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Protein profile and amino acid profile of *Vacuum drying* and freeze-drying of family channidae collected from central kalimantan, indonesia

Firlianty[1*], E. Suprayitno[2], Hardoko[2], H. Nursyam[2]

[1]*Faculty of Agriculture, Palangkaraya University, Central Kalimantan, Indonesia*

[2]*Faculty of Fisheries and Marine Science, Brawijaya University, Malang, East Java Indonesia*

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Abstract

Snakeheads are one of animal protein sources having high protein content and complete amino acid quality. Central Kalimantan possesses several species of dominant snakeheads that so far have not been extensively known yet, *Channa striata*, *C. micropelthes, C. Pleuropthalmus*. These three species are potential in wound healing process due to low cost and easy utilization. Results showed that the *vacuum drying* and freeze-drying powder products possessed 14 complete and important amino acids. Dominant amino acids are leucine, alanine, aspartic acid and glutamic acid known highly beneficial in wound healing process. Based upon SDS Page analysis, each sample has similar protein molecular weight range.

* **Corresponding Author:** Firlianty ✉ firli_1980@yahoo.com

Introduction

Since long time ago Central Kalimantan communities have hereditarily had snakeheads to meet nutritional needs and to cure wounds, especially post-partum recovery. The fish are usually steam-cooked. Snakehead (*Channa striata*) potency in wound healing has been known, since this fish possesses high albumin concentration and the fish albumin is expected to be able to be an albumin source as a substitute *Human Serum albumin* (HSA) whose price is still relatively expensive (Suprayitno, 2003; Santoso, 2009). Preliminary study showed that snakeheads have high albumin, 8.93 g/dl for *Channa Micropelthes*, 8.26 g/dl for *Channa Pleuropthalmus* and 6.78 g/dl for *Channa striata*, respectively. For people of Central Kalimantan, *Channa striata* is believed to be able to give fast effect in post-partum healing process. Central Kalimantan possesses very large fisheries potency, one of which is snakeheads (Channidae) (Fisheries and Marine Services of Central Kalimantan Tengah, 2009). Higher use of snakeheads as albumin source for health benefit, particularly wound healing and post-surgery, makes a variety of processing diversification of the fish be able to utilize as maximum as possible as albumin source, cheap and easily processed. One of the snakehead divertifications is to have in powder types, either *vacuum drying* or freeze drying products. The powder product is intended to extend the storage period and ease its utilization. So far there is no study on vacuum-drying and frezee-drying fish powder of the three snakehead species used in wound healing process. Snakehead group is one of the protein sources with high protein concentration and complete amino acids (Muchtadi, 2010). Some traditional processess in food preservation could damage or take out some important nutrition so that it could reduce the absorbed nutritive value (Antwi, 2006). Therefore, fish filtrate processing to make albumin powder is done through moderate heating, at 49°C in order to prevent protein denaturation of the powder (Yuniarti *et al*, 2013). The freeze-drying-based powder processing is conducted through -20°C freezing. Protein profile and amino acid composition are important nutritive value indicators to determine the quality of food materials to make high nutritive products beneficial to human health. Fish is one of food materials possessing high protein content, and therefore it is highly beneficial for human.

This study used *Channa micropelthes, Channa pleuropthalmus* and *Channa striata* as major materials for fish powder production, based on the highest content of proximate, albumin, zn and amino acid through vacuum-drying and freeze-drying process. It was aimed at measuring protein profile and amino acid profile of vacuum- and freeze-drying powder of *Channa micropeltes*, *C. pleuropthalmus*, and *C. striata*.

Materials and method

Protein profile was obtained using SDS PAGE with laemli method and amino acid profile with HPLC. Fish flesh of family Channidae was extracted using a vacumn extractor at 35°C for 12.5 minutes at 1 atm., then processed to be powder through vacuum- and freeze-drying.

Vacuum-Drying Powder Processing

Fifty percent of fish filtrate was added with 75% gum arabic and 25% gelatin, 5% lecithin and 10% CMC, and homogenized in a homogenizer at 2000 rpm for 15 minutes (Mariyana, 2007). The homogenous mixture was poured into a pan and inserted into a vacumn drier at 49°C for 5 hours until the sample was dry, blended to fine size and sieved through 60 mm-sieve (Yuniarti *et al*, 2013).

Freeze-Drying Powder processing

Fish filtrate protein was separated using ammonium sulphate in order to obtain products of pure albumin content**.** Fish filtrate was separated from fat using cold organic solvent (n-hexane) in filtrate-solvent ratio of 3:1, then water fraction was separated using cold separator funnel, and stored at -4°C for deposition in 50% ammonium sulphate for one night until deposit and supernatant were formed, then the supernatant was precipitated in 80% ammonium sulphate (Rahman, 2007). The deposit was then dialyzed using membrane cellophane, refrozen at –

20°C and freeze-dried to gain the product.

Results and discussion

The present study found amino acid profile and protein albumin profile of the three fish species as freeze drying and *vacuum drying* products :

Asam amino profile

Amino acids are major components for protein development, and it is separated in two groups, essential and non-essential amino acids. The former cannot produced in the body so that it should be added from food materials, while the latter can be produced in the body (Sitompul, 2004). The amino acid content of the freeze-drying and vacuum-drying powder of family Channidae are given in Table 1.

Table 1. Amino Acids of freeze-drying and vacuum-drying powder of the snakeheads (%).

Amino acids	Freeze-drying Powder			Vacuum-drying Powder		
	C. micropelthes	*C. pleurophthalmus*	*C. striata*	*C. micropelthes*	*C. pleurophthalmus*	*C. striata*
			Essential			
Histidine	1.85	1.71	1.75	2.01	0	0
Arginine	3.41	3.09	3.55	4.04	5.29	4.30
Methionine	1.99	1.43	1.78	2.15	0	1.93
Valine	7.07	5.85	7.58	6.09	3.24	3.55
Phenylalanine	6.14	6.86	5.93	6.36	1.63	3.13
Isoleucinee	5.01	4.55	5.36	4.14	1.97	2.97
Leucine	9.43	9.31	9.27	9.37	4.40	7.37
Lysine	4.38	4.90	3.56	5.36	1.16	1.79
Aspartic acid	15.11	15.10	Non-essential 16.09	12.10	9.67	8.45
Glutamic acid	12.43	12.58	11.60	11.31	11.81	11.78
Serine	6.64	5.84	6.05	7.35	5.42	7.32
Glycine	8.85	9.45	7.98	10.71	40.53	29.10
Alaninee	10.03	11.85	10.94	11.47	14.89	15.62
Tyrosine	1.68	1.58	1.99	1.80	0	2.68

Table 2. Migration distance of SDS marker, Rf value and molecular weight of the bands.

Marker Distance	Rf marker	BM marker (kDa)	Log BM
33	0.056604	140	2.146128
57	0.097770	100	2.000000
91	0.156089	70	1.845098
143	0.245283	50	1.698970
199	0.341338	40	1.602060
251	0.430532	35	1.544068
323	0.554931	25	1.397940
467	0.801029	15	1.1760913

Table 1 shows that in the freeze-drying powder, *Channa micropelthes* possesses the highest essential amino acid content, histidine, methionine, leusine, lysine and non-essential one, serine. *Channa pleuropthalmus* has the highest phenylalanine, glutamic acid, glycine, and alanine. In *Channa striata*, the highest content occurs in arginine,valine, isoleucine,aspartic acid (16.09%) and tyrosine.

In *vacuum-drying* powder (Table 1), *Channa micropelthes* possesses the highest content of histidine, methionine, valine, phenylalanine, isoleusine, leusine, and lysine, aspartic acid, and serine. C. pleuropthalmus has the highest arginine, glutamic acid, and glysine. Whereas, *C. striata* possesses the highest content of alanine and tyrosine.

Moreover, *vacuum drying* and freeze drying powder derived from C. micropeltes have higher number of the highest amino acid than the other two species. Glycine content is higher in the *vacuum drying* powder than that in the freeze drying powder. This amino acid is highly important in wound healing process. Glycine and arachidonic acid synergize with other amino acids in tissue healing process.

Table 3. Migration distance of *freeze-drying C. micropelthes* for SDS, Rf value and molecular weight of each band.

Migration distance	Rf	BM (kDa)	Log BM
111	0.187817	71.83734	1.856350
125	0.211506	67.21031	1.827436
161	0.272420	56.63496	1.753085
207	0.350254	45.50721	1.658080
241	0.407783	38.71324	1.587860
275	0.465313	32.93358	1.517639
301	0.509306	29.1032	1.463941
321	0.543147	26.46272	1.422635
363	0.614213	21.67162	1.335891
373	0.631134	20.66513	1.315238
439	0.742809	15.09828	1.178928
459	0.776650	13.72844	1.137621

Tabel 4. Migration distance of *vacuum-drying C. micropelthes* for SDS, Rf value and molecular weight of each band.

Migration distance	Rf	BM (kDa)	Log BM
119	0.208406	67.79837	1.831219
133	0.232925	63.28374	1.801292
153	0.267951	57.35074	1.758539
191	0.334501	47.56727	1.677308
211	0.369527	43.10773	1.634555
229	0.401051	39.45276	1.596077
287	0.502627	29.65470	1.472094
307	0.537653	26.87450	1.429340
441	0.772329	13.89616	1.142895

Leucine is the amino acid mostly found in protein and absolutely needed in children development as brain function trigger and for nitrogen equilibrium of the adults, preventing muscle retraction, and helping wound healing of skin and bone (Liputo *et al* 2013). Non-essential amino acids, such as aspartic acid, glycine and glutamic acid play important role in wound healing process (Tan and Azhar, 2014).

Table 5. Migration distance of *freeze-drying C. pleuropthalmus* for SDS, Rf value and molecular weight of each band.

Migration distance	Rf	BM (kDa)	Log BM
103	0.174873	74.49957	1.872150
123	0.208829	67.71797	1.830704
159	0.269949	57.02958	1.756100
201	0.341256	46.67265	1.669062
233	0.395586	40.06342	1.602748
289	0.490662	30.66887	1.486698
313	0.531409	27.35029	1.436962
335	0.568761	24.62469	1.391371
359	0.609508	21.96013	1.341635
375	0.636672	20.34594	1.308478
427	0.724958	15.87511	1.200717
455	0.772496	13.88966	1.142692

SDS PAGE

The SDS PAGE analysis reflected protein band profile variations in *C. striata*, *C. micropelthes* and *C. pleuropthalmus*. The vacuum-drying and freeze-drying products of these species had protein profile of nearly same molecular weight. This result is expected to be able to use as a reference for further study in family channidae product development for wound healing.

Table 6. Migration distance of *vacuum-drying C. pleuropthalmus* for SDS, Rf value and molecular weight of each band.

Migration distance	Rf	BM (kDa)	Log BM
123	0.213913	66.75714	1.824498
141	0.245217	61.13468	1.786288
161	0.280000	55.44112	1.743832
201	0.349565	45.59536	1.658921
215	0.373913	42.57962	1.629202
237	0.412174	38.23847	1.582501
293	0.509565	29.08203	1.463625
309	0.537391	26.89430	1.429660
431	0.749565	14.81429	1.170681

Table 7. Migration distance of *freeze-drying C. striata* for SDS, Rf value and molecular weight of each band.

Migration distance	Rf	BM (kDa)	Log BM
43	0.073756	98.98580	1.995573
55	0.094340	93.42198	1.970449
95	0.162950	77.03763	1.886703
113	0.193825	70.63455	1.849017
147	0.252144	59.95604	1.777833
199	0.341338	46.66196	1.668963
223	0.382504	41.56380	1.618715
239	0.409949	38.47838	1.585217
265	0.454545	33.94547	1.530782
287	0.492281	30.52962	1.484721
309	0.530017	27.45750	1.438661
325	0.557461	25.41924	1.405163
371	0.636364	20.36360	1.308855
381	0.653516	19.40519	1.287918
447	0.766724	14.11682	1.149737
467	0.801029	12.81929	1.107864

Based on Fig. 1, protein band profile of *vacuum drying* and freeze drying powder product of family channidae varied. The molecular weight ranged between 66.81-68.67 kDa. Protein profiles in *C. micropeltes*, *C. Pleurophthalmus*, and *C. striata* are slightly different. The protein band profile of the *vacuum drying* powder is different from that of the freeze drying. It could result from heating causing the protein breakdown. Processing through heating, freezing, high salt concentration exposure results in chemical and physical changes so that the absorption will increase due to protein denaturation (Chukwu and Shaba, 2009).

Table 8. Migration distance of *vacuum-drying C. striata* for SDS, Rf value and molecular weight of each band.

Migration distance	Rf	BM (kDa)	Log BM
169	0.289880	53.9228	1.731773
205	0.351630	45.3316	1.656401
223	0.382504	41.5638	1.618715
241	0.413379	38.1092	1.581030
297	0.509434	29.0928	1.463785
313	0.536878	26.9331	1.430286
409	0.701544	16.9549	1.229296
519	0.890223	9.97686	0.998994

The albumin protein band was recorded in freeze drying and *vacuum drying* products of the three fish species. Several protein types with varied molecular weight were also found in *vacuum drying* and freeze drying fish product. Nevertheless, the powder products of these species had lower albumin density in vacuum-drying product than in freeze drying product. According to Iwan (2006), heating in processing could decrease the protein band thickness with large molecule and increase number of protein parts left in protein separation, but Mahasri (2007) found that thick or thin protein band from SDS-PAGE could result from genetic difference between the proteins. Immunogenetic protein has bigger molecular weight than 1000 Dalton (Tizard, 1987).

Table 9. Molecular weight of *vacuum drying* and freeze-drying powder of *C. micropelthes*, *C. pleuropthalmus* and *C. striata*.

Band	Treatment molecular weight (kDa)					
	C. micropelthes		*C. pleuropthalmus*		*C. striata*	
	Vacuum drying powder	Freeze drying powder	*Vacuum drying* powder	Freeze drying powder	*Vacuum drying* powder	Freeze drying powder
1	67,798	71,837	66,757	74,499	53,922	98,985
2	63,283	67,210	61,134	67,717	45,331	93,421
3	57,350	56,634	55,441	57,029	41,563	77,037
4	47,567	45,507	45,595	46,672	38,109	70,634
5	43,107	38,713	42,579	40,063	29,092	59,956
6	39,452	32,933	38,238	30,668	26,933	46,661
7	29,654	29,103	29,082	27,350	16,954	41,563
8	26,874	26,462	26,894	24,624	9,976	38,478
9	13,896	21,671	14,814	21,960		33,945
10		20,665		20,345		30,529
11		15,098		15,875		27,457
12		13,728		13,889		25,419
13						20,363
14						19,405
15						14,116
16						12,819

Increased albumin density in *C. micropeltes* plays important role in synthesis and degradation rate and intravascular and extravascular distribution. If infection occurs, it will impact on extravascular and intravascular albumin distribution that will eventually alter the protein synthesis and degradation rate. The use of exogenic albumin is assumed to be able to lift up the intravascular albumin concentration, but during inflammation, the albumin serum will reduce (Nicholson *et al*, 2000).

Data above show that albumin band density of each fish species is different. Albumin band density in freeze drying product was 19.43% for *C. micropelthes*, 9.27% for *C. pleuropthalmus*, and 13.35% for *C. striata*, respectively. In *vacuum drying* product, it was 8.28% for *C. micropelthes*, 7.20% for *C. pleuropthalmus*, and 3.01% for *C. striata*, respectively. Figure 5.5. reflects that *C. micropelthes* has highest albumin band density.

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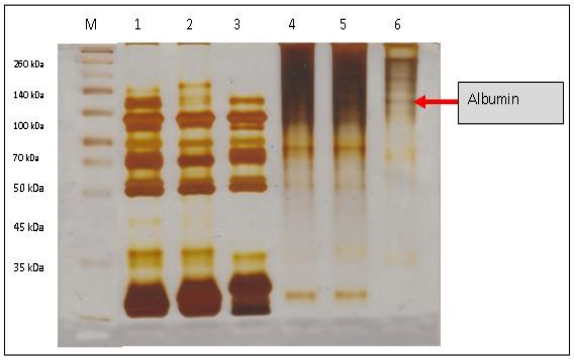
able to lift up the intravascular albumin concentration, but during inflammation, the albumin serum will reduce (Nicholson *et al*, 2000).



Note: M=protein marker

1 = *freeze drying C. micropelthes*

2 = *freeze drying C. pleuropthalmus*

3 = *freeze drying C. striata*

4 = *vacuum drying C. micropelthes*

5 = *vacuum drying C. pleuropthalmus*

6 = *vacuum drying C. striata*

← = albumin

Fig. 1. Protein profile of family Channdae using SDS PAGE.

Data above show that albumin band density of each fish species is different. Albumin band density in freeze drying product was 19.43% for *C. micropelthes*, 9.27% for *C. pleuropthalmus*, and 13.35% for *C. striata*, respectively. In *vacuum drying* product, it was 8.28% for *C. micropelthes*, 7.20% for *C. pleuropthalmus*, