# Characteristics of Nano Concentrate Protein Dispersion of Snakehead Fish (Channa striata) by Addition of Tween 80

*by* Zaenal Zaenal

# Characteristics of Nano Concentrate Protein Dispersion of Snakehead Fish (*Channa striata*) by Addition of Tween 80

# Characteristics of Nano Concentrate Protein Dispersion of Snakehead Fish (*Channa striata*) by Addition of Tween 80

**M Mahendradatta[1], YL Imran[2], Zainal[1], AB Tawali[1] and M Asfar[1]**

[1]Food Science and Technology Study Program, Department of Agricultural Technology, Faculty of Agriculture, Hasanuddin University, Makassar
[2]Master Program of Food Science and Technology, Department of Agricultural Technology, Faculty of Agriculture, Hasanuddin University, Makassar

E-mail: metamahendradatta@gmail.com

**Abstract**. This study aimed to obtain the characteristic of nanoparticles concentrate protein of snakehead fish (*Channa striata*) by addition of tween 80. The research consisted of two phases. First phase was the manufacture of nano concentrate protein dispersion from snakehead fish and second phase was the drying process of the concentrate using *freeze dryer* and characterization of physic-chemical properties of nano concentrate protein snakehead fish. The results of particle size measurement after using the sonication method and homogenization with the addition of tween 80 ranged from 331.6 to 353.0 nm. Based on the results of the *particle size analyzer* (PSA) the best value in the treatment of adding of 2% tween 80 with a polydispersity index value of 0.470, particle size distribution based on volume obtained by 2 size categories namely 101.8 nm and 449.4 nm and the average of smallest particle size ranged from 223.6 to 363.2 nm based on the results of the analysis of *electron microscope scanning* (SEM). The morphology of concentrate protein of snakehead fish based on the results of SEM analysis before the addition of tween 80 has abstract molecule, compact molecular structure (the molecules stick together) and the size was still in micrometer scale. While the addition of tween 80 showed that the particle structure of granules was not compact so the possibility of being broken down was still very high and in nanometer scale particle size. The physical characteristics of nano concentrate protein of snakehead fish before adding tween 80 included bulk density (0.52 g/ml), emulsion capacity (14.72%), water absorption (2.49 ml/g) and oil absorption (2.16 ml/g). After adding tween 80 the physical characteristics included density bulk (0.46 g/ml), emulsion capacity (48.15%), water absorption (0.42 ml/g) and oil absorption (2.00 ml/g). The chemical characteristics of nano concentrate protein of snakehead fish before the addition of tween 80 included, water (6.98%), ash (6.03%), fat (2.27%) and protein (85.10%). After adding tween 80 the chemical characteristics were water content (5.13%), ash (4.08%), fat (6.48%) and protein (88.28%).

## 1. Introduction

The application of nanotechnology in food sector in Indonesia will be very effective due to the abundant raw materials. One of those was snakehead fish (*Channa striata*) that has many benefits because it contained high protein, particularly albumin as much as 15-20% [11].

Snakehead fish has been processed into fish protein concentrate (SFPC) in form of powder as food supplement and as food ingredient for functional food [3]. Research on reducing of particle size of SFPC has been carried out by homogenization [6]. The result showed that the average particle size obtained with heterogeneous and unstable values was around 869.2nm - 2014µm. [11] has combined the ultra sonication and the homogenization to reduce the particle size. The best value was obtained after ultra sonication treatment for 5 minutes and homogenization at speed of 6500 rpm for 5 minutes. The particle size was 636.8 nm and not in nanoparticle size yet. These might be due to the aggregation of particles after being broken down.

To overcome this problem, a treatment was needed to prevent and reduce SFPC particle aggregation to remain stable with nanoparticle size (nm). One treatment that often used in preventing and reducing the aggregation was the use of tween 80 from the non-ionic surfactant group [8]. Non-ionic surfactants have no charge, but contain groups that have high affinity for water due to strong interactions of dipoles arising from hydrogen bonds.

Based on the description above, this research studied the effect of using tween 80 in preventing the aggregation of SPFC particle after treated by sonication and homogenization methods.

## 2. Methodology

### *2.1 Material*

The materials used were snakehead fish protein concentrate, tween 80 with concentrations of 1%, 2%, 3%, aquades corn oil, peanut oil, and chemicals such as sulfuric acid (HCl), sodium hydroxide (NaOH), buffer sodium acetate 1.0M, mercury (II ) oxide (HgO), potassium sulfate ($K_2SO_4$), sulfuric acid ($H_2SO_4$).

### *2.2 Methods*

This research has been conducted in two phases. The first phase of research was the manufacture of snakehead fish protein nano-concentrate (SFPnC). The second phase has been conducted to determine the physico-chemical of SFPnC selected from the best result of the first phase.

*2.2.1 The first phase*. The 10 g of SFPC was weighed and the aquades was added as much as 100 mL. The sonication of samples was carried out with a 405 power sonic device for 15 minutes with a frequency of 50 Hz then followed by the addition of tween 80 with a concentration of 1%, 2% and 3% (in aquadest solvents). Further, the sample was homogenized using Ultra turax T 25 basic tool with a speed of 6,500 for 5 minutes. After that, the particle size of SFPC was analyzed using Particle Size Analizer (PSA), particle size distribution based on the polidispersity index and particle morphology with scanning microscope electrone (SEM) analysis. After getting the best treatment the next phase of research was conducted.

*2.2.2 The second phase*. The SFPnC was treated using freeze dryer. Firstly the SFPnC was put into petri-dish using a measuring cup, which each dish containing as much as 20 mL. The dispersion was wrapped using plastic wrap and frozen for 24 hours at -20°C. The sample was put into the freeze dryer for 48 hours at temperature of -40°C. The SFPnC flakes was then crushed and weighed.

*2.2.3 Physico-chemical characteristic of SFPnC*. The characteristics of SFPnC were determined, including using scanning electron microscope (SEM), bulk density, emulsion capacity, water absorption and oil absorption for physical characteristic and protein content, fat content, moisture content and ash content for chemical characteristic.

## 3. Results and Discussion

*3.1 Selection of tween 80 concentration*

The first phase of this research aimed to obtain the best treatment of SFPnC after sonication – homogenization and addition of tween 80. The best treatment indicator was based on the particle size, particle size distribution based on the polydispersity index and particle morphology with scanning microscope electrone (SEM) analysis (figure 1, figure 2, figure 3). The application of tween 80 aimed to prevent aggregation after breaking process of the particle using sonication and homogenization. The concentration of tween 80 used referred to the study of [8].

The result of the first phase was displayed in figure 1, figure 2 and figure 3.

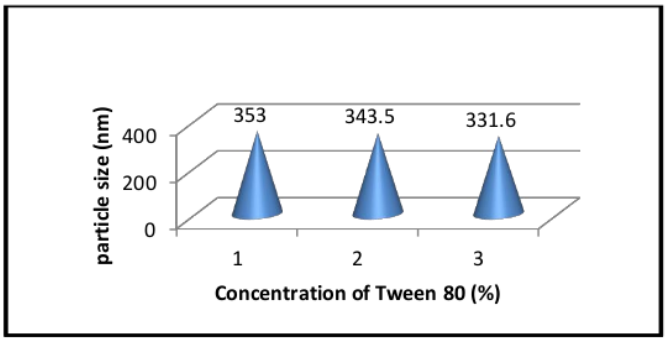


**Figure 1.** Particle size of SFPnC in various concentration of Tween 80.

The higher the concentration of adding tween 80, the smaller the particle size obtained. This indicated that tween 80 was functioning properly to reduce the occurrence of aggregation after reduction in size using the sonication method and homogenization. Allegedly when the process of breaking down the SFPnC particles, tween 80 coated the molecules so that there was no aggregation or reunification of molecules that have been broken down.

This was in accordance with the theory of interparsial films on the mechanism of emulsion formation. This theory said that the emulgator (tween 80) will be absorbed at the boundary between water and oil, so that a film layer was formed which will wrap the dispersion phase particles. With the wrapper of the particle, the effort between similar particles to join became blocked. In other words, the dispersion phase became stable [5].

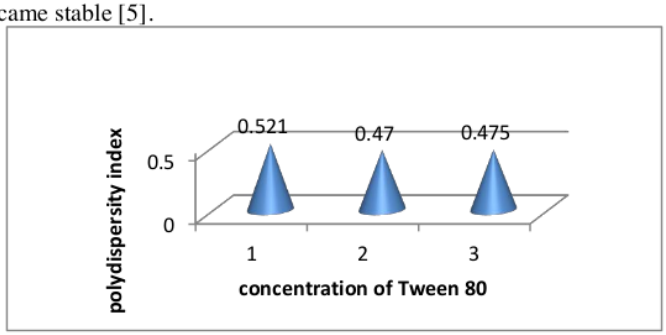


**Figure 2.** Polydispersity index of SFPC in various concentration of Tween 80.

The polydispersity index was a parameter that states the particle size distribution of a nanoparticle system [7], where the range of values from 0.1 to 0.25 shows a narrow size distribution, while a value of more than 0.7 indicates a wide distribution. The results of the analysis of the polydispersity index of

particle size of SFPnC showed that the lowest value was obtained after adding of 2 % of tween 80 by 0.470 and the highest value after adding of 1% of tween 80 which was 0.521 (figure 2). The value obtained showed the distribution of homogeneous particles because the average value obtained was <0.7.

According to [4], samples with polydispersity index values more than 0.7 have a very wide size distribution. The smaller the polydispersity index number, the more uniform the particle size because if the size difference between particles were greater than the results will affect particle characterization.

*3.2 Morphology of SFPnC particle size*

Enlargement of SFPnC nano particle size was carried out at 10,000x magnification to find out more about the particle structure formed and to know the particle size values that were read randomly by an electron microscope (SEM) scanning device. Based on the morphology particle of SFPnC in 10,000x enlargement presented in figure 3, there was a very contrasting difference from the 4 treatments. Without addition of tween 80, the SFPnC particle looked like an abstract molecule, compact molecular structure (the molecules stick together) and particle sizes vary between 1241-1543 µm. While the SFPnC particles with the addition of tween 80 looked like round, dense granules (covered by tween 80) and particle sizes vary. By the concentration of 1% and 3% of tween 80 the particle structure was more compact with the aggregate shape and had particle sizes ranging from 324.6 and 302.3 nm (by 1% concentration) and 341.6-347.5 nm (by 3% concentration).

While the treatment of 2% of tween 80, the particle structure has incompact granules so that the possibility of being broken down was still very high and has the smallest particle value of 223.6-363.2 nm. The morphology and size of SFPnC particles will affect the processed products. The particle size will affect the level of water and fat absorption. Protein in powder form with small particle size and low density absorbed and trapped more water and oil than proteins with high density [14].

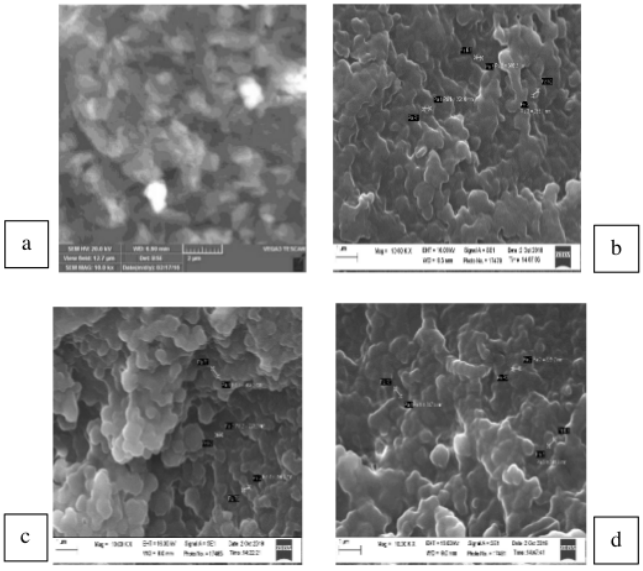


**Figure 3.** Morphology of SFPC particle size without addition of Tween 80 (a), with addition of Tween 80 (1%) (b), Tween 80 (2%) (c) and Tween 80 (3%) (d) at 10,000x magnification.

*3.3 Physical characteristics of SFPnC flour*

The physical properties of food ingredients were important in the process of designing and manufacturing food products. Most of these properties showed the changes in the chemical composition and structure of food from molecular to macroscopic level. The physical characteristics of SFPnC without and with adding of tween 80 were presented in table 1.

**Table 1.** Physical characteristic of SFPC before and after using of sonication-homogenization and tween 80

| Parameters | SFPC | SFPC nanoparticle |
|---|---|---|
| Bulk density (g/ml) | 0.520 ± 0.035 | 0.46 ± 0.021 |
| Emulsion capacity (%) | 14.717 ± 2.657 | 48.15 ± 1.282 |
| Water adsorption (ml/g) | 2.490 ± 0.010 | 0.42 ±0.010 |
| Oil adsorption (ml/g) | 2.160 ± 0.286 | 2.00 ± 0.100 |

Bulk density was determined by measuring the weight of a product needed to fill a certain volume. The density of a material indicated the density of the material in a certain volume (space). Based on the results of the T test obtained a 2-tailed significance value of 0.053 (P> 0.05) so that there were no significant differences in the two treatments. This showed that the addition of tween 80 can reduce the density value of bulk. The higher the bulk density means the smaller the volume of packaging needed. A material was stated if it had small density value, meaning that for a light weight a small volume (space) was needed [9].

Emulsion capacity was the ability of a protein solution or suspension to emulsify oil. Based on the T-test statistical test, the 2-tailed significance value was obtained at 0,000 (P> 0.05) so that the results were significantly different for the two treatments. This showed that the addition of 2% of tween 80 increased the emulsion capacity of SFPnC. The tween 80 can draw oil and water phases at once and place themselves between the two phases. It worked by forming a film or layer around the droplets of dispersed droplets and serves to prevent coalescence and separation of the dispersion liquid.

Water absorption was defined as the ability of food to hold water added and that was in the food itself during processing of food. Based on the statistical test of the T test obtained 2-tailed significance value of 0,000 (P>0.05), that resulted the significant differences in the two treatments. The result showed that the addition of 2% of tween 80 can reduce the ability of SFPnC to absorb water given. It was suspected that this was allegedly due to the fact that tween 80 was easier to bind oil molecules than water molecules. This was supported by the value of emulsion capacity of SFPnC of 48.15%. It can be concluded that tween 80 has a hydrophilic group less than the lipophilic group. Hydroxyl groups have the ability to bind water from the environment by forming hydrogen bonds.

Oil absorption was a property that can indicate the interaction of a material with oil. Based on the statistical test of the T test, the 2-tailed significance value was obtained at 0.412 (P> 0.05) so that the results did not show any significant differences in the two treatments. This was allegedly due to the types of oil used in the testing the oil that was from the group of vegetable oil. Tween 80 had soluble properties in water and ethanol but was insoluble in vegetable oil.

*3.4 Chemical characteristics of SFPnC*

The chemical characteristics measured were the content of protein, fat, moisture, and ash. The chemical composition of SFPC was presented in table 2.

**Table 2.** Chemical characteristic of SFPC with and without addition of Tween 80

| Compounds | SFPC | SFPC nano particle |
|---|---|---|
| Moisture (%) | 6.983 ± 0.455 | 5.13 ± 0.233 |
| Ash (%) | 6.03 ± 0.573 | 4.08 ± 0.044 |
| Fat (%) | 2.27 ± 0.563 | 6.483 ± 0.202 |
| Protein (%) | 85.31 ± 0.220 | 88.28 ± 0.095 |

Determination of moisture content was an important and most extensive analysis carried out in the processing and testing of food. The moisture content of the material was also related to the quality and stability of the ingredients. It needed to be known in determining the nutritional value of food. The results of the T-test statistical test obtained a 2-tailed significance value of 0,000 (P> 0.05) so that the results were significantly different in the two treatments. The addition of tween 80 decreased the moisture content of product. It was allegedly due to the formation of hydroxyl groups between the molecules of SFPnC and tween 80 when solving particle size. It said that the more number of tween 80 was used, the greater the surface area and the porous structure of the material, which will cause the drying process speed, because the transportation system was accelerated in removing moisture contained in the material during evaporation process. The low moisture content in products, especially flour, was a requirement that have to be fulfilled.

Ash content was generally expressed as a mineral contained in a material. Minerals were not significantly affected by chemical and physical treatment during processing, in the presence of oxygen, some minerals may be oxidized to higher-valence minerals, but did not affect their nutritional value. Although some components of food were damaged in the process of heating food, the process did not affect the mineral content in food [1]. indicating that there were significant differences in the two treatments. This showed that the addition of tween 80 caused the decrease in ash content of SFPnC. As a consequent, the quality of SFPnC was getting better. According to [2] , that the higher the ash content the worse the product quality and vice versa, although this is not related to the quantity and quality of food protein. The intended nano SFPnC ash content quality was in quality I because it has an average value of 4.08%. According to [10] the maximum quality I of ash was 10%, quality II was 15% and quality III was 20%. Based on this, the lower level of ash the better quality of the protein concentrate.

The results of the T-test statistical test obtained a 2-tailed significance value of 0,000 (P> 0.05) indicating that there were significant differences in the two treatments on fat. This showed that the addition of tween 80 increased the fat content of SFPnC. This was presumably because of the large HLB (Hydrophilic Lipophilic Balance) value of tween 80 which results in the oil emulsion properties in water. This was in line with the value of the SFPnC emulsion capacity obtained after the addition of tween 80 that increased from 14.72% to 48.15%. It was assumed that tween 80 formed a thin layer which prevents aggregation by a mechanism to reduce the oil/water surface tension. Tween 80 was a non-ionic surfactant whose description is in the form of a yellow oil solution, has an HLB value of 15. Tween 80 was used as a surfactant because of the HLB value that corresponds to the combined HLB of palm oil and VCO which is 15.5. The SFPnC can be categorized in fatty fish flour because it has an average value of 6.48%. According to Windsor (2001) that low-fat fish have less than 3% fat content, medium fat has 3-5% fat content and high fat content has a fat content of 7%. From the results of the T-test statistical test obtained a 2-tailed significance value of 0,000 (P> 0.05) so that there were significant differences in the two treatments.

The addition of 2% of tween 80 increased the levels of SFPnC protein after being broken down. This showed that tween 80 worked well in protecting protein molecules that have been broken down

during sonication and homogenization. It was suspected that when the molecular size reduction process tween 80 protects nano-sized amino acids so as not to re-unite (aggregation).

Based on the nano quality requirements, SFPnC was included in the quality category I, because it contained 88.28% protein. According to [10] that the quality requirements of fish protein concentrate are based on crude protein (%), which is a minimum quality of 80% I, quality II is at least 75% and quality III is at least 55%.

## 4. Conclusion

It can be concluded that The results of scanning electrone microscope (SEM) showed that 2% concentration of tween 80 gave the incompact particle structure granules so that the possibility of being broken down was still very high with the smallest average particle size (223.6-363.2 nm) and polydispersity index 0.470.

The result of physical characteristics showed that the bulk density, water and oil absorption decreased after addition of tween 80 while the emulsion capacity increased whereas the result of chemical characteristics showed that moisture and ash content decreased after addition of tween 80 while fat and protein increased.

## 5. References

[1] Antia B S, E J Akpan, P A Okon and I U Umoren 2006 Nutritive and anti-nutritive evaluation of sweet potatoes (*Ipomoea batatas*) Leaves *Pakistan J. of Nutrition*. **5** (2) 166-168

[2] Apriliyanti T 2010 Physicochemical properties and sensory studies of purple sweet potato flour (*Ipomoe batatas Blackie*) with various drying process *Thesis*. (Surakarta: University of Sebelas Maret)

[3] Asfar M, Tawali A B and Mahendradatta M 2014 Potency of snakehead fish (*Channa striata*) as source of healthy food (a review) *Proc. of National Seminar on Industrial Tech. II*. 175 – 180

[4] Avadi M R, Assal M M S, Nasser M, Saideh A, Fatemeh A, Rassoul D and Moreza R 2010 Preparation and characterization of insulin nanoparticles using chitosan and arabic gum with ionic gelation method *Nanomedicine; Nanotechnology, Biology and Medicine 6*.

[5] Dewita dan Syahrul 2010 Study on diversification of Patin fish (*Pangasius sp*) in form of Fish Protein Concentrate and its application on snack food to overcome malnutrition in infants in Kabupaten Kampar, Riau (Pekanbaru: Result Report of Hibah Kompetensi, Research Center of Riau University)

[6] Mahendradatta M, N Anisa, J Langkong, A B Tawali, M Asfar and N K Sukendar 2017 Snakehead fish (*Channa striata*) protein concentrate dispersion as food supplement with addition of brown sugar and herbs extract *Proc. of National Seminar PATPI* (Lampung: Faculty of Agriculture Universitty of Lampung) 604-611

[7] Nidhin M, R Indhumaty, K J Sreeram and B U Nair 2009 Synthesis of iron oxide nanoparticels of narrow size distribusion on polysaccharide templates *Buletin Material Sci*. **31** (1) 93-96

[8] Purwantiningsih S, Napthaleni, K Mersi and W Tuti 2010 Encapsulation of ketoprofen with kitosan-alginat based on type of concentration of tween 80 and span 80 *Makara, Sains*. **14** (2)

[9] Rieuwpassa F 2005 Biscuit made from fish protein concentrate and probiotic as food supplement to increase antibody IgA and nutrition status of infant *Dissertation*. (Bogor: Postgraduate School IPB)

[10] Ruiter A 1995 Fish and Fishery Products *Compotition, Nitrition Properties and Stability*. (Singapore: CAB International)

[11] Tawali A B, Roreng M K, Mahendradatta M and Tawali S 2012 Difussion of production technology of protein concentrate from snakehead fish as food supplement in Jayapura *Proc. of Ristek Insinas*. 243-247

[12] Tawali A B, Asfar M, Mahendradatta M and Tawali S 2018 Comparison of proximate composition, amino acid, vitamin and mineral contents of whole fish powder and fish protein concentrate from local Indonesian snakehead fish (*Channa striata*) *Carpathian J. of Food Sci*. **10** (3) 40–46
[13] Windsor M L 2001 Fish Protein Concentrate *Ministry of Technology Torry Advisory Note* **39**
[14] Zayas J F 1997 *Functionality of Proteins in Food* (New York: Springer)

# Characteristics of Nano Concentrate Protein Dispersion of Snakehead Fish (Channa striata) by Addition of Tween 80

ORIGINALITY REPORT

| %13 | %9 | %10 | %8 |
|---|---|---|---|
| SIMILARITY INDEX | INTERNET SOURCES | PUBLICATIONS | STUDENT PAPERS |

MATCHED SOURCE

| | | |
|---|---|---|
| 1 | Submitted to Udayana University<br>Student Paper | %4 |

4%

★ Submitted to Udayana University

Student Paper

| | | | |
|---|---|---|---|
| EXCLUDE QUOTES | ON | EXCLUDE MATCHES | < 5 WORDS |
| EXCLUDE BIBLIOGRAPHY | ON | | |