The protein and albumin contents in some species of marine and brackishwater fish of South Sulawesi, Indonesia

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Abstract. The purpose of this study was to explore sources of fish albumin from marine and brackishwater fish species in search of alternatives to the snakehead fish albumin. The study used seven species of marine and brackishwater fish namely Indian scad (*Decapterus russelli*), Indian mackerel (*Rastrelliger kanagurta*), red fusilier (*Pterocaesio chrysozona*), Japanese threadfin bream (*Nemipterus japonicus*), eeltailed catfish (*Paraplotosus albilabris*), white snapper (*Lates calcarifer*) and milkfish (*Chanos chanos*), and a freshwater species, snakehead fish (*Channa striata*), for comparison. All fish samples were analyzed for their meat and water soluble protein, and albumin contents. For water soluble protein and albumin determination, as much as 50 g of fish meat were extracted by homogenizing with distilled water (1:4), heated in a waterbath at 50ºC for 1 h, filtered with a filter paper under reduced pressure and frozen storage until used for analysis. The protein of the meat and extract was determined following micro Kjeldahl method and the albumin content was measured using Lowry method. The results showed that the total protein content of the fish meat was between 21.32 and 27.97% (w/w), whereas the water-soluble protein content was between 16.67 and 28.78% of the total meat protein. The albumin content of fish samples ranged from 1.08 to 5.04% of the meat wet weight. The highest albumin content (5.04%) was found in the milkfish (*Chanos chanos*).
Key Words: albumin, brackishwater fish, *Channa striata*, milkfish, protein.

Introduction. The increase knowledge and awareness about benefits of consuming snakehead fish (*Channa striata*) has resulted in high demand and put the natural stock of this species under high pressure. The success of snakehead fish albumin in substituting or even replacing the expensive Human Serum Albumin (HSA) and its efficacy in raising albumin level in hypoalbumin patients have made this particular fish albumin attracting even more attention. Fish albumin is a sarcoplasmic of high water solubility. According to Masuelli (2013), albumin is a type of globular protein that is easily soluble in water and diluted saline solvents and is coagulated by heat.

Albumin plays important roles in the processes of metabolism in the fish body. Albumin functions to maintain blood osmotic pressure, helps transport metabolites (fatty acids, hormones, bilirubin), and filters fluids in body tissues (De Smet et al 1998; Baker 2002; Andreeva 2011; Kovyrshina & Rudneva 2012). The benefit of consuming snakehead fish has long been realized by ancestors of Asian communities. In the past, the juice of steamed snakehead fish was given to post-delivering women because it was believed capable of accelerating the wound healing process and recovery (Mohd & Abdul Manan 2012). The snakehead fish extract was also used to treat wounds due to its role in plastic process of new cell tissue in the body (Putri et al 2016). However, the snake-like appearance and the fishy smell of the snakehead fish have caused many people unwilling to consume this fish as such. Nonetheless, the advanced of science and technology has made it possible for people to experience the benefits of the snakehead fish through various innovative products in the form of liquid, powder or capsules.

The increase use of the snakehead fish albumin has inducing fears on the sustainability of natural stock of the snakehead fish. To balance the high demand, various efforts to cultivate this species of fish have been taken but with a very limited success. Unfortunately, there exist believes that wild fish is much better as compared to the cultured one. Research to determine chemical composition and albumin levels of wild and cultivated snakehead fish has been carried out by some researchers. Chasanah et al (2015) reported that the total protein content of wild and cultivated snakehead fish was similar, but the albumin content was higher in the wild fish. These authors also found that amino acid profiles of wild and cultured snakehead fish were similar, but they differed in the quantity of individual amino acid. They stated further that amino acids play a role in wound healing. Considering the high diversity of fish species in Indonesia as well as the already heavy pressure on the snakehead fish natural stock, the aim of the present study was to explore the marine and brackishwater fish species in search of alternative sources of albumin other than the snakehead fish.

Material and Method

Materials and chemicals. The types of fish used in this study consisted of seawater fish and brackishwater fish. Four species of seawater fish taken were the Indian scad (*Decapterus russelli*), Indian mackerel (*Rastrelliger kanagurta*), red fusilier (*Pterocaesio chrysozona*) and Japanese threadfin bream (*Nemipterus japonicus*). The brackishwater fish consisted of three species namely eeltailed catfish (*Paraplotosus albilabris*), white snapper (*Lates calcarifer*) and milkfish (*Chanos chanos*). The seawater fish were obtained from Paotere Fish Landing Center in Makassar, while the brackishwater fish were obtained from brackishwater ponds in Barru and Pangkap Districts. The snakehead fish (*Channa striata*) was obtained from wet market in Makasar. All samples were immediately ice-cold in a coolbox to maintain freshness. Subsequently the samples were brought to the laboratory and frozen after cleaning. Other materials used were bovine serum albumin (BSA) as a standard albumin, distilled water, H_2SO_4, NaOH, HCl, H_3BO_3, methyl red indicator, $NaHCO_3$, Na-K tartaric 1%, $CuSO_4$, phenol reagent, filter paper. This study was conducted from June to August 2019.

Sample preparation and extraction. Prior to extraction of soluble fraction, the fish sample was thoroughly washed under running tap water, filleted and bone and skin separated. The fillet was then pre-homogenized using a commercial blender, transferred into zipped high density polyethylene (HDPE) plastic bags and frozen-storage until used for further analyses. Extraction of soluble protein, including albumin, was performed by homogenizing 50 g of the pre-homogenized fish meat with 4 portion of distilled water (1:4 w/v) using a laboratory homogenizer (WiseTis® HG-15D Homogenizer). Then the sample was subsequently heated in a waterbath at 50°C for 1 h. The sample was then cooled and filtered using W*hatman* no. 1 filter paper to separate the filtrate.

Determination of total protein of fish meat and extract. Total protein of meat and meat extract was determined following the micro Kjeldahl method (Muchtadi 1989). Briefly, an approximately 1 g of fish meat (2 mL in the case of fish extract) was transferred into a 100 mL Kjeldahl flask and 10 mL of concentrated sulfuric acid was added into the flask and then shaken gently. Two grams of selenium mixture catalyst were added to assist the destruction process. The sample destruction was performed at ± 400°C until the solution turned clear. After cooling, the digested sample was diluted to 100 mL with distilled water and thoroughly mixed. Five milliliter of the mixture were transferred into a distillation flask and 100 mL of distilled water and 10 mL of 10% sodium hydroxide were added. Distillation was carried out and the distillate was collected in an Erlenmeyer flask containing 2% of boric acid to which 3-4 drops of methyl red indicator have been added. Titration was performed using 0.1 N H_2SO_4 until a light pink color of the distillate appeared. The volume of H_2SO_4 used was recorded and the protein content was calculated as follows:

$$\text{Protein content of meat (\%)} = \frac{\text{Fd x V x N x 14 x 6.25}}{\text{weight of sample (g)}} \text{ x 100}$$

$$\text{Protein content of extract (\%)} = \frac{\text{Ev x V x N x 14 x 6.25}}{\text{voulme of sample (mL)}} \text{ x 100}$$

where: V = volume of H_2SO_4 used for sample titration;
N = normality of H_2SO_4 solution;
Fd = dilution factor;
Ev = total volume of extract.

Albumin content determination. The albumin levels in fish extract were determined following the Lowry method as described by Muchtadi (1989):

1. Reagents preparation
a. Reagent 1, sodium carbonate 2% in 0.1 N NaOH solution;
b. Reagent 2, 0.5% copper sulfate in Na-K tartrat 1% solution (freshly prepared prior to use);
c. Reagent 3, mix 50 mL of reagent 1 with 1 mL of reagent 2 (freshly prepared prior to use);
d. Reagent 4 (Folinciocalteau reagent or phenol reagent), mix 100 g of sodium tungstate, 25 g of sodium molybdate, 500 mL of distilled water, 50 mL of 85% phosphoric acid and 100 mL of concentrated HCl in a 2 L flask. Carefully refluxe the mixture for 10 h using a condenser. After coolling, add 150 g lithium sulfate, 50 mL distilled water and a few drops of bromine (Br_2). Boiling of the mixture was continued for another 10 min without a condenser to eliminate excess of bromine. After cooling the volume of the solution was adjusted to 100 mL and filtered, if necessary. If the solution shows a greenish color, then the boiling process must be continued. The result is a stock reagent which must be dissolved in distilled water (1:1) before use;
e. Standard albumin solution, bovine serum albumin 0.25 mg mL^{-1}.

2. *Preparation of standard curve.* Transfer into in test tubes: 0 (blank); 0.1; 0.2; 0.4; 0.6; 0.8; and 1.0 mL of standard albumin. Adjust the total volume to 4 mL with distilled water and then add 5.5 mL of reagent 3, mix evenly and leave for 10-15 min at room temperature. Add 0.5 mL of reagent 4 into each tube, and again mix evenly. Allow it to stand for about 30 min until the blue color is formed. Measure the absorbance at a wavelength of 650 nm using a UV-Vis spectrophotometer (PerkinElmer Lambda 35) and create the standard curve.

3. *Measurement of albumin content in sample.* As much as 1.5 mL of the fish extract was transferred into a clean test tube and then treated as for the standard albumin. The optical density of the sample was then measured at 650 nm using a UV-Vis spectrophotometer. The albumin content was then calculated using a linear equation generated from the standard albumin.

Data analysis. All data obtained were subjected to one-way analysis of variance (ANOVA) using SPSS 17 statistical software. Significant difference was determined at 95% level of probability ($\alpha = 0.05$). Where ANOVA indicated the presence of a significant difference, Tukey test was employed to determine different values of the respected parameters.

Results and Discussion

Protein contents of fish meat. The total protein contents in the fish meat varied between 21.32% in white snapper (*L. calcalifer*) and 27.97% in Indian scad (*D. russelli*). Five out of seven species of marine and brackishwater fish analyzed displayed protein content similar to that of the snakehead fish (*C. striata)*, a fresh water species (Figure 1). Majority of the fish analyzed has protein content at around 21-23% with a narrow range of variation. The highest protein content was found in two fish species of Scombroid family namely the Indian Scad (*D. russelli*) (27.97%) followed by the Indian mackerel (*R. kanagurta*) (26.07%). ANOVA results showed that variations ($p < 0.05$) existed in the total protein content of meat among the fish species studied. Tukey test showed that the Indian scad and the Indian mackerel contained higher protein ($p < 0.05$) as compared to the rest of the species.

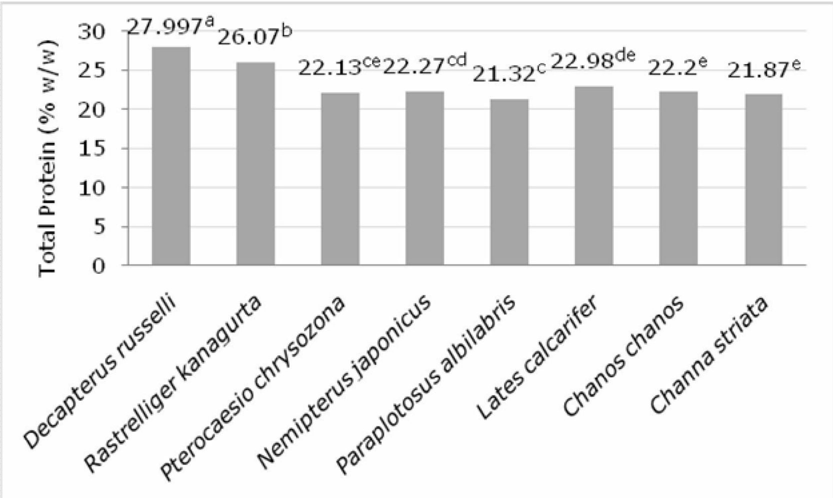

Figure 1. Total protein contents of marine, brackishwater and freshwater fish studied (Note: different superscripts indicate significant differences).

