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Potency of Catfish (*Clarias* sp.) Protein Hydrolysates as Candidates Matrices for Microbiology Reference Material

Eti Kurniawati[1], Bustami Ibrahim[2] and Desniar[2]
[1] National Center for Examination of Fisheries Product Implementation
Jalan Raya Setu No. 70 Cipayung Jakarta Timur 13880, Indonesia
[2]Department of Aquatic Product Technology, Bogor Agricultural University
Kampus Dermaga Bogor, Indonesia

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Abstract

Fish protein hydrolysate (FPH) is a derivative product of fish proteins containing smaller peptides and amino acids. FPH products have high water solubility, good emulsion capacity, and large expanding ability. With its functional properties, it allows FPH to be used as a raw material in the manufacturing of secondary microbiological reference materials. This study was intended to characterize catfish (*Clarias* sp.) FPH as a candidate for the matrix of microbial secondary reference. The FPH was prepared through enzymatic hydrolysis, freeze-drying and milling. The hydrolysis processes were carried out using 5% (w/w) papain, 55 °C for 5 hours, then the papain activity was stopped by increasing the temperature to 80 °C for 20 minutes.The FPH was combined with gelatine, sodium glutamate, glucose solution, and was spiked with *Salmonella enteritica* sv *Enteritidis* and freeze-dried. Results showed that catfish FPH was yellowish-white powder with a FPH yield of 11.05%. The proximate analysis of FPH revealed the moisture content of 3.77 ± 0.12%, ash content of 7.26 ± 0.03%, protein content of 86.09 ± 0.17%, and fat content of 1.38 ± 0.07%. The protein content of the FPH was greater than skim milk (33.42%). Carbohydrate levels of catfish FPH and skim milk were 1.56% and 57.46%, respectively. The best concentration of catfish FPH to perform as a microbiological reference material was 14%, obtained from highest viability of *Salmonella* bacteria and homogeny. The candidate for reference material were stable at storage temperatures of -20 °C.

Keywords: fish protein hydrolysate, FPH, catfish, reference material, microbiology

1. Introduction

Fish protein hydrolysate (FPH) is a derivative product of fish proteins containing smaller peptides and amino acids. FPH is obtained by treating fish meat with enzymatic or chemical under controlled conditions of pH and temperatures. Studies on the fish protein hydrolysates have been done previously, namely FPH of catfish (*Clarias batrachus*) using alcalase and papain (Seniman, Yusop & Babji, 2014) and FPH of African catfish using papain (Nurhayati, Nurjanah & Sanapi, 2013). FPH is potential for functional food ingredients as they possess numerous important and unique properties such as water-holding capacity, oil absorption capacity, protein solubility, gelling activity, foaming capacity and emulsification ability (Chalamaiah, Rao, & Jyothirmayi, 2010). FPH can be used as an excellent source of nitrogen for maintaining the growth of different microorganisms. Ghorbel et al. (2005) used various FPH from *Sardinella aurita* as nitrogen sources for the growth of *Rhizopus oryzae* and the production of lipase. In another study, Safari, Motamedzadegan, Regenstein, Gildberg and Rasco (2012) used hydrolysates from yellowfin tuna (*Thunnus albacares*) heads as a complex nitrogen source for lactic acid bacteria.

Reference materials refer to the subject that have one or more homogeneous and stable substances to be used in calibration equipment, testing methods or as standards in confirmation or sample analysis (ISO, 2008). Reference materials play an important role in

*Corresponding author.
E-mail: ethie_tea@yahoo.com

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national and international standardization activities as well as laboratory accreditation. Testing laboratories that have been accredited ISO / IEC 17025 are required to be able to meet the requirements related to the test result's quality assurance. Reference material is one of the tools used to assess laboratory performance on an ongoing basis, as quality control in conducting testing and can be used to calibrate equipment and to validate or verify the testing method. Reference materials must meet some requirements specified in the ISO Guide 34 which needs to be homogeneous and stable within the specified limit for certain time and must be representative according to the intended use. Representative means that the reference material should resemble the routine sample or the matrices must be following the tested sample. Microbiological reference material with fish meat matrices is available primarily for microbiological proficiency testing such as *Listeria monocytogenes* in smoked fish and *Vibrio parahaemolyticus* in fish products, used by Fapas, a world-renowned accredited proficiency testing provider. Currently, microbiological reference materials with fish matrices in Indonesia are not yet available so that there is a need from fisheries products laboratory for reference materials with specific fisheries matrices.

