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Microbiological Evaluation and Storage Stability of Snakehead Fish Extract (*Channa striata*) using Steaming Method

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Abstract

The snakehead fish (SHF) has commercial value due to its health benefit. Steaming extraction method using distilled water within temperature below 60°C is the one of extraction methods to obtaining the SHF extract with high yield of valuable soluble protein albumin, as well as relatively simple to applied at home industries. Recently, the recommended storage for these conventional steamed SHF extract (SSHFE) product is in freeze temperature (-20°C), not in room temperature. Evaluation to find scientific reason related to the SSHFE storage stability is necessary required. The series of storage stability and microbiological test toward SSHFE had investigated included total plate count (TPC); most probable number (MPN) of coliform and Eschericia coli test using Gram staining and Indol, Methyl Red, Voges Praskauer, Citrate (IMVIC) at different storage stability treatments. This research found that SHF extract during storage of 0 week after out from freezer were negatively contaminated (TPC= $<10^2$ CFU/ml). While, TPC at storage of 1; 4; 8 weeks after out from freezer showed contamination in level of $1.722x10^3$ CFU/ml; $1.327x10^4$. CFU/ml and $6.839x10^4$ CFU/ml, respectively which still below threshold that stipulated by Indonesian Food Standardization ($5x10^5$ CFU/ml). While MPN index were exceed the standard, i.e. 24/g (1 week), 350/g (4 weeks), and <1600/g (8 weeks). Moreover, the gram stain and IMVIC test indicated that they were positively contaminated by *Eschericia coli*. Accordingly, SSHFE is recommended to store at freeze temperature and immediately consumed after melt in room temperature. Further research of sterilizing methods for SSHFE processing is suggested.

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Keywords

Microbiology test; Storage stability; snakehead fish (*Channa striata*) steaming extraction.

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Introduction

Snakehead fish (*Channa striata*) is the one of abundant tropical freshwater fish in almost region Indonesia which traditionally known as medicine. It also has been developed in pond cultures due to its commercial value and health benefit (FAO, 2000).

Scientific studies revealed that the snakehead fish (SHF) contains albumin which potentially increases the albumin levels in patients with hypoalbuminemia and postoperative surgery and liver function recovery (Santoso, 2009), contains antioxidant glutathione and its precursors i.e. amino acids glutamine, cysteine and glycine that essential for tissue repair, including pancreas (Sunarno, 2015; Esrefoglu, 2012; Robertson and Harmon, 2007). The previous pharmacological studies proved that using steamed SHF extract has recovery potential for pancreatic and testicular damaged in diabetic mice as well as lowering their blood sugar toward the normal levels. Furthermore, refer to the malondealdehide indicated that SHF extract has role to increase antioxidant capacity in the testis, pancreas and plasma of diabetic mice (Hidayati 2017; Sekartaji and Hidayati, 2016, Abdulgani *et al.,* 2016)

Steam extraction using distilled water within temperature below 60°C is the one of extraction methods to obtaining the SHF extracts with high yield of valuable soluble protein included albumin (Romadhoni *et al.,* 2016; Nugroho, 2012; Mustafa,2012; FAO, 1986). Albumin content in snakehead fish was reported at value of 6.4% (Romadhoni *et al. ,*2016) . Those conventional steamed SHF extract (SSHFE) product is recommended to store in freeze temperature (-20°C), not in room temperature. Evaluation to find scientific reason related to the SSHFE storage stability necessary required.

Accordingly, SSHFE is potential to be developed as a nutraceutical, food /part of a food that safe to eat orally and provides health benefits (Palthur *et al.,* 2010). included for anti-diabetes. Therefore, it is necessary to conduct a series of optimization analyzes to obtain SHF nutraceutical products that are feasible to consume before commercial production. The microbiological storage stability evaluation should be investigated for safety purposes, to provides important information how to store and expiring date labeling (Bensley, 2008).

Materials and Methods

The processing of steam extraction adopted from Romadhoni *et al.,* (2016). The fins, scale, gill and digestive organs were removed from SHF body. Then, the remaining tissues primarily consist of flesh were rinsed in running water, cut into smaller pieces and stored in icy container prior to steam extraction. The steaming process was conducted in sterile room using modified steam extractor which consist of the stainless steel steamer- pan with the outlet pipe beneath to collect and directing the evaporated water to the collecting flask. The steamer is digitally connected with temperature and time controller equipment. Steaming extractor was adjusted at 50±10°C for 30 minutes. During steaming process, the droplets of concentrate were directed into the vessel that connected into the sterilized flask. Then, the flasks were capped and stored in the freeze refrigerator (-20°C) prior to microbiological analysis. In this report, the yielded of steamed SHF extract is called as SSHFE.

The triplicate SSHFE were grouped into four storage stability treatments: 0; 1; 4 and 8 weeks after out from freezer. The culture media without SSHFE were used as control samples. The series of microbiological and the storage stability test were employed to each sample included total plate count (TPC); most probable number (MPN) of coliform and Eschericia coli test using Gram staining and Indol, Methyl Red, Voges Praskauer, Citrate (IMVIC) .

Total Plate Count (TPC) testing was performed in accordance with the procedure of microbial contamination test of Indonesia National Standard: SNI No.01-2897-1992. The 10 mL SHF extract sample were homogenized in 90 ml of peptone dilution fluid (PDF), and serial of 10-fold dilutions (from 10-1 until 10-7) were prepared. The 1 mL aliquot of each dilution was placed into a sterilized petri dish then added with 15-20 mL of plate count agar (PCA) then shaken homogeneously and incubated for 48 hours at 37°C. The estimated number of bacteria were computed by multiplying the average number of colonies that appear on each plate with the dilution factor recorded and reported

as colony forming units (CFU) (Bambang, 2014; Damongilala, 2009).

Enumeration of Total Coliform Count was conducted using five tubes of most probable number (MPN) method according to procedure of analysis colifom and E. coli in fish product that stipulated by Indonesian National Standard SNI 01-2332.1, 2006; Bartram and Pedley, 1996).

The Presumptive Test

10 ml of SHF extract samples was homogenized with 90 ml of Butterfield's Phosphate Buffered diluent solution, then three consecutive of 10-fold dilutions (10-1; 10-2; 10-3) were prepared. Using sterile pipettes , transfer 1 ml aliquot of each dilution to each of 5 tubes of Lactose Broth (LB) which containing the Durham's tube. The tube was incubated for 48 hours ± 2 hours at 35°C ± 1°C and the gas production during 24 hours in Durham's tube and color change of the media (more turbid) were observed. The positive tube is characterized by turbidity and gas production in Durham's tube. Furthermore, the enumeration of Coliform was determined based on the number of positive results from each tube set and compare with MPN standard chart that stipulated by SNI (2006), to give presumptive coliform count per gram sample.

Confirmed Test of *Escherichia coli*

A positive presumptive test were inoculated in selective media of eosin methylene blue agar (EMBA) and incubated at 35°C for 24 hours. The presence of *Escherichia coli* colony in the media showed the distinctive (typical) characteristic (the black color in the middle with or without metallic green). Presence of typical colonies confirms positive coliform test. Each culture that positive coliform bacteria was inoculated on Eosin Methylene Blue Agar (EMBA) medium, and then incubated at 37°C for 24 hours. Then, the green colonies with metallic luster and greenish spots on EMBA media were inoculated on nutrient agar (NA) medium (SNI, 2006). After incubation at 45 °C for 24 hours, medium were tested with gram staining and IMViC assay according to Harley and Prescott (2002).

Results

All Result of evaluation the bacterial colony growth using TPC method is presented at Table 1.

Table 1: Result of TPC: Bacterial Colony Growth

Media	Total Plate Count (CFU/ml)				
	Storage stability treatments (week)	Replication			Average (CFU/ml)
		1	2	3	
Control (media without SSHFE)	0	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	1	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	4	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	8	$<10^2$	$<10^2$	$<10^2$	$<10^2$
Media with SSHFE	0	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	1	$1,146x10^3$	$2,315x10^3$	$1,711x10^3$	$1,722x10^3$
	4	$1,593x10^4$	$1,101x10^4$	$1,287x10^4$	$1,327x10^4$
	8	$5,691x10^4$	$7,513x10^4$	$7,313x10^4$	$6,839x10^4$

The control media which not added by SSHFE at storage stability treatments of 0;1;4,8 weeks were negatively contaminated by bacteria ($<10^2$ CFU/ml). It is indicated that the media that used in this research were not contaminated. Media with SSHFE at storage of 0 week after out from freezer also negative ($<10^2$ CFU/ml). Whereas on the media that added with SSHFE at storage of 1; 4; 8 weeks

after out from freezer, were positively contaminated in level of $1.722x10^3$ CFU/ml; $1.327x10^4$. CFU/ml and $6.839x10^4$ CFU/ml, respectively. Refer to National Standardization Agency (BSN) these bacterial colony number were still below threshold that stipulated at level of $5x10^5$ CFU/ml. However, bacterial colonies in SHF extract in the storage of 1; 4; 8 weeks possibly contain coliform bacteria, hence the coliform test is necessary required.

The MPN test were performed toward the media that containing the bacterial colony , the media that containing SSHFE at the storage of 1;4;8 weeks. According to the presumptive test, showed that SSHFE at the storage of 1;4;8 weeks were positive results of coliform. These status were indicated by the gas formation and turbidity in Durham's tube within lactose broth media (LB) that containing SSHFE. Gas and turbidity indicated that coliform bacteria had used lactose as a carbon source and did the fermentation process that produced acid and gas (Dad, 2000).

Result of coliform enumeration based on the number of positive results in presumptive test that comparing with MPN standard chart is exhibited at Table 2. The MPN index in media with SSHFE is follow: 24/g (1 week), 350/g (4 weeks), and <1600/g (8 weeks), which all are exceed the Indonesian National standard that stipulated at level of <3/g. Furthermore, the confirmation test of coliform and E coli also shown the positive result. The gram stain and IMVIC test indicated that they were positively contaminated by *Escherichia coli*.

Table 2: Enumeration result of most probable number (MPN). The MPN index in media with SSHFE are exceed the Indonesian National standard that stipulated at level of <3/g

The storage stability treatments (week)	Dilution															Positive tube	MPN per gram	Confidential limit 95%	
	1 ml					0,1 ml					0,01 ml								
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5			Upper	Lower
1	+	-	+	-	+	+	-	+	-	+	+	-	+	-	-	3-3-2	24	9,8	70
4	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	5-4-4	350	100	710
8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	5-5-5	>1600	700	-

Discussion

The media with SSHFE at room temperature with storage of 0 week was negatively contaminated by bacteria (table 1). Furthermore, during storage of 1; 4; 8 weeks were observed positively contaminated. The freezing storage prior to storage experiment influence the bacterial behavior. Several studies reported that freezing technique (-20°C) can decreasing microorganisms in storing foods (Storper *et al.,* 1982; Pankey *et al.,* 1987; Schukken *et al.,* 1989), decrease in the number of samples positive for *Escherichia coli* and *Arcanobacterium pyogenes* (Schukken *et al.,* 1989); lowering the number of salt tolerant bacteria from $9.8x10^5$ CFU/ml to $7.6x10^3$ CFU/ml and *Staphylococcus s*pp from ($3.6x10^3$ CFU/ml) to $8.2x10^1$CFU/ml (Alrabadi, 2015). Therefore, the appearance colony at 0 week storage cannot observed yet.

The prolonging of storage treatments is followed by the increasing of bacterial colony growth indicated that media with SSHFE were being suitable substrate for bacteria. Bacterial growth is affecting by several factors included the composition of substrate, initial contamination and temperature (Koutsoumanis, 2006). SSHFE consist of high nutrient uch as

proteins with albumin as a major fraction, fat, glucose and some minerals Zn,Cu, and Fe (Nugroho, 2012; Mustafa, 2012) hence supporting for bacterial growth. The *E. coli* initial contamination in SSHFE also considerable since the SHF lives in river bottom substrate (Sea Grant, 2008). The temperature influences to bacterial growth were shown when SSHFE were placed out from the freezer to room temperature for 1; 4; and 8 weeks. In the other hand, the contamination during the extraction process also considered. Steam extraction at 60^{0}C for 30 minutes have risk not sufficient to kill bacteria. To avoid the presence of *E.coli* and *Salmonella* spp. in meat, it have been promoted to cook at temperatures ranging from 63 to 85°C (Juneja *et al.,* 1998; USDA, 2011) Based on this research, SSHFE is recommended to stored at freeze temperature and immediately consumed after melt in room temperature. Further sterilizing methods for SSHFE processing is suggested.

The research found that snakehead fish extract (SSHFE) that yielded from distilled water steam extraction using temperature of 60^{0}C for 30 minutes have risk contaminated by *E coli* bacteria. SSHFE at the storage of 1;4 and 8 weeks after out from freeze temperature contain coliform with MPN range of 24/g -1600/ g which exceeded Indonesian National Standard.

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