

## Characteristic of Allergen Protein of Nila Baby Fish (*Oreochromis niloticus*) and Its Processed Product

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### Abstract

Allergen protein is a protein that could triggers allergy reaction. This study purposes to examine the characteristic of allergen protein of unripe, boiled, and fried nila baby fish. Characteristic of allergen protein were observed by electrophoresis and immunoblotting method, while the determination of protein concentration was observed by Bradford method. Identification of the used nila baby fish was accomplished in Faculty of Fishery and Marine Science of IPB. The result of identification showed that the used nila baby fish was *Oreochromis niloticus*. The proximate analysis of unripe, boiled, and fried nila baby fish resulted water content ranged from 19.16%-23.68%, fat content ranged from 1.03%-20.44% and carbohydrate content ranged from 0.16%-20.27%. Protein concentration of unripe, boiled, and fried nila baby fish extract respectively were 1963.45 mg/L; 699.82 mg/L; 607.79 mg/L. The band amount of allergen protein of unripe, boiled, and fried nila baby fish which was detected by electrophoresis, respectively were 16 of protein band, 26 of protein band and 16 of protein band. The immunoblotting showed that the sum of respondent who contained specific IgE that can bind with allergen protein of boiled and fried nila baby fish were more than allergen protein of unripe nila baby fish. It indicated that the processing process by boiling and frying would increase allergenicity toward nila baby fish.

**Keywords:** Allergen, nila baby fish, SDS-PAGE, immunoblotting.

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### 1. INTRODUCTION

Nila Fish (*Oreochromis niloticus*) is one of high valuable fish species in economic. Consumption need either seed or fish from year to year tends to increase along with cultivation plant expansion (Darwisito *et al.*, 2008). Moreover, food processing products of nila fish also start developing. One of them is food processing product comes from nila baby fish. This product has umami flavor and contains nutrient especially essential amino acid, such as leucine, histidine, glycine, alanine, and tyrosine as well as high protein and sufficiently low level of lipid (Ogunji *et al.*, 2005, Justi *et al.*, 2003).

World Allergy Organization states that 22% of the world population suffer from allergies and continues to increase every year.

Ebo *et al* (2010) reported that consumed nila fish could potentially causing an allergy to sensitive people toward nila fish. Allergy could trigger light symptoms such as pruritis, runny nose, and puffiness even evoked an enough heavy response like anaphylaxis that may cause death (2016). Some foods which contained allergen are fish, shell, egg, soybean, cow milk, wheat, peanut, tree nuts (as canary, walnut, hazelnut) (Gupta *et al.*, 2013). Type of common allergen protein contained in seafood, mollusk, and fish is *tropomyosin* (Boye and Godefroy, 2010).

Foodstuff contained allergen protein could be detected by molecular weight and its reaction with IgE antibody, like allergen protein in goatfish (*Oreochromis mossambicus*) that has molecular weight about

17 kDa-114 kDa (Liu *et al.*, 2012). Allergen protein of mackerel fish has molecular weight about 20 kDa (Misnan *et al.*, 2005), and tuna fish's about 27-38 kDa (Hamada *et al.*, 2001). Food processing process with heat could influence allergenicity characteristic of the foodstuff (Nowak-wegrzyn and Fiocchi, 2009). Besides the unripe nila baby fish, this study also used fried and boiled nila baby fish. The study aim is to examine the characteristic of allergen protein of unripe, fried, and boiled nila baby fish

## 2. MATERIALS AND METHODS

### Materials

The materials that used in this study were nila baby fish (bought from fish market in Cengkareng, West Jakarta), blood serum taken from 9 respondents (8 seafood allergy sufferers and 1 normal respondent), phosphate buffer saline (PBS) pH 7.5, ammonium persulfate (APS), methanol, glacial acetate acid, NaCl, aquadest, tween-20, Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) 10%, TEMED (N,N,N',N'-tetramethyl-ethane-1,2-diamine), tris base, SDS (sodium dodecyl sulphate), BSA (bovine serum albumin), acrylamide, glycine, glycerol, bromphenol blue, 2-merkaptoethanol, skim milk, aquabidest, coomassie brilliant blue G-250, PBST (phosphate buffer saline – Tween 20), anti IgE human antibody labeled HRP enzyme (Horseradish Peroksidase), DAB substrate (3,3-Diaminobenzidine), N,N-methylene-bisacrylamide, high molecular weight protein (HMW) Fermentas® (contained 10 kinds of standard protein with molecular weight 250 kDa, 150 kDa, 100 kDa, 75 kDa, 50 kDa, 37 kDa, 25 kDa, 20 kDa, 15 kDa, and 10 kDa).

The equipments and instruments that used in this study were blender, centrifuge, pan, wok, stove, SDS-PAGE Bio-Rad Mini-Protean II, immunoblotting Mini Trans-Blot® Electrophoretic Transfer, spectrophotometer UV-VIS, freeze drier, nitrocellulose membranes for blotting pore size 0.45 µm, size 15 cm x 15 cm (Sigma N8267), analytic scale, pH meter, vortex, stirrer, thermometer, laboratory flask, measuring cup, beakers, Eppendorf tube, micropipet 5 µL to 1000 µL, filter paper Whatman No.1, general filter paper, and other glass instruments.

### Proximate Analysis (AOAC, 1990)

The proximate analysis carried out consisted of water content, ash content, protein concentration, fat content and carbohydrate content as describe in AOAC 1990.

### Protein Extraction (Hashimoto *et al.*, 1979)

Sample of nila baby fish treated with 3 different treatments, i.e. unripe, fried, and boiled. The fried nila baby fish obtained from frying process at 130 °C-140 °C for 10 minutes, whereas the boiled nila baby fish obtained from boiling process at 100 °C for 5 minutes. 20 grams of each nila baby fish sample were weighed, added phosphate buffer saline (PBS) pH 7.5 with ion power (I) = 0.05 (15.6 mM Na<sub>2</sub>HPO<sub>4</sub>, 3.5 mM KH<sub>2</sub>PO<sub>4</sub>) and blended for 3 x 1 minutes, next centrifuged at 4000 rpm for 25 minutes at 4 °C. The obtained supernatant separated and kept in freezer at -20 °C for the further analysis.

### Protein Analysis (Bradford, 1976)

Bradford reagent made of the following manners: 100 mg of coomassie brilliant blue G-250 dissolved in 50 mL of ethanol 95%. Next, 100 mL phosphate acid 85% added and diluted until 1 L precisely with aquadest. Then filtered the solution using filter paper Whatman No.1, afterwards kept the solution in dark bottle at refrigerate temperature. The solution is the Bradford reagent. An amount of protein isolate dissolved with aquadest then 100 µL was taken into dark test tube. Added 5 mL of Bradford reagent, then shaken it by vortex and incubated for 5 minutes. Protein concentration was measured by spectrophotometer at 595 nm wavelength. The used standard was BSA with various concentration of 0-1000 ppm.

### Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) (Laemmli, 1970)

Optimum concentration of acrylamide in separating gel was 12 % while 4% for stacking gel. Added 40 µl of protein extract with sample buffer (1:1), heated for 5 minutes, then put into wells coincide with marker protein that had been heated for 15 minutes. Run SDS-PAGE for 135 minutes at 90 Volt. Gel was colored by staining solution consist of coomassie brilliant blue R-250, methanol, glacial acetate acid, and aquadest. Then washed the colored gel three times using

aquadest, next rinsed by destaining solution consist of methanol, glacial acetate acid, and aquadest.

Protein molecular weight of sample could be calculated by regression equation, which obtained from curve of relation between relative mobility of marker protein ( $R_f$  or retention factor) and molecular weight logarithme of marker protein. Relative mobility of marker protein was counted in comparing protein migration rate that measured from start line of separating gel till end of protein band, and dye migration rate.

#### **Serum Preparation (Zakaria et al., 1998)**

Blood serum were taken from 8 allergy sufferers of seafood or fish and 1 normal respondent who known by respondent quisioner and agreement. Blood of respondent which taken was 10 mL. Medical personels of Science and Technology Faculty of UIN Syarif Hidayatullah Jakarta collected the respondent bloods. The blood placed in tube without EDTA. The blood of allergy sufferer carried to laboratorium for separating its serum by centrifuge. The blood incubated for 1 hour, then sentrifuge at 2500 rpm for 20 minutes.  $\pm$  5 mL of supernatant taken then kept in freezer at  $-20$  °C for the further analysis.

#### **Immunoblotting (Bollag and Edelstein, 1991)**

Uncolored gel of electrophoresis result transferred to other container. Afterwards, gel and nitrocellulose membrane stacked in transblotting device. The arranging of transblotting device stained with transfer buffer solution using filter paper and spon as an aid. Then the arranged device included in blotting container and filled with transfer buffer solution. Blotting was held at 90 Volt for 90 minutes. The transferred membrane released from the device series and performed fixation with methanol 50% for 2 minutes, then blocked using skim milk 5% in PBST for 1 hour at room temperature. Later, membrane washed with PBST 3 x 5 minutes.

300  $\mu$ L of allergy sufferer serum with dilution rasio 1: 10 in PBST added to membrane, then incubated for 2 hours at room temperature. The washery used PBST for 3 x 5 minutes, next given 2  $\mu$ L of HRP antibody

conjugated monoclonal mouse anti-human IgE (with dilution rasio 1:3000 in PBST) and incubated while shaking for 1 hour. Membrane rewashed with PBST for 3 x 5 minutes, and added DAB substrate. The positive result marked by forming brown band in nitrocellulose membrane.

### **3. RESULT AND DISCUSSION**

#### **Characteristic of Raw Material**

Nila baby fish attained the age of 2 until 4 weeks with size 3-6 cm were used at this study. Identification result from Faculty of Fishery and Marine Science of IPB showed that nila baby fish implied in family of *Cichlidae* with Latin name *Oreochromis niloticus*. Figure 1 showed the appearance of nila baby fish, colored golden-black with black ribbon or line in vertical artwart at tail fin till body. Amount of spines at dorsal fin ranged from 16-18. The pectoral fin colored black. Fish's mouth aimed up with unbulged head. Nila baby fish conformed to ovale body, with golden-cream underside and bright eye colour.



**Figure 1.** Baby fish Nila (*O. niloticus*)

European Medicines Agency Evaluation of Medicines for Human Use (EMA) in The Guideline on Allergen Products: Production and Quality Issues (EMA/CHMP) BWP/304831/2007, explained that concentration of protein, carbohydrate, fat, and water constituted parameters used to value the raw material of protein isolate. Allergen in foodstuff commonly formed of protein or glycoprotein (Cianferoni and Spergel, 2009).

**Table 1.** Chemistry composition of nila *Baby fish (O. niloticus)*

No	Sampel	Parameter (%)				
		Water	Ash	Protein	Fat	Carbohydrate
1	Unripe	76.83	2.50	19.31	1.20	0.16
2	Boiled	75.47	3.42	19.16	1.03	0.92
3	Fried	27.45	8.56	23.68	20.44	20.27

Table 1 showed that the boiled nila baby fish had protein concentration 19.16% that similar to unripe nila baby fish (19.31%), otherwise protein concentration of fried nila baby fish increased 23.68%. Justi *et al.* (2003) identified 4 weeks old-unripe *O. niloticus* possessed 17.2 % of protein concentration, whereas according to Asia *et al.*, (2015) the fried nila baby fish had 20.27 % of protein concentration. The difference of proximate value, included protein concentration of nila baby fish in every study caused by environment condition, fish habitat, nutrition or nutrient, and different fish food. (Soekendarsi *et al.*, 2016). Meanwhile, fluctuation of nutrient decreasing of foodstuff caused by cooking process depended on kind of foodstuff and temperature (Sundari *et al.*, 2015).

The highest of fat content acquired at fried nila baby fish 20.44%, that caused by presence of absorbed cooking oil in the fish. Besides that, the frying process engendered the decreasing of water content of nila baby fish product become 27.45%. It happened because frying process effected the movement of mass, which signed of movement oil into product (Saguy and Pinthus, 1995). Carbohydrate content obtained by difference of calculation, so if there were one or more of content from proximate parameter changed, the carbohydrate content would also changed. In this matter, if the water content declined thus the carbohydrate content would be huge in foodstuff.

#### Extract of Nila Baby Fish (*O. niloticus*)

Table 2 showed the protein concentration of extract from unripe, boiled, and fried nila baby fish. The protein concentration of unripe nila baby fish extract was higher than protein concentration of boiled and fried nila baby fish extract. Boiling and frying process could affect protein solubility in phosphate buffer saline solution as solvent. The decline of concentration was suspected of related to protein loss or modification because

of heating. Protein heating triggered the denaturation, colour changing, derivatization of amino acid, dissolution of peptide bond, and forming of active compound in censoring (Sundari *et al.*, 2015).

Figure 2 showed the protein profile of unripe, boiled, and fried nila baby fish. The amount of detected protein bands of unripe nila baby fish were 16 protein bands with molecular weight 10 kDa, 11 kDa, 12 kDa, 15 Da, 18 kDa, 27 kDa, 34 kDa, 40 kDa, 46 kDa, 65 kDa, 80 kDa, 99 kDa, 108 kDa, 119 kDa, 250 kDa, and more than 250 kDa. The boiled nila baby fish resulted 26 protein bands with molecular weight 10 kDa, 11 kDa, 14 kDa, 16 kDa, 27 kDa, 32 kDa, 38 kDa, 44 kDa, 59 kDa, 65 kDa, 69 kDa, 72 kDa, 84 kDa, 88 kDa, 93 kDa, 99 kDa, 102 kDa, 108 kDa, 113 kDa, 119 kDa, 125 kDa, 132 kDa, 138 kDa, 145 kDa, 250 kDa, and more than 250 kDa. The amount of detected protein bands of fried nila baby fish were 16 protein bands with molecular weight 10 kDa, 13 kDa, 15 kDa, 32 kDa, 44 kDa, 56 kDa, 65 kDa, 75 kDa 84 kDa, 99 kDa, 113 kDa, 119 kDa, 125 kDa, 132 kDa, 250 kDa and more than 250 kDa. The protein band amount of boiled nila baby fish was bigger than unripe and fried nila baby fish.

**Table 2.** Protein concentration of nila baby fish extract

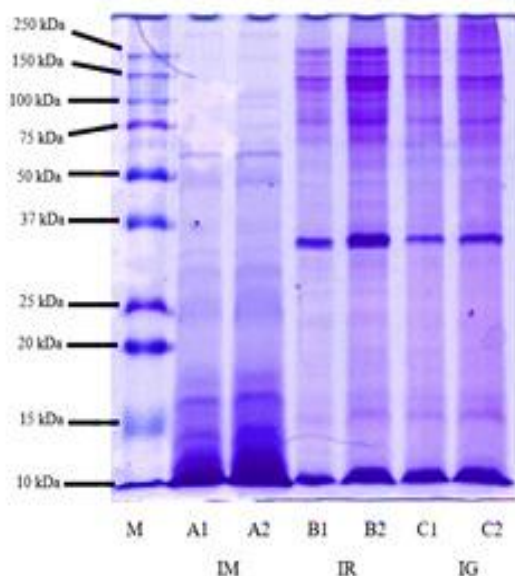
No.	Sample	Concentration (mg/L)
1	Unripe	1963.45
2	Boiled	699.82
3	Fried	604.79

#### SDS-PAGE

The protein bands of nila baby fish which resistant of boiling process were 10 kDa, 11 kDa, 27 kDa, 99 kDa, 108 kDa, 250 kDa and more than 250 kDa, whereas the protein bands of nila baby fish which resistant of frying process were 10 kDa, 65 kDa, 99 kDa, 250 kDa and more than 250 kDa. The protein bands of nila baby fish which resistant

of boiling and frying process were 10 kDa, 99 kDa, 250 kDa and more than 250 kDa.

Heating process caused changing of protein solubility, thus affecting the amount and type of extracted protein in process of protein extraction (Palupi, *et al* 2015)



**Figure 2.** SDS-PAGE marker result (M) of nila baby fish unripe (IM), boiled (IR), and fried (IG)

### Immunoblotting

Immunoblotting is a standard method to detect an allergen or allergy from food using respondent serum. The chosen respondent was a respondent who has allergic to fish, seafood, or bug according to interview result. The interview of 20 respondents resulted that 8 respondents expressed allergy symptom while 12 respondents didn't express allergy symptom. Table 3 showed respondent data whose allergy symptoms, that would be taken their serum for immunoblotting test of allergen protein extract from nila baby fish. Immunoblotting test result on Figure 4 indicated that 8 respondents possessed specific IgE which could bind to allergen protein in unripe, boiled, and fried nila baby fish. It was expressed by forming brown band at nitrocellulose membrane. The brown band formed because of reaction between anti IgE human antibody labeled HRP (Horseradish Peroxidase) enzyme and DAB (3,3-Diaminobenzidine) substrate.

Respondent (1), (2), (3), (5), (6), (7) and (8) had specific IgE that could bind to allergen protein in boiled and fried nila baby fish, whereas the mentioned respondents didn't

have specific IgE that could bind to allergen protein in unripe nila baby fish. Respondent (4) had specific IgE that could bind to allergen protein in unripe nila baby fish but didn't have specific IgE that could bind to allergen protein in boiled and fried nila baby fish. It is showed that 7 respondents from 8 respondents positively had allergic to boiled and fried nila baby fish but had not allergic to unripe nila baby fish, while only one respondent had allergic to unripe nila baby fish. The result indicated that the processing food by boiling and frying triggered the allergenicity increasing of nila baby fish.

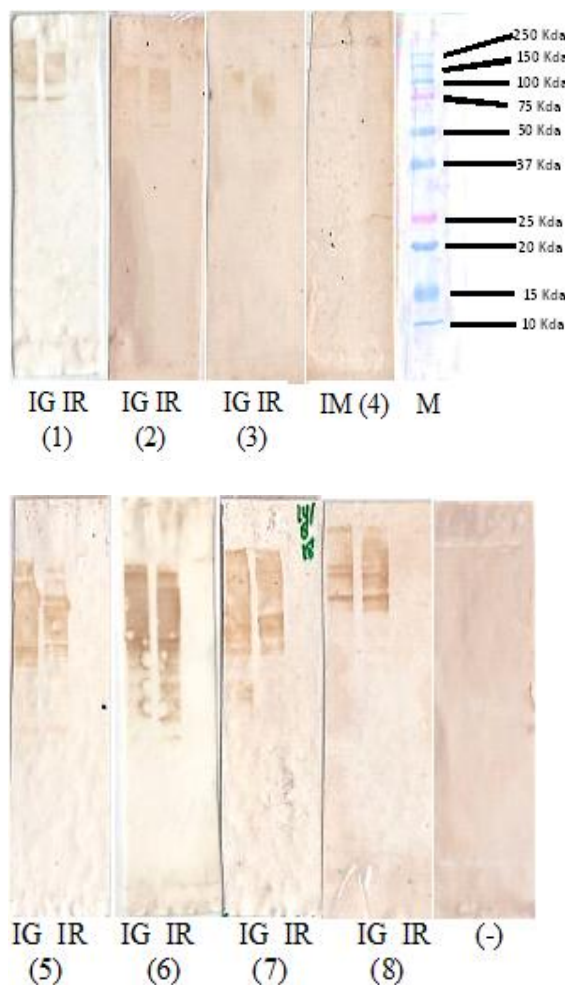
According to research by Toda *et al.*, (2014) exposed that respondents more allergic to processing food product than unripe food, e.g. extract of fried peanut linked IgE tighter than unripe peanut. The increasing of allergenicity caused by boiling and frying process which was suspected of related to tridimensional structure transformation of protein, so the epitope that formerly closed and unreactive with specific IgE antibody turned it open and reactive. The food processing product could string out the amount rise of protein epitope that reacted to specific IgE antibody in respondent serum (Boye and Godefroy, 2010). Allergenicity of allergen protein could influence on its owned epitope (Shreffler *et al.*, 2004). Link between IgE and allergen commonly happened if allergen protein contained more than one epitope (Nowak-wegrzyn, 2003).

Molecular weight of allergen protein of boiled nila baby fish that bound to IgE antibody's respondent (1) were 95 kDa, 115 kDa, 145 kDa 250 kDa and more than 250 kDa, whereas IgE antibody's respondent (1) could bind to allergen protein of fried nila baby fish had molecular weight 93 kDa, 115 kDa, 145 kDa, 250 kDa and more than 250 kDa. IgE antibody's respondent (2) that linked with allergen protein of boiled nila baby fish had molecular weight 84 kDa, 99 kDa and more than 250 kDa, while that linked with allergen protein of fried nila baby fish had molecular weight 93 kDa, 133 kDa, 250 kDa and more than 250 kDa.

Respondent (3) had IgE antibody that bound to allergen protein of boiled nila baby fish had molecular weight 127 kDa and more than 250 kDa, whereas those linked with allergen protein of fried nila baby fish had

molecular weight 127 kDa, 144 kDa, 250 kDa dan lebih dari 250 kDa.

IgE antibody's respondent (4) who only could bind to allergen protein of unripe nila baby fish had molecular weight 31 kDa. IgE antibody's respondent (5) could linked with allergen protein of boiled nila baby fish had molecular weight 86 kDa, 91 kDa, 96 kDa, 128 kDa, 250 kDa and more than 250 kDa. Molecular weight of allergen protein of fried nila baby fish that bound to IgE antibody's respondent (5) were 77 kDa, 81 kDa, 86 kDa, 91 kDa, 250 kDa and more than 250 kDa.



**Figure 4.** The immunoblotting result of 8 respondents towards nila baby fish

Respondent (6) had IgE antibody that bound to allergen protein of boiled nila baby fish had molecular weight 35 kDa, 52 kDa, 55 kDa, 65 kDa, 72 kDa, 81 kDa, 90 kDa, 101 kDa, 107 kDa, 120 kDa, 134 kDa, 250 kDa, and more than 250 kDa, while those linked with allergen protein of fried nila baby fish had

molecular weight 65 kDa, 76 kDa, 96 kDa, 101 kDa, 113 kDa, 127 kDa, 134 kDa, 142 kDa, 150 kDa and more than 250 kDa. Respondent (6) assumed had the strongest binding between IgE and allergen protein, thus resulted the most allergen protein among others.

Molecular weight of allergen protein of boiled nila baby fish that bound to IgE antibody's respondent (7) were 69 kDa, 80 kDa, 94 kDa, 110 kDa, 116 kDa, 250 kDa and more than 250 kDa, whereas those linked with allergen protein of fried nila baby fish had molecular weight 43 kDa, 47 kDa, 67 kDa, 74 kDa, 83 kDa and more than 250 kDa.

IgE antibody's respondent (8) could bind to allergen protein of boiled nila baby fish had molecular weight 91 kDa, 105 kDa, 110 kDa, 116 kDa, 128 kDa, 134 kDa, 222 kDa and more than 250 kDa, while those linked with allergen protein of fried nila baby fish had molecular weight 95 kDa, 116 kDa, 121 kDa and more than 250 kDa.

The difference of molecular weight in each respondent showed that respondent 4 only sensitive to unripe nila baby fish, meanwhile respondent (1), (2), (3), (5), (6), (7) and (8) only sensitive to processed nila baby fish. The negative respondent towards nila baby fish showed to normal person that happened unbinding between IgE and allergen protein, so there wouldn't result a brown band.

Allergen come from animal (include fish and shell) commonly possessed a high thermostability and so the allergenicity of the protein would not disappear but moreover increasing (Boye & Godefroy, 2010). Heating process to nila baby fish suspected would change the protein conformation include the protein epitope, so that caused the increasing of protein allergenicity character of nila baby fish.

In other study, *O. mossambicus* was identified had allergen protein with molecular weight 17 kDa till 114 kDa and major protein at 32 kDa. The mentioned protein named Ore m4 implied in tropomyosin group (Liu, *et al.*, 2012). Allergen protein in respondent (4) with molecular weight 31 kDa approached molecular weight of *O. mossambicus* at 32 kDa (Ore m4). The molecular weight 31 kDa suspected of allergen protein in tropomyosin group.

**Table 3.** The quisioner result of allergy sufferer respondents

Code of Respondent	Gender/Age	Allergy Trigger	Appeared Symptom	Time for Appearing Symptom
1	P/21 yo	Shrimp, crab	Pruritis	Hours
2	P/22 yo	Seafood	Pruritis	Hours
3	P/20 yo	Fish, Shrimp	Pruritis	Hours
4	L/22 yo	Shrimp	Pruritis	Hours
5	P/31 yo	Grasshopper, Beef	Pruritis	Days
6	P/ 23 yo	Grasshopper, Beef, and Goat meat	Pruritis and stomachache	Hours
7	P/41 yo	Grasshopper, Beef	Pruritis	Hours
8	L/23 yo	and black Crab Shrimp, Grasshopper, and Bee	Pruritis, dizzy,vomit and diarrhea	Days

**Table 4.** Molecular weight of nila baby fish's allergen protein

Resp.	Sample	Molecular weight of protein band (kDa) number-												
		1	2	3	4	5	6	7	8	9	10	11	12	13
(1)	IG	>250	250	145	115	93								
	IR	>250	250	145	115	95								
(2)	IG	>250	250	133	93									
	IR	>250	99	84										
(3)	IG	>250	250	144	127									
	IR	>250	127											
(4)	IM	31												
(5)	IG	>250	250	91	86	81	77							
	IR	>250	250	128	96	91	86							
(6)	IG	>250	150	142	134	127	113	101	96	76	65			
	IR	>250	250	134	120	107	101	90	81	72	65	55	52	35
(7)	IG	>250	83	74	67	47	43							
	IR	>250	250	116	110	94	80	69						
(8)	IG	>250	121	116	95									
	IR	>250	222	134	128	116	110	105	91					

Information: Resp.: Respondent, IG: Fried nila Baby fish, IR: Boiled nila Baby fish, IM: Unripe nila Baby fish

#### 4. CONCLUSION

Type of nila baby fish used in this study was *Oreochromis niloticus*. Proximate characteristic of unripe nila baby fish changed through process of boiling and frying. Also, allergen protein profile of nila baby fish was influenced by boiling and frying process. The amount of protein bands of boiled nila baby fish were more than unripe and fried nila baby fish.

Nila baby fish supposed contained allergen protein which resistant of heating process with molecular weight 10 kDa, 99 kDa, 250 kDa and more than 250 kDa. The process of boiling and frying to nila baby fish would increase the allergenicity character of nila baby fish.

#### REFERENCES

- AOAC. 1990. AOAC: Official Methods of Analysis (Volume 1). (K. Helrich, Ed.) (15th ed., Vol. 1). USA: Association of Official Analytical Chemists.
- Asia N, Suparmi, Sumarto. 2015. Studi Penerimaan Konsumen Terhadap fried Baby Nila (*Oreochromis niloticus*) dengan ukuran berbeda. Universitas Riau.
- Bollag D, Edelstein S. 1991. Protein Method. New York: Willey-Liss.
- Boye JI, Godefroy SB. 2010. Allergen Management in the Food Industry. John Wiley & Sons, Inc., Hoboken, NJ.
- Cianferoni A, Spergel JM. 2009. Food allergy:

- review, classification and diagnosis. *Allergology International*. 58(4): 457–466.
- Darwisito S, Junior MZ, Sjafei DS, Manalu W, Sudrajat AO. 2008. Pemberian pakan mengandung vitamin e dan minyak ikan pada induk memperbaiki kualitas telur dan larva ikan nila. *Jurnal Akuakultural Indonesia*. 7(1): 1-10.
- Ebo DG, Kuehn A, Bridts CH, Hilger C, Hentges F, Stevens WJ. 2010. Monosensitivity to pangasius and tilapia caused by allergens other than parvalbumin. *Journal of Investigational Allergology & Clinical Immunology*. 20(1): 84–88.
- [EMA] European Medicines Agency (EMA/CHMP)BWP/304831/2007). The Guideline on Allergen Products: Production and Quality Issues.
- Gupta RS, Dyer AA, Jain N, Greenhawt MJ. 2013. Childhood for allergies : current diagnosis, treatment and management strategies. *Mayo Clinic Proceedings*. 88(5): 512-526
- Hashimoto K, Watabe S, Kono M, Shiro K. 1979. Muscle protein composition of Sardine and Mackerel. *Bulletin of the Japanese Society of Scientific Fisheries*. 45(11): 1435-1441.
- Justi K, Hayashi C, Visentainer J, de Souza N, Matsushita M. 2003. The influence of feed supply time on the fatty acid profile of Nile tilapia (*Oreochromis niloticus*) fed on a diet enriched with n-3 fatty acids. *Food Chemistry*. 80(4): 489–493.
- Laemmli SDS PAGE Fanglian He Carnegie Institution at Stanford. 1970. 1: 3-6.
- Liu R, Holck AL, Yang E, Liu C, Xue W. 2013. Tropomyosin from tilapia (*Oreochromis mossambicus*) as an allergen. *Clinical & Experimental Allergy*. 43(3): 365–377.
- Liu R, Yang E, Liu C, Xue W. 2011. Tilapia (*Oreochromis mossambicus*) allergens characterized by ELISA, SDS-PAGE, 2D gels, Western blotting and MALDI-TOF mass spectrometry. *International Journal of Food Sciences and Nutrition*. 63(3): 259–266.
- Misnan R, Murad S, Arip M, Mohamed J. 2005. Characterization of Immunoglobulin E-Binding Proteins (IgE) of *Scomberomorus commerson* Lacepede. *Jurnal Sains Kesihatan Malaysia*. 3(2); 79-87.
- Misnan R, Murad S, Jones M, Taylor G, Rahman D, Arip M, Mohamed J. 2008. Identification of the major allergens of Indian scad (*Decapterus russelli*). *Asian Pacific Journal of Allergy and Immunology*. 26(4): 191-198.
- Nowak-wegrzyn A. 2003. Future approaches to food allergy. *Pediatrics*. 111: 1672-1680.
- Nowak-Wegrzyn A, Fiocchi A. 2009. Rare, medium, or well done? The effect of heating and food matrix on food protein allergenicity. *Current Opinion in Allergy and Clinical Immunology*. 9(3): 234–237.
- Ogunji JO, Wirth M, Rennert B. 2005. Assessing the dietary amino acid requirements of tilapia. *Oreochromis niloticus* fingerlings. 1988: 1–9.
- Saguy IS, Pinthus EJ. 1995. Oil uptake during deep-fat frying: factors and mechanism. *Food Tech*. 4: 142-145.
- Shreffler WG, Beyer K, Chu TT. 2004. Microarray immunoassay: association of clinical history, in vitro IgE function, and heterogeneity of allergenic peanut epitopes. *J Allergy Clin Immunology*. 113: 776–782.
- Soekendarsi E, Hasyim Z, Hasan S. 2016. Perbandingan Kandungan Gizi Ikan Nila *Oreochromis niloticus* asal Danau Mawang Kabupaten Gowa dan Danau Universitas Hasanuddin Kota Makassar. 1: 39–46.
- Sundari D, Almasyhuri, Lamid A. 2015. Pengaruh proses pemasakan terhadap protein. *Media Litbangkes*. 25(4): 235–242.
- Suseno R, Palupi NS, Prangdimurti E. 2016. Alergenisitas sistem glikasi isolat protein kedelai-fruktooligosakarida. *Agritech*. 36(4): 450–458.
- Toda M, Heilmann M, Ilchmann A, Vieths S. 2014. The maillard reaction and food allergies: is there a link?. *Clinical Chemistry and Laboratory Medicine*. 52(1): 61-67.
- Zakaria FAK, Ispurwanto, Rahman. 1998. Telaah sifat alergenitas protein udang putih (*Penaeus merguensis*) untuk produksi isolat alergen. *Bulletin Teknologi dan Industri Pangan*. 2(IX): 54–59.