According to ISO (2017), to produce reference materials, it requires comprehensive studies, covering from the production stage, testing, evaluation and management aspects. In details, it is includes the selection of raw materials, feasibility studies for the processing and characterization of materials, preparation of candidate reference materials, determination of the value of certification and uncertainty, homogeneity studies, stability studies, and evaluation of the results (Sujarwo & Nuryatini, 2013).

The production of microbiological reference materials is often hampered by the instability of living organisms such as bacteria and the statistical variability that attached the microbiological procedures. According to Jarvis (2014), microbiological reference materials can be made by inoculation and standardization of food ingredients such as skim milk or preserved cultures of certain types that can be added to food ingredients. Basically, reference materials production is an encapsulation process, which is a packaging process (coating) of core material (in this case is microbes), using certain encapsulation materials. Coating materials that commonly used as encapsulants are gum, carbohydrates and proteins such as skim milk, gelatin, lactose, sucrose, maltodextrin, alginate, gum arabic, starch, agar, gelatin, carrageenan, albumin, and casein (Sumanti, Lanti, Hanidah, Sukarminah, & Giovanni, 2016). The use of FPH as a candidate for matrices of microbiological secondary reference materials is expected to provide protection against microbes and representative, and appropriate for candidate microbiological reference material with fish matrices. Encapsulation material serves to maintain viability and protect microbes from damage due to unfavourable environmental conditions. According to Rizqiati, Jenie, Nurhidayat, and Nurwitri (2006), the use of protein as a coating material can maintain bacterial resistance. According to Triana, Yulianto, and Nurhidayat (2005), isolates of *Lactobacillus* sp. Mar 8 has higher viability when encapsulated using skim milk at a concentration of 10% than 5%.

In this study, fish protein hydrolysates were characterized and compared to skim milk which was commonly used in the production of microbiological reference materials. The purpose of this study was to characterize catfish protein hydrolysates as matrices for the production of microbiological reference materials.

2. Materials and Methods

2.1 Materials

Raw catfish (weight range of 800-900 g per head) used in this research were obtained from fish suppliers in Cilangkap, East Jakarta, Indonesia. Water-soluble papain enzyme 30000 USP-U/mg (EC 3.4.22.2) was purchased from Merckmillipore with specific activity 500 U/mg. A strain of *Salmonella enteritica* sv *Enteritidis* (ATCC® 13076) from American Type Culture Collection was used as reference material.

2.2 Methods

2.2.1. Preparation of raw materials

The catfish samples were weighed, skin-removed, and filleted. The catfish meat (skinless fillet) was then minced using a food processor. The proximate compositions (moisture content, ash content, protein content and fat content) were analysed.

2.2.2. Fish protein hydrolysates preparation

In this study, fish protein hydrolysate production was conducted according to Nurhayati et al. (2013) that had been modified, namely using catfish sample and 5% (w/w) papain enzyme with hydrolysis time of 5 hours. Fish meat that has been minced, then homogenized with distilled water (ratio 1 : 2) using a shaker for 2 minutes. The pH value of the mixture was adjusted to reach the optimal pH of 7.0 by adding 1 M NaOH solution or 1 M HCl solution. Furthermore, papain enzyme at a concentration of 5% (w/w) was

added to the fish meat mixture. The hydrolysis was carried out at 55°C using waterbath shaker for 5 hours. After the hydrolysis process was completed, the papain enzyme was inactivated at 80 °C for 20 minutes to stop the hydrolysis process. Samples were centrifuged at a speed of 5000 rpm for 20 minutes at 4 °C to separate the supernatant and pellet. The supernatant was dried using freeze dryer for 96 hours at -40 ± 2 °C.

2.2.3. Analysis of proximate and amino acid composition

Proximate analysis was carried out on fish protein hydrolysates and commercial skim milk for microbiology. The amino acid compositions and proximate of FPH and skim milk were analyzed. The total carbohydrate was determined by difference. The proximate analysis was carried out referring to AOAC (2005). Amino acid composition was analyzed by ultra performance liquid chromatography (UPLC).

2.2.4. Preparation of bacterial strains

Preparation of bacterial culture was preceeded by a preliminary test to determine the purity and population of *Salmonella*. The population of *Salmonella* was carried out by mean of inoculation in a 10 ml BHI broth and incubated for 18-24 hours at 35±2 °C, then the population was calculated by the total plate count method on PCA media (BSN, 2015). The number of bacterial populations inoculated into the reference material was 10^2 CFU/ml.

2.2.5. Processing of microbiology secondary reference material

Processing of microbiology secondary reference material referred to BBP2HP (2017). After the bacterial culture was ready, the candidate of microbiological reference material of fish matrices (FPH of catfish) with five different concentrations (10, 11, 12, 13, and 14%) was produced. Catfish protein hydrolysate was weighed accordingly to each concentration and dissolved in distilled water. The solution was added by sodium glutamate, 40% glucose solution, gelatin and homogenized using a magnetic stirrer. The pH of the homogenate solution was set up to pH 7.2 and it was sterilized using an autoclave at 110 °C for 15 minutes. The sterile homogenate was inoculated by *Salmonella* bacterial culture (10^2 CFU/mL) and then 2 ml of the solution was pipetted and put into vials using a sterile pipette. The bottles were frozen using dry ice and 95% absolute ethanol for ± 1.5 hours then freeze-dried at -40±2 °C for 36-48 hours. Candidate of microbiological secondary reference materials that have been freeze dried were then tested for homogeneity and stability.

2.2.6. Viability estimation

Viability test was conducted by calculating the bacterial population before the freeze-drying process and immediately after the freeze-drying process was completed. Viability of bacterial cells was calculated using Total Plate Count (TPC) method with a series of dilutions in phosphate-buffered solution, then grown in PCA culture media. The survival bacteria was determined by calculation of colony-forming units per ml (CFU/ml). Percentage of bacterial viability was calculated using the following equation (Rizqiati et al., 2006):

$$\text{Viability (\%)} = \frac{\text{Log A}}{\text{Log B}} \times 100\%$$

with: A : colony forming in dry basis after freeze-drying (CFU/mL)

B : colony forming in dry basis before freeze-drying (CFU/mL)

2.2.7 Homogeneity study

Ten vials were chosen randomly from a batch produced and two independent sample preparations, then the measurement of total plate number (TPC) was performed on each vials according to SNI 2332-3-2015 (BSN, 2015). Homogeneity evaluation was carried out on several units of reference material to determine the standard deviations between units. The distribution was checked using normal probability plots and histograms. Finally, an analysis of variance (ANOVA) was performed to quantify the within-bottle standard deviation and the between-bottle standard deviation.

2.2.8. Stability study

Two types of stability tests of the candidate of microbiological reference material, i.e. a stability test at storage temperature (-20 °C) and a stability test at a higher temperature which was determined at two different temperatures, i.e. 30 °C, and 37 °C. Once a week, for 4 weeks, three vials from each storage temperature were examined in duplicate on PCA medium then the measurement of total plate number (TPC) was performed on each vials according to SNI 2332-3-2015 (BSN 2015). The stability was evaluated using the T test at 95% confidence level.

3. Results and Discussion

3.1. Proximate Analysis of Catfish Raw Material

The proximate composition of raw material is important for the assessment of the potential

development and application of reference materials. Proximate composition of catfish meat (*Clarias* sp.) are shown in Table 1.

As shown in Table 1, the raw material of catfish (wet weight basis) contained 74.77% of moisture, 1.36% ash, 17.11% protein and 6.72% fat. These results were not much different from the study conducted by Seniman *et al*. (2014) which had moisture, ash, protein and fat contents 76.85%, 1.09%, 20.32% and 4.75%, respectively. On the other hand, the chemical composition of catfish on dry basis weight contained ash, protein and fat 5.38%, 67.82% and 26.65%, respectively.

