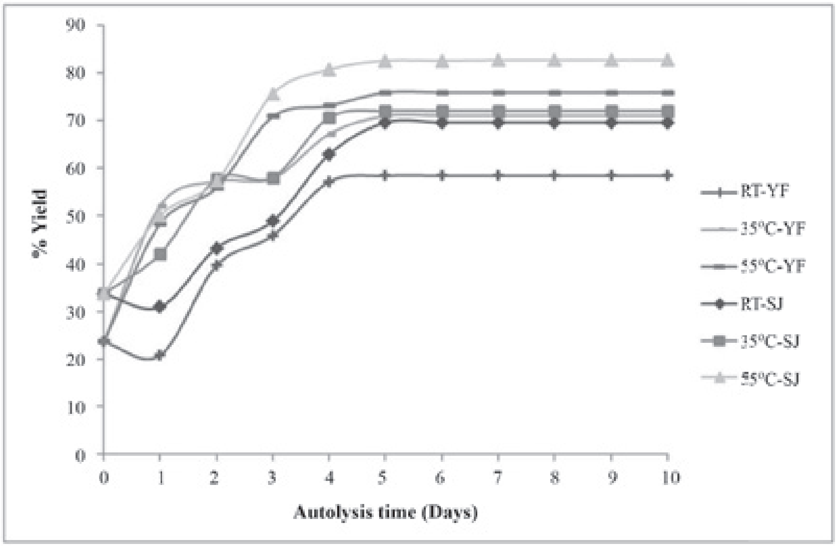

Figure 1. Yields of protein hydrolysates from yellowfin and skipjack tuna viscera at different conditions of autolysis (33°C (room temperature: RT), 35°C and 55°C for 10 days). Abbreviations: YF = yellowfin; SJ = skipjack.

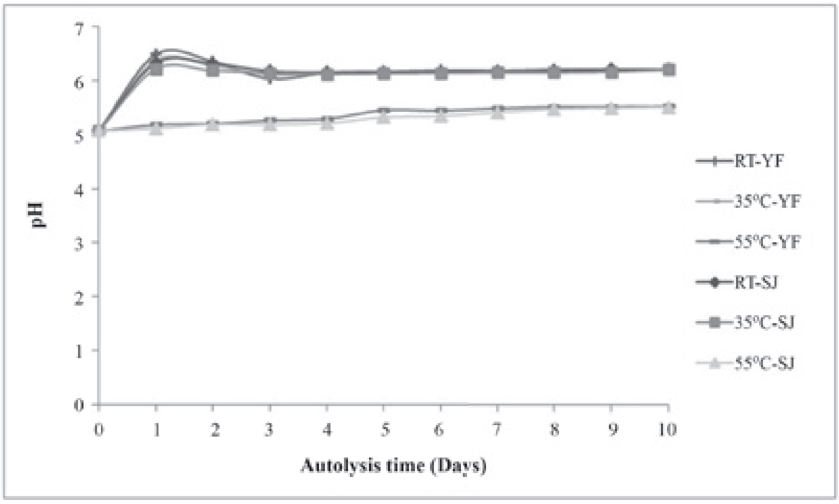

Figure 2. pH of protein hydrolysates from yellowfin and skipjack tuna viscera at different conditions of autolysis (33°C (room temperature: RT), 35°C and 55°C for 10 days). Abbreviations: YF = yellowfin; SJ = skipjack.

Total soluble protein content

Protein hydrolysate samples at 33ºC (room temperature) at day 0 had a high total soluble protein content and strong smell as it had not yet been autolyzed. After autolysis, the total soluble protein content in protein hydrolysate increased. The result of total soluble protein contents showed a similar pattern among the three optimal temperatures. Protein hydrolysate at 55ºC for 5 days had a higher protein content than at other conditions for both SJ and YF tuna viscera ($P < 0.05$). Therefore, the protein hydrolysates from both YF and SJ tuna viscera at this condition were selected for further analysis in the subsequent experiments

During the autolysis process, the total soluble protein content of protein hydrolysates increased due to the degradation of proteins (Figure 3). The low amount of total soluble protein content at the initial autolysis stage was related to osmosis that led to increasing water content (Xu *et al*., 2008). Protein hydrolysates at 33°C (room temperature) and 35°C were autolyzed from day 7 onwards, resulting in a rotten smell but no change in color.

Color

The color of protein hydrolysates at 33°C (room temperature) and 35°C after 5 day autolysis were a lighter than the 55°C after 5 day autolysis (Table 2). The color of protein hydrolysate from YF tuna viscera at 55°C after 5 day autolysis was brownish-red in color ($L^* = 60.76$, $a^* = 30.12$ and $b^* = 44.83$) (Table 2). In contrast, protein hydrolysate from SJ tuna viscera at the same conditions had a brownish-yellow color ($L^* = 60.94$, $a^* = 35.31$ and $b^* = 79.48$). The color of these protein hydrolysates was similar to commercial soy sauce (Ruchikachorn *et al*., 2005) and seasoning sauce (Miyagi *et al*., 2013).

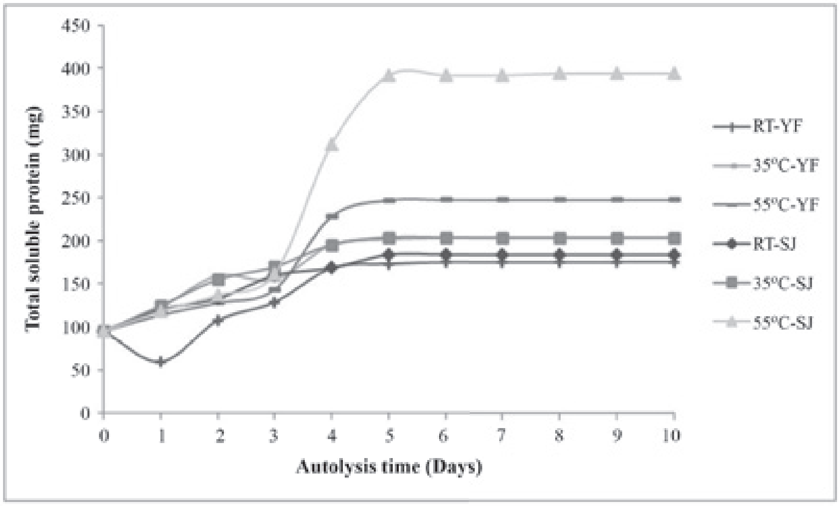

Figure 3. Total soluble protein content of protein hydrolysates from yellowfin and skipjack tuna viscera at different conditions of autolysis (33ºC (room temperature: RT), 35ºC and 55ºC for 10 days). Abbreviations: YF = yellowfin; SJ = skipjack.

Table 2. Color of protein hydrolysates from yellowfin and skipjack tuna viscera at different conditions of autolysis (33°C (room temperature: RT), 35°C and 55°C for 5 days). Abbreviations: L* = lightness, a* = the red/green value and b* = the yellow/blue value.

Yellowfin at room temperature	**Yellowfin at 35°C**	**Yellowfin at 55°C**	**Skipjack at room temperature**	**Skipjack at 35°C**	**Skipjack at 55°C**
L* = 63.14	L* = 62.95	L* = 60.76	L* = 63.14	L* = 64.33	L* = 60.94
a* = 68.23	a* = 67.18	a* = 23.12	a* = 43.61	a* = 41.82	a* = 35.31
b* = 33.54 (brownish-red color)	b* = 34.70 (brownish-red color)	b* = 44.83 (dark brownish-red color)	b* = 72.57 (brownish-yellow color)	b* = 75.09 (brownish-yellow color)	b* = 79.48 (dark brownish-yellow color)

Degree of hydrolysis

The DH of protein hydrolysates from SJ tuna viscera (61.73 % of DH) was higher than protein hydrolysate from YF tuna viscera (52.98 % of DH) ($P < 0.05$) (Table 3). The DH of protein hydrolysate from YF tuna viscera (52.98 % of DH) was higher than protein hydrolysate from yellowfin tuna head (34.00 % of DH) (Ovissipour *et al*., 2010), whereas the DH of protein hydrolysate from SJ tuna viscera (61.73 % of DH) was similar to skipjack tuna viscera (62.90 % of DH) (Chotikachinda *et al*., 2013). The heating process (at 55 ºC) can activate endogenous enzyme resulting in denatured protein, which may improve the ability of enzymes to hydrolyse proteins and thus influence DH and yield of protein hydrolysate (Xu *et al*., 2008; See *et al*., 2011).

Total nitrogen content

As shown in Table 4, the total nitrogen content of protein hydrolysate from SJ and YF tuna viscera (36.24 and 24.10 g l^{-1}, respectively) was higher than the total nitrogen content of fish sauce standard (20.0 g l^{-1}) (Codex Alimentarius Commission, 2011). The high total soluble nitrogen was caused by the endogenous enzymes and high temperature. They can stimulate the breaking down, there by releasing water soluble proteins and soluble nitrogen compounds from the viscera cells (Jiang *et al*., 2007; Xu *et al*., 2008).

Table 3. The degree of hydrolysis of protein hydrolysates from yellowfin and skipjack tuna viscera at 55 °C for 5 days autolysis

Treatments	**Degree of hydrolysis (%)**
Yellowfin	52.98 ± 0.74
Skipjack	61.73 ± 0.38

The data are presented as mean ± standard deviation and were statistically analyzed for mean differences between the species of each composition using T-test (T - values, $P<0.05$).

Table 4. The total nitrogen, formaldehyde nitrogen, ammonia nitrogen and amino acid nitrogen of protein hydrolysates from yellowfin and skipjack tuna viscera at 55 °C for 5 days autolysis

Nitrogen compound	Yellowfin	Skipjack
Total nitrogen ($g \cdot l^{-1}$)	24.10 ± 0.03	36.24 ± 0.11
Formaldehyde nitrogen ($g \cdot l^{-1}$)	15.53 ± 0.12	23.10 ± 0.17
Ammonia nitrogen ($g \cdot l^{-1}$)	1.83 ± 0.06	1.57 ± 0.06
Amino nitrogen / total nitrogen (%)	56.85 ± 0.40	59.42 ± 0.42

The data are presented as mean ± standard deviation and were statistically analyzed for mean differences between the species of each composition using T-test (T - values, $P<0.05$).

Formaldehyde nitrogen content

As shown in Table 4, the formaldehyde nitrogen content of protein hydrolysates from SJ and YF tuna viscera (23.10 and 15.53 g l^{-1}, respectively) was higher than commercial fish sauce (3.88-17.94 g l^{-1}) (Somboonyarithi *et al*., 2000). In fermented foods, formaldehyde nitrogen content plays an important role as the indicator of the degree of hydrolysis (Byun *et al*., 2000). The proteolytic enzyme of tuna viscera was involved in the conversion of insoluble materials to soluble protein. Therefore, the degree of hydrolysis of SJ protein hydrolysate was higher than YF protein hydrolysate (Table 3).

Ammonia nitrogen content

The ammonia nitrogen content is caused by the breakdown of soluble protein and peptides into free amino acid and volatile nitrogen compounds which represented precursors for amine formation used by spoilage microorganisms. (Lopetcharat *et al*., 2002; Xu *et al*., 2008). The ammonia nitrogen contents of protein hydrolysates from YF and SJ tuna viscera were 1.83 and 1.57 g l^{-1}, respectively (Table 4). These values were lower than that of concentrated anchovy fish sauce (2.08 g l^{-1}) (Somboonyarithi *et al*., 2000). Therefore, the decomposition of nitrogenous compounds in these samples was also lower than concentrated anchovy fish sauce.