The protein content of fish varies among species and is influenced by internal and external factors. These factors include eating habits, species, sex, size, level of maturity (age), season, reproduction cycle, environmental conditions, geographical location, food abundance, quality of feed, and the digestible energy content in the feed (Irianto & Susilo 2007; Mol et al 2008; Ayas & Ozogul 2011; Fawole et al 2013). The protein contents of fish in this study were higher than those of the swamp water fish previously reported by Susilowati et al (2016). Susilowati et al (2016) found that the protein content of swamp water fish of Merauke namely stripped snakehead (*C. striata*) was 18.31%, Nile tilapia (*Oreochromis niloticus*) was 17.26%, gray mullet (*Liza tade*) was 18.90%, catfish (*Clarias batrachus*) was 18.43%, *L. calcarifer* was 17.11%, and climbing perch (*Anabas testudineus*) was 18.92%. According to Huss (1995), the protein contents in fish ranged from 16 to 22% of the total muscle mass. Variation of the protein content of fish inhabiting different ecosystems was noticed by Ravichandran et al (2011). They reported that marine fish showed much variation in their protein contents (17.04-28.01%) as compared to those of freshwater fish (19.72-22.84%) and of brackishwater fish (18-19%). Dörücü (2000) argued that protein content within a fish population tends to be relatively constant, but starvation and gonad development cause the depletion of muscle protein, especially in non-fatty fish species. Dawson & Grimm (1980) reported the breakdown of body protein during development of the ovaries in female plaice.

Soluble protein. Based on the total protein of the meat, the proportion of total soluble protein of the fish extracts was between 16.67 and 28.78%. Except for the Indian scad (*D. russelli*) which showed the lowest soluble protein (16.67%), all fish studied displayed the content of soluble protein higher than 23% (Figure 2). The Japanese threadfin bream (*N. japonicus*) and eeltailed catfish (*P. albilabris*) were found to have the highest total soluble protein. ANOVA showed the presence of significant difference ($p < 0.05$) in the total soluble protein in extracts of fish studied. Tukey test indicated that the Japanese threadfin bream and eeltailed catfish contained a similar total soluble protein ($p > 0.05$) but significantly higher ($p < 0.05$) than those of other species studied.

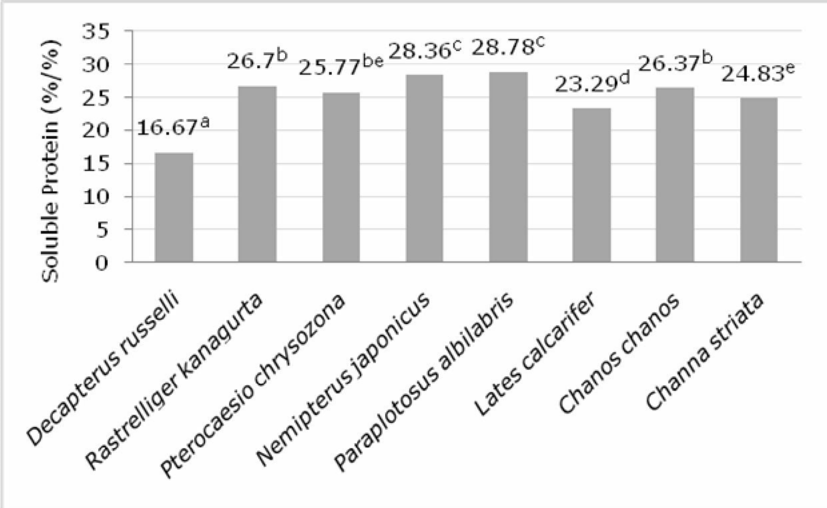

Figure 2. Total soluble protein (% of meat protein) of fish used in the study (Note: different superscripts indicate significant differences).