3.2. Proximate Analysis of Fish Protein Hydrolysate of Catfish

The hydrolysis of catfish meat was carried out using the commercial papain enzyme (Merck) with the activity of 500 U/mg. The yield of catfish protein hydrolysate obtained was 11.05%. In the process of hydrolysis, not all substrate was hydrolyzed but some substrate were precipitated. According to Nurhayati, Salamah, and Amalia (2007), the bigger addition of the papain enzyme caused fewer precipitation produced which indicated that more meats hydrolyzed into smaller molecules. Wijayanti, Romadhon, and Rianingsih (2015) stated that the enzyme concentration had a significant effect on the yield of hydrolyzed milk-fish FPH using the enzyme papain.

The hydrolysate in the form of supernatant were freeze-dried to obtain protein hydrolysate powder. The freeze-drying process was carried out at a temperature of –40 ± 2 °C for 96 hours. The hydrolysis process resulted in a clean yellowish-white colour powder of catfish protein hydrolysate as seen in Figure 1.

This study also carried out the proximate analysis and amino acid composition for both catfish FPH and skim milk that commonly used in the microbiological reference materials production (BBP2HP, 2017). The proximate contents of catfish protein hydrolysate and skim milk can be seen in Table 2. Several studies of fish protein hydrolysates produced from catfish as raw materials have been carried out with various result values, influenced by the concentration of enzymes and the type of fish used.

The catfish protein hydrolysate contained 3.77 ± 0.12% moisture. It does not differ from the moisture content of catfish protein hydrolysate produced by Nurhayati et al. (2013) and Seniman et al. (2014), but lower than that of Salamah et al. (2012) (Table 2). The difference in the moisture level of catfish protein hydrolysates is very dependent on the drying method. The drying method used in this study was freeze-dry similar to that conducted by Nurhayati et al. (2013),

Table 1. Proximate composition of raw catfish (*Clarias* sp.)

Proximate composition	% wet basis	% dry basis	Catfish (% wet basis)*
Moisture	74.77 ± 0.73	-	76.85 ± 0.17
Ash	1.36 ± 0.01	5.38	1.09 ± 0.04
Protein	17.11 ± 0.13	67.82	20.32 ± 1.42
Fat	6.72 ± 0.03	26.65	4.75 ± 0.61

Reference: *Seniman et al. (2014)

Figure 1. Protein hydrolysate from catfish

Table 2. Proximate compositions of protein hydrolysates from catfish (*Clarias* sp.)

Parameters	FPH of *Clarias* sp.	FPH of *Clarias gariepinus**	FPH *Clarias gariepinus***	FPH *Clarias batracus****
Moisture content (%)	3.77 ± 0.12	3.45 ± 2.05	5.46	3.17 ± 0.46
Ash content (%)	7.26 ± 0.03	2.47 ± 0.67	5.71	0.74 ± 0.08
Protein content (%)	86.09 ± 0.17	69.26	53.29	83.09 ± 0.55
Fat content (%)	1.38 ± 0.07	0.50	1.94	1.09 ± 0.05

Reference: *Nurhayati et al. (2013); **Salamah *et al*. (2012); ***Seniman *et al*. (2014)

while the drying method conducted by Salamah et al. (2012) was the spray drying method. The low moisture content of catfish protein hydrolysates was due to the freeze-drying method used. Some samples lost moisture contents during the drying process (Seniman et al., 2014).

The ash content of catfish protein hydrolysate in this study was 7.26%, greater than that resulted by Nurhayati et al. (2013), Salamah et al. (2012) and Seniman et al. (2014). Several studies have reported that FPH ash content varies depending on the type of fish and the process. Salamah et al. (2012) reported that high ash content in FPH caused by the addition of alkali compounds such as NaOH and/or acid compounds such as HCl, in the process of protein hydrolysis to achieve the optimum pH of the enzyme and keep the pH constant during the hydrolysis process. The mixture of acid and alkali compounds in the protein hydrolysate solvent causes the formation of salt compounds that increase the ash content of the protein hydrolysate.

The protein content of catfish protein hydrolysate is greater than the raw material of meat. It showed that the process of hydrolysis of meat into smaller molecules went well. Wijayanti, Romadhon, and Rianingsih (2016) reported that protein levels increase following the concentration of enzymes increment, thus the hydrolysis reaction becomes faster. However, the addition of excess enzymes, on a certain level, resulted in a constant amount of hydrolysate since the addition of the enzyme is no longer active. The high protein content of fish protein hydrolysates also caused by the dissolved protein that occurs during the hydrolysis process and the removal of solids which are not dissolved during the centrifugation process (Seniman et al., 2014).

On the other hand, the fat content of catfish protein hydrolysate was low i.e. 1.38%. According to Wijayanti et al. (2016), low fat content hydrolysate products were generally more stable and durable compared to high fat protein products. Furthermore, low fat content in catfish protein hydrolysates could be caused by the removal of insoluble fat following centrifugation at 4°C (Seniman et al., 2014).

3.3. Proximate Analysis of Fish Protein Hydrolysate of Catfish and Skim Milk

The percentage of chemical composition between catfish protein hydrolysate and skim milk can be seen in Figure 2.

Skim milk is milk with a fat content of 1.5% (w/w) and has high protein 34% (w/w) (Kemenkes, 2012). Based on the percentage of dry weight basis, there are differences in protein levels and carbohydrate levels between catfish protein hydrolysates and commercial skim milk. Catfish FPH protein content (db) was 89.46% while skim milk was 33.42%. Carbohydrate levels are determined by difference≤showing carbohydrate levels of catfish FPH was 1.56% and skim milk was 57.46%.

Reference materials production is an encapsulation process. Encapsulation is a technology used mainly to package sensitive bioactive materials and microorganisms in a miniature capsule. The microbial cells have typical sizes ranges from 1 to 5 μm in diameter. Therefore, they can be entrapped by microencapsulation. The purpose of micro-encapsulation of microbial is to provide a protective barrier between them and the destructive factors prevalent in the surrounding environment such as heat, oxygen, and moisture. Skim milk is commonly used as a raw material used in production microbiological reference materials (Jarvis, 2014; In't Veld, 1998). According to El-Salam and El-Shibiny (2015), milk protein has been widely used single or in combination with other biomaterials as capsules for probiotics. According to Dianawati, Vijay Mishra, and Shah (2013) compared to soy protein isolates, milk protein provides better protection for *Bifidobacterium longum* 1941 after freeze-drying and during exposure to the acid and bile

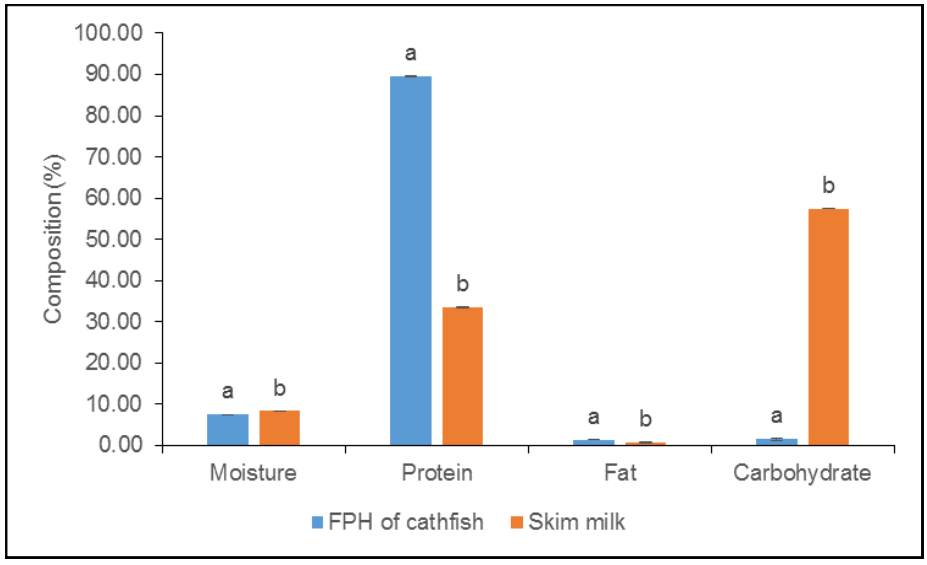

Figure 2. Proximate composition (% dry basis) of catfish FPH and skim milk

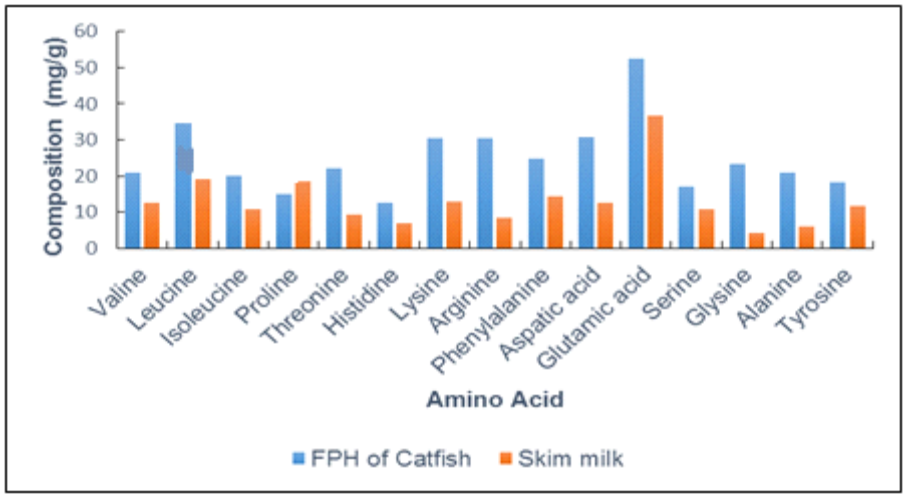

Figure 3. Amino acid composition of catfish protein hydrolysate and skim milk

environment. Previous study by Lopez (2013) found that whey protein increased the resistance of probiotic *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* against acid and bile salts. Other studies have shown that in addition to whey protein, casein was also capable of encapsulating *Lactobacillus* F19, *Bifidobacterium* Bb12, and *L. rhamnosus* GG and improved cell survival for up to 90 days (Burgain, Gaiani, Linder, & Scher, 2011). Chen, Remondetto, and Subirade (2006) reported that proteins have the ability to interact, protect, and reverse bonds with various active compounds through their functional groups. In addition, protein may also have the desired stabilizing effect on food texture.

3.4. Amino Acid Composition of FPH and Skim Milk

The amino acid composition of fish protein hydrolysates is important because of the nutritional value and the influence on the functional properties. Amino acids are the main constituent components of protein. Catfish protein hydrolysate contains 9 types of essential amino acids, namely valine, leucine, isoleucine, proline, threonine, histidine, arginine, lysine, and phenylalanine. While the non-essential amino acids contained in the catfish protein hydrolysate are aspartic acid, glutamic acid, serine, glycine, alanine and tyrosine.

In this study, the enzyme papain was used for the hydrolysis process. Sari, Ekantari, and Ustadi (2008) reported that the enzyme papain could break peptide bonds in asparagine-glutamine residues, glutamate-alanine, valine and leucine-phenylalanine-tyrosine. The amino acid composition of a catfish protein hydrolysate is presented in Figure 3. The highest amino acid content was glutamic acid leucine and aspartic acid.

There are similarities between amino acid composition of the cat fish protein hydrolysate and the skim milk that commonly used as an ingredient for the microbiological reference materials production. The highest amino acid composition in skim milk is glutamic acid and leucin at concentration of 36.83 mg/g and 19.18 mg/g, respectively. The composition of glutamic acid which is higher than other amino acids is thought to have an important in the process of bacterial encapsulation. Dimitrello, Kandylis, and Kourkoutas (2016) stated that monosodium glutamate was considered as optimal lyoprotectant for *L.casei* cells because it gives cell viability values up to 80% after freeze drying. This is supported by Carvalho et al. (2003), which stated that monosodium glutamate has the ability to protect various lactic acid bacteria during freeze drying.

3.5. Viability Test of *Salmonella* sp.

Viability test was carried out to determine the resistance of *Salmonella* in the process of making microbiological reference material after the freeze drying process. Viability test was carried out at each concentration of FPH and 12% skim milk was used as a control. The viability of *Salmonella* after freeze-drying of each concentration can be seen in Figure 4.

The results showed that the viability of *Salmonella* at 5 different FPH concentrations of catfish was above 50%. The highest viability of *Salmonella* was obtained at a concentration of 14% matrices, i.e. 66.24 ± 0.73%. Whereas the viability of *Salmonella* using 12% skim milk was 55.74 ± 1.04%. *Salmonella* viability tends to increase with the amount of catfish FPH concentration used. According to Martin, Lara-Villoslada, Ruiz, & Morales (2015), during the freeze-drying process (lyophilization) ice crystals formed could damage bacterial cells, dissolving substances in the fraction, consequently, harm the bacteria (Maltesen & van de Weert, 2008). Water plays an important role in cell integrity and stability, and its removal from bacterial cells can cause extensive damage to surface proteins, cell walls and cell membranes, and decreases its viability after drying. Various protectors (cryoprotectants) were reported to be added to the drying media before the freeze-drying process to protect the survival of probiotics during dehydration (Martin et al., 2015). The positive effect of the use of protein on dry microorganisms due to its capacity to protect cells by stabilizing cell membrane constituents and to make porous structures in freeze-dried products that make rehydration easier (Selmer-Olsen, Birkeland, & Sorhaug, 1999).

3.6. Homogeneity Study of Microbiological Reference Material Candidate

Homogeneity is an important requirement for reference materials including homogeneity within and between units. Homogeneity between units and within units is important to ensure that each unit of reference material carries the same value so that if a subsample is taken by the user, it has the same value. The results of the homogeneity test of the reference material in fish matrices (FPH of catfish) are presented in Table 3.

Homogeneity is an important requirement for all reference materials and includes both within- and

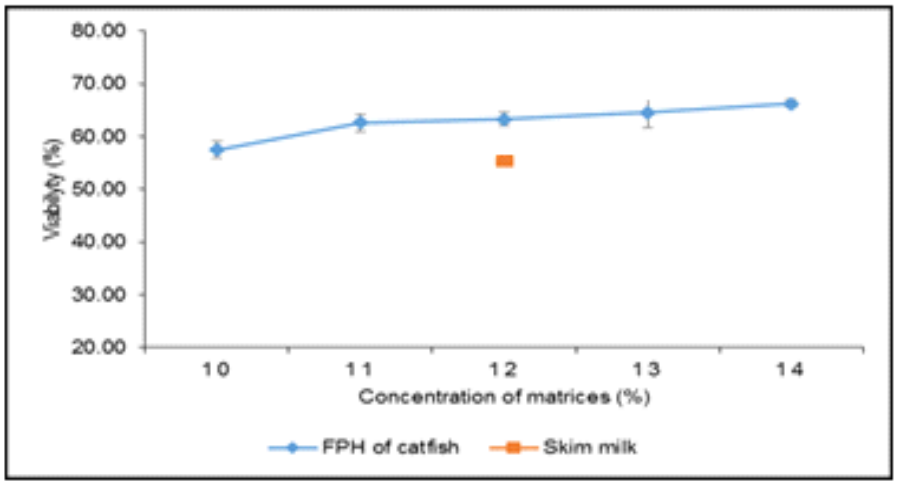

Figure 4. Viability of *Salmonella* sp in candidate microbiological reference material after freeze-drying

Table 3. Homogeneity test of candidate of microbiological reference materials with fish matrices

Matrices	Concentration	$M_{between}$	M_{within}	F	F Crit
FPH	10%	0.1201	0.0118	10.1414	3.0204
	11%	0.1376	0.0075	18.3132	
	12%	0.0635	0.0152	4.1696	
	13%	0.0076	0.0019	3.9867	
	14%	0.0031	0.0019	1.5800	
Skim milk	12%	0.0327	0.0320	1.0223	

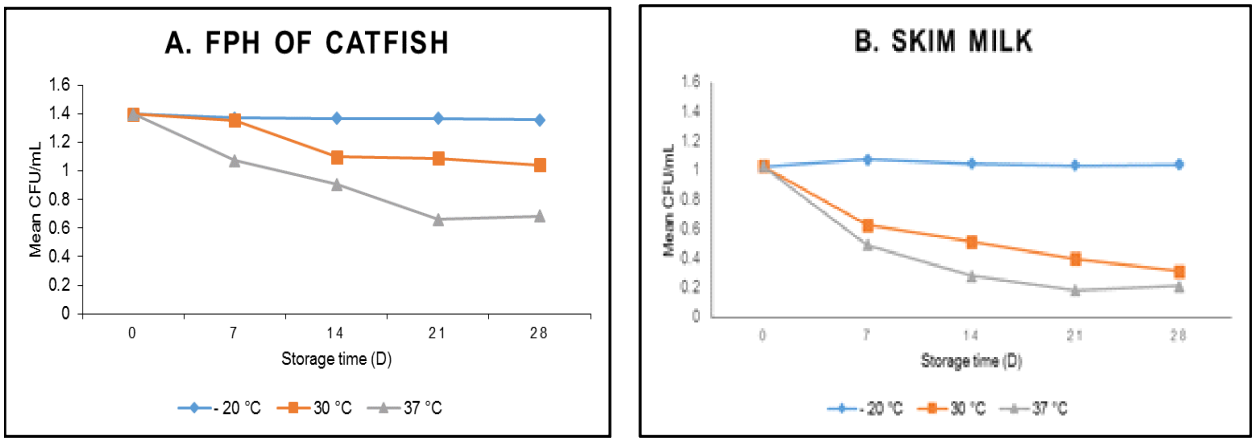

Figure 5. Result of stability tests of candidate microbiological reference material with fish matrices (A) and skim milk (B) at various temperatures: -20°C (♦), 30°C (■), and 37°C (▲)

between-unit homogeneity. Between-unit homogeneity is important to ensure that each reference material unit carries the same value for each property; within-unit homogeneity is important to ensure the subsample taken for measurement by users of the material has the same value. ISO (2016) accordingly requires the assessment of the homogeneity of reference material. Homogeneity can be determined by confirmation using an F test, where the value in between unit is not statistically significant at the 95% confidence. The results showed that only at 14% concentrations of FPH of catfish showed homogeneous conditions (F<Fcrit). With higher concentrations of catfish FPH, *Salmonella* bacteria can be encapsulated better than lower concentrations of catfish FPH so that the distribution of bacteria in the reference material is better. Sugindro, Mardliyati, and Djajadisastra (2008) reported that the efficiency of encapsulation increases with increasing concentration of the coating material. The wall layer is getting better and stronger so that it can protect the core material well.

3.7. Stability Study of Microbiological Reference Material Candidate

Reference materials should be sufficiently stable for their intended use so that the end-user can rely on the assigned value at any point within the period of validity of the certificate. Such materials cannot remain stable indefinitely because they contain living organism which continues to grow or die during the storage period. The results of the stability test of reference material candidates with the fish matrices and skim milk as a control can be seen in Figure 5.

An isochronous short-term stability study, t-Test: two samples assuming equal variance analysis revealed no significant trends for storage temperature of -20°C (Pvalue> α= 0.05). During storage condition at 30°C, the reference material was stable for only

one week, while at 37°C storage was not stable. The same thing happens with microbiological reference materials in skim milk. Mooijman and Havelaar (1997) reported a CRM certification study for *Escherichia coli*, showing good stability at storage temperatures of -20ºC and low stability with increasing temperatures. Jarvis (2014) reported that the microbiological reference material for *Salmonella* was stable for several years at -20 ºC, but for only about 6 months at 5 ºC. Maier, Griepink, In't Veld, Mooijman, and Havelaar (1993) reported that the addition of sucrose into skim milk had a good effect and increased the stability of the *Salmonella* reference material.

4. Conclusion

The production of catfish protein hydrolysate using 5% (w/w) enzyme papain resulted in the formation of yellowish-white powder and yield of 11.05%. The protein content of catfish FPH was 86.09 ± 0.17% with the highest amino acid of glutamic acid 52.42 mg/g. Using 14% catfish FPH, it produced the highest viability of *Salmonella* bacteria, homogeny and stable at a storage temperature of -20 ºC for 28 days. Candidates for microbiology secondary reference material with FPH of catfish matrices were stable until day 7 at 30ºC and unstable at 37ºC storage temperatures.

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