Amino nitrogen content

The amino nitrogen content of the protein hydrolysates from YF and SJ tuna viscera had 56.85 and 59.42 % of total nitrogen, respectively (Table 4). These values were higher than concentrated fish sauce (49.13% of total nitrogen) (Somboonyarithi *et al*., 2000). However, the amino nitrogen content of these protein hydrolysates was within the range of high quality fish sauce (40 to 60% of total nitrogen) (Codex Alimentarius Commission, 2011).

Chemical compositions of protein hydrolysate composition

Protein hydrolysate from SJ tuna viscera autolyzed at 55°C for 5 days had more protein content and less fat content than that from YF tuna viscera ($P < 0.05$) (Table 5).

Table 5. Chemical composition of protein hydrolysates from yellowfin and skipjack tuna viscera at 55°C for 5 days autolysis

Chemical analysis (%)	Yellowfin	Skipjack
Protien	15.09 ± 0.03	22.65 ± 0.13
Fat	0.59 ± 0.02	0.48 ± 0.05
Salt	1.95 ± 0.08	1.88 ± 0.04

The data are presented as mean ± standard deviation and were statistically analyzed for mean differences between the species of each composition using T-test (T - values, $P<0.05$).

It was also found that it contains a greater amount of proteins than the commercial fish sauce (11.00 - 14.00%) (Somboonyarithi *et al*., 2000). Therefore, SJ tuna viscera could be a good material of protein hydrolysate with a nutritionally important source of dietary protein (Kilinc *et al*., 2005).

The pH of the protein hydrolysates from SJ and YF tuna viscera was 5.45 and 5.62, respectively. The pH of the liquid samples was higher than in the raw material due to the production of TVB-N (9.52-45.39 mg $100g^{-1}$ of SJ and 9.55-47.38 mg $100g^{-1}$ of YF) during autolysis (Hernández-Herrero *et al*., 1999). The salt content of the protein hydrolysates from SJ and YF was 1.88 and 1.95 %, respectively since tuna viscera absorbed salt from ice seawater during post-harvest. Therefore, these protein hydrolysates were found salt content without salt addition.

The histamine contents increased when autolysis time increased. It is because histidine is converted to histamine by the histidine decarboxylase from bacteria (Brillantes *et al*., 2002). The histamine contents in the protein hydrolysates from SJ (276.87 mg kg^{-1}) and YF (289.95 mg kg^{-1}) tuna viscera were lower than the maximum levels (400 mg kg^{-1}) of histamine in fish sauce set by the Codex Alimentarius Commission (2011) meaning that the sauces would be safe for consumers.

Amino acids

Protein was hydrolyzed into free amino acids and peptides by endogenous protease from the tuna viscera (Lalasidis *et al*., 1978; Rebeca *et al*., 1991). The major amino acids of protein hydrolysates from SJ and YF tuna viscera were glutamic acid, aspartic acid, leucine, lysine, arginine, alanine and glycine (Table 6). The essential amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine) must be taken every day because these amino acids cannot be synthesized in the human body (Brown, 2008). The protein hydrolysates from SJ and YF tuna viscera had high levels of essential amino acids (Table 6). Therefore, these protein hydrolysates are an important source of dietary nutrition. Hydrophobic amino acids (glycine, leucine, alanine, valine, isoleucine, proline, phenylalanine, methionine, and tryptophan) and aromatic amino acid (histidine, isoleucine phenylalanine and tryptophan) are the main contributors to the antioxidant activities (Pownall *et al*., 2010; You *et al*., 2010). The protein hydrolysates from SJ and YF tuna viscera were rich in hydrophobic and aromatic amino acids (Table 6). Therefore, these protein hydrolysates had high antioxidant activities (Table 7).

DPPH and ABTS radical scavenging activity

As shown in Table 7, DPPH radical scavenging activity of protein hydrolysates from SJ and YF tuna viscera (EC_{50} of 1.93 and 3.00 mg protein ml^{-1}, respectively) was higher than those from skipjack dark muscle (EC_{50} of 4.54 mg protein ml^{-1}) (Chi *et al*., 2015).

As shown in Table 7, ABTS radical scavenging activity of protein hydrolysates from SJ and YF tuna viscera (IC_{50} of 0.43 and 0.44 mg protein ml^{-1}, respectively) was higher than those from anchovy (*Clupeonella engrauliformis*) with IC_{50} of 0.60 mg protein ml^{-1} (Ovissipour *et al*., 2013).

Antioxidant activity of protein hydrolysate depends on enzyme specificity, hydrolysis conditions, levels and compositions of amino acids and small peptides (Klompong *et al*., 2007).

Therefore, protein hydrolysate from skipjack tuna viscera contains substances, which might be electron donors, that could react with DPPH and ABTS radicals resulting in a production of stable products and a reduction of the radical chain reaction.

Table 6. Amino acid composition of protein hydrolysates from yellowfin and skipjack tuna viscera at 55°C for 5 days autolysis

Amino acid (mg · 100 g^{-1})	Yellowfin	Skipjack
Essential amino acid	1,400.02	1,087.07
Arginine	539.57	635.10
Histidine	935.92	788.50
Isoleucine	1,500.15	1,293.65
Leucine	1,487.11	1,254.46
Lysine	637.51	580.97
Methionine	820.59	706.11
Phenylalanine	1,101.00	839.11
Tryptophan	256.46	279.44
Valine	1,087.28	894.18
Total	9,765.72	8,358.59
Non-essential amino acid		
Alanine	1,485.18	1,075.66
Aspatic acid	2,240.73	1,652.29
Cysteine	290.40	395.58
Glutamic acid	3,125.67	2,384.96
Glycine	1,425.02	1,050.35
Proline	1,088.47	803.61
Serine	1,204.30	923.83
Tyrosine	237.07	228.86
Total	11,096.84	8,515.14

The data were statistically analyzed for mean differences between the species of each amino acid composition using T-test (T - values, $P<0.05$).

Table 7. DPPH and ABTS radical scavenging activity of protein hydrolysates from yellowfin and skipjack tuna viscera at 55°C for 5 days autolysis

Treatments	Scavenging activity	
	DPPH (EC_{50} mg protein· ml^{-1})	ABTS (IC_{50} mg protein· ml^{-1})
Yellowfin	3.00 ± 0.08[a]	0.44 ± 0.02[a]
Skipjack	1.93 ± 0.02[b]	0.43 ± 0.01[b]

The data are presented as mean ± standard deviation and were statistically analyzed for mean differences between the species of each radical using T-test (T - values, P<0.05).

Sensory property

All of the panelists satisfied and accepted protein hydrolysates produced from YF and SJ tuna viscera. The likeness scores of odor, saltiness, sweetness, and bitterness of these protein hydrolysates were not significantly different (Table 8). The panelists described that the color of these protein hydrolysates was similar to soy sauce and seasoning sauce. They claimed that the samples had smell and taste of steamed fish but did not have smell and taste of fishy or rotten. The salty, sweet, and bitter tastes of these protein hydrolysates were evaluated based on panelists' preference (Table 8).

As shown in Table 6, the sweetness of these protein hydrolysates came from amino acids (of glycine, alanine, threonine, proline, hydroxyproline, serine, and glutamine) (Femaroli, 1975). On the other hand, threonine, proline, asparagine, histamine, methionine, valine, isoleucine, arginine, phenylalanine, tryptophan, and leucine contributed for the bitterness taste (Femaroli, 1975). Although these protein hydrolysates highly consisted of amino acids for the bitter taste, the panelists accepted them because the overall taste was similar to the well-known Thai commercial fish sauce produced from tuna. However, the protein hydrolysate from SJ tuna viscera had high preference scores of color and

Table 8. Sensory analysis of protein hydrolysates from yellowfin and skipjack tuna viscera at 55°C for 5 days autolysis

Character	Score	
	Yellowfin	Skipjack
Color	8.79 ± 0.34	8.97 ± 0.71
Odor	7.88 ± 0.4	8.01 ± 0.62
Saltiness	6.45 ± 0.27	6.53 ± 0.49
Sweetness	6.89 ± 0.83	6.92 ± 0.58
Bitterness	6.83 ± 0.83	6.87 ± 0.76
overall acceptance	7.09 ± 0.72	7.72 ± 0.94

The data are presented as mean ± standard deviation and were statistically analyzed for mean differences between the species of each character using T-test (T - values, P<0.05).

overall acceptance more than YF tuna viscera since Thai people preferred brownish-yellow color than to brownish-red color (Table 8). Based on this result, skipjack tuna viscera is proposed as an alternative source with high qualities to use in the protein hydrolysate production. Consequently, protein hydrolysates from SJ tuna viscera might be acceptable and potentially adopted by the fish sauce industry.

Microbiological analysis

The protein hydrolysates from YF and SJ tuna viscera had fewer microbiological contaminants (Table 9) than standard traditional Thai products did (Fish Inspection and Quality Control Division Department of Fisheries, 2013). Therefore, these protein hydrolysates are safe for human consumption. The low microbial loads might be caused by the simultaneous pasteurization at 55°C for 5 days since the time and temperature for controlling bacterial pathogen growth is 55°C for 1 h (Weiss, 1973; Sumontha, 2002.). Therefore, these protein hydrolysates produced by autolysis processes at 55°C for 5 days are safe from bacterial contamination and toxin formation. The low microbial loads of these protein hydrolysates might refer to a long shelf-life of products.

Table 9. Microbiological analysis of protein hydrolysates from yellowfin and skipjack tuna viscera at 55°C after autolysis for 5 days

Treatments	Time (Day)	Total bacterial count (CFU g^{-1} or ml^{-1})	*Salmonella* spp. 25ml^{-1}	*Staphylococcus aureus* (MPN g^{-1} or ml^{-1})	*Vibrio Cholerae* 25ml^{-1}	*Escherichia coli* (MPN g^{-1} or ml^{-1})	Yeast & Mold (CFU g^{-1} or ml^{-1})
Yellowfin	0	6.2 x 10^2	Not found	9.49 x 10	Not found	<3	<1
	1	<1	Not found	<3	Not found	<3	<1
	2	<1	Not found	<3	Not found	<3	<1
	3	<1	Not found	<3	Not found	<3	<1
	4	<1	Not found	<3	Not found	<3	<1
	5	<1	Not found	<3	Not found	<3	<1
	6	<1	Not found	<3	Not found	<3	<1
	7	<1	Not found	<3	Not found	<3	<1
	8	<1	Not found	<3	Not found	<3	<1
	9	<1	Not found	<3	Not found	<3	<1
	10	<1	Not found	<3	Not found	<3	<1
Skipjack	0	4.9 x 10^2	Not found	6.21 x 10	Not found	<3	<1
	1	<1	Not found	<3	Not found	<3	<1
	2	<1	Not found	<3	Not found	<3	<1
	3	<1	Not found	<3	Not found	<3	<1
	4	<1	Not found	<3	Not found	<3	<1
	5	<1	Not found	<3	Not found	<3	<1
	6	<1	Not found	<3	Not found	<3	<1
	7	<1	Not found	<3	Not found	<3	<1
	8	<1	Not found	<3	Not found	<3	<1
	9	<1	Not found	<3	Not found	<3	<1
	10	<1	Not found	<3	Not found	<3	<1

CONCLUSION

This research suggested that SJ tuna viscera could be a suitable material for protein hydrolysate production with acceptable qualities, nutrient contents, and strong DPPH and ABTS radical scavenging activities. Protein hydrolysate from SJ tuna viscera contained about 22.65% of protein, so it could be a nutritionally important source of dietary protein. Protein hydrolysate from SJ tuna viscera contained salt (1.88%) and histamine (276.87 mg kg^{-1}) that were at safe levels for consumption. The color of the protein hydrolysate from SJ tuna viscera was brownish-yellow. Therefore, the protein hydrolysate from SJ tuna viscera might be a good source of protein that would be good for consumer's health. Furthermore, it could potentially be accepted by the fish sauce industry in the future.

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