A slightly different picture was observed when considering the total soluble protein based on the weight of the fish meat. The Indian mackerel (*R. kanagurta*) was found to have the highest ($p < 0.05$) total soluble protein (6.96% of the meat weight, w/w) compared to other species. Based on the meat weight, the total soluble protein in this study was between 4.66 and 6.96%, the lowest being in the Indian scad (*D. russelli*) (Figure 3). Figures 2 and 3 also showed that, in general, the amount of soluble protein of fish shared a similar pattern when calculated on the basis of the total meat protein content and the meat wet weight.

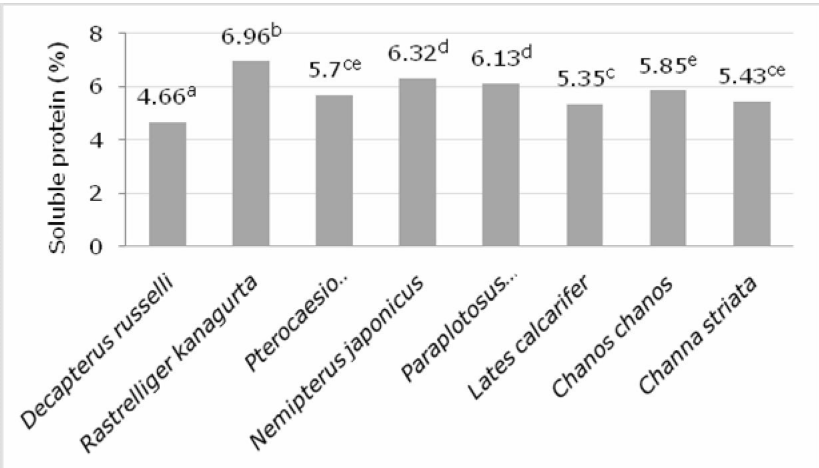

Figure 3. Total soluble protein (% of meat weight) of fish used in the study (Note: different superscripts indicate significant differences).

The present results were higher than the values reported by Rahmawati (2014) for soluble protein content of cultured Pangas catfish (*Pangasius pangasius*) which was between 1.7 and 3.4%, and by Mustafa et al (2012) for snakehead fish (3.34%). In the snakehead fish powder, the water soluble protein was found to be between 2.34 and 10.88%, depending on the method of extraction used (Fatmawati & Mardiana 2014).

Albumin content. The albumin content of the fish studied ranged from 1.08 to 5.04% of the meat weight (w/w). Statistical analysis (ANOVA) showed that there were significant variations ($p < 0.05$) in the albumin content of the fish studied. Among the fish analyzed, pond raised milkfish (*C. chanos*) was found to have the highest ($p < 0.05$) albumin content while the lowest ($p < 0.05$) was shown by two demersal fish species of the marine water origin, the red fusilier (*C. chrysozona*) and the eeltailed catfish (*P.*

albilabris) (Figure 4). The Indian scad (*D. russelli*), Japanese threadfin bream (*N. japonicas*) and white snapper (*L. calcalifer*) displayed a similar level ($p > 0.05$) of the albumin content. A freshwater fish, *C. striata*, was taken as a reference fish for albumin and showed that its albumin content was significantly lower than that of the milkfish, was similar to that of the Indian mackerel, but was significantly higher than the albumin content of five other species used in this study.

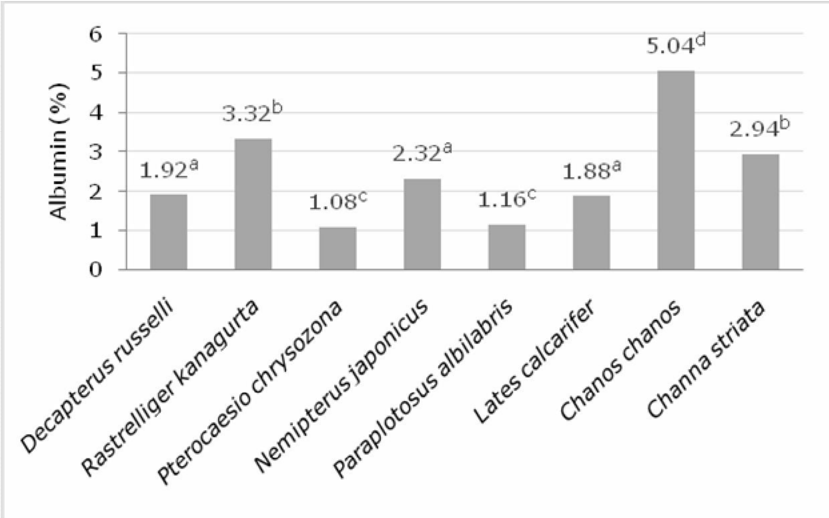

Figure 4. Total albumin content (% of meat weight) of fish used in the study (Note: different superscripts indicate significant differences).

Figure 5 showed that the proportion of albumin in the protein of fish meat was between 4.87 and 22.71%, and its value varied ($p < 0.05$) depending on species. The highest proportion was shown by the milk fish, while majority of the fish studied has less than 10% albumin in their total meat protein, being lowest in the red fusilier and eeltailed catfish (±5%).

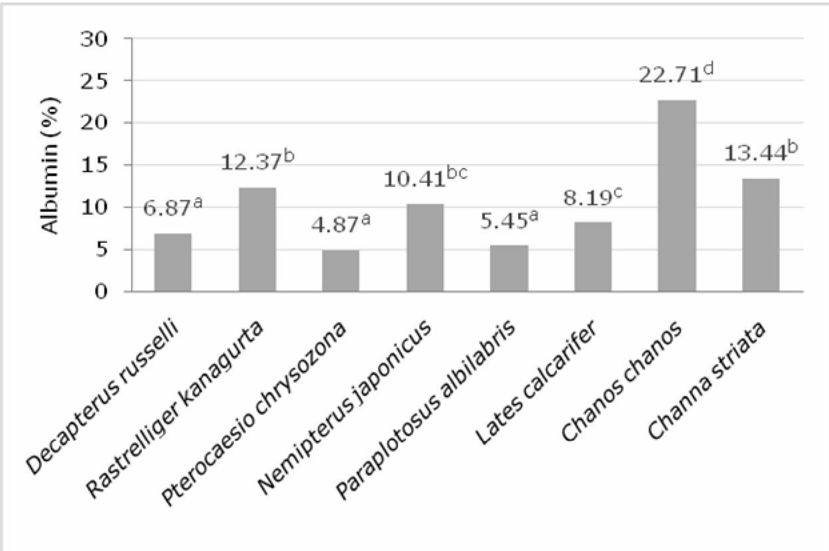

Figure 5. Total albumin content (% of meat protein) of fish used in the study (Note: different superscripts indicate significant differences).

A similar pattern was also noticed for the proportions of albumin in the soluble protein (Figure 6). The albumin fraction in the soluble protein varied significantly ($p < 0.05$) among the fish species studied. In milkfish the soluble protein was almost exclusively made up by albumin (86.15%). In the snakehead fish, over a half of its soluble protein was constituted by the albumin fraction (54.14%) which was lower than the value

(64.61%) reported by Mustafa et al (2012). The rest of the fish species showed that less than 50% of their total soluble protein was made up by the albumin. In the red fusilier (*C. chrysozona*) and Japanese threadfin bream (*N. japonicus*), albumin constituted only about 19% of the total soluble protein. Lower proportion of albumin in the fish extract indicate that majority of the extracted nitrogen is composed of non-protein nitrogen, such as trimethylamine and its oxide derivative, nucleotide, peptide and free amino acids, among others.

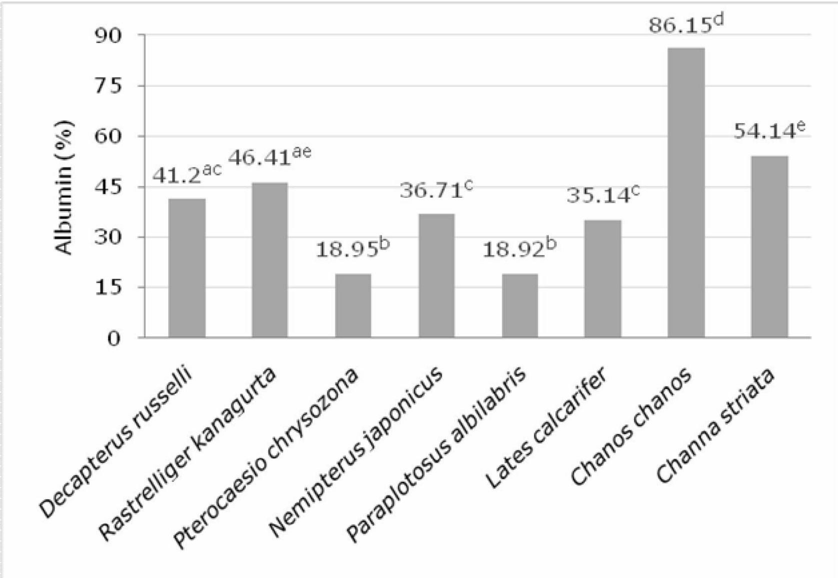

Figure 6. Proportion (%) of albumin in the total soluble protein (Note: different superscripts indicate significant differences).

The albumin contents of fish in this study were higher than those reported by Susilowati et al (2016). These authors found that the albumin concentration of *C. striata* was 1.39 g/100 g, and of barramundi it was 0.57 g/100g. Previously, it has been reported that the albumin content of *C. striata* from West Java was 1.07 g/100g (Susilowati et al 2015), *C. striata* from Central and East Java were 0.76 g/100g and 0.91 g/100g, respectively (Chasanah et al 2015). Variation of the albumin concentration depends on fish species, size, diet consumption rate, dietary availability and digestibility rate (Wiramiharja et al 2007). On the other hand, chemical composition of fish depends on the species, age, sex, habitat, and environment condition (Irianto & Susilo 2007).

The scientific data on albumin concentration in teleost fish vary greatly among species as well as for the same species. Concentration of albumin (albumin-like proteins) in fish plasma of teleosts can vary from 10 to 50%, while in terrestrial vertebrates albumin accounts for more than 50% of the total serum proteins (McDonald & Milligan 1992). In few fish species, specific properties of albumin were characterized and identified as so-called albumin-like proteins (Hasnain et al 2004). Albumin-like protein was found in different bony fish and lamprey, while it was absent in some species of elasmobranchs (Metcalf & Gemmell 2005).

The amount of albumin extracted and recovered from fish meat is affected by extraction temperature, fish meat quality, size reduction of meat and extraction solvents (Suprayitno 2003). As a polar compound, albumin requires polar solvents to dissolv it (Winarno 2004; Yuniarti et al 2013).The temperature used during extraction facilitates the opening of albumin structure and so the use of a proper temperature on protein is needed (Stryer 1981; Slavik 1982; Arakawa et al 1991; Wicker et al 1986; Arntfield et al 1989). Nugroho (2013) explained that heating affected permeability of cell wall and hence accelerating the release of cell plasma. Heating at high temperature will cause protein to coagulate and therefore difficult to be extracted (de Man 1997).

Conclusions. All fish samples examined contained high level of protein in their meat. Of the marine and brackishwater fish species analyzed, the albumin content was highest in the milkfish (*C. chanos*), which is nearly double than the albumin content of the snakehead fish (*C. striata*), a very popular source of fish albumin of freshwater origin. Contradicting to the very high protein content in its meat, the Indian scad (*D. russelli*) is inferior to most of the species in this study in terms of the albumin content. Among the marine fish species analyzed, the Indian mackerel (*R. kanagurta*) displayed the highest albumin, which is comparable to the albumin content of the snakehead fish. Therefore, the milkfish and the Indian mackerel are highly potential as alternative to the snakehead fish as sources of fish albumin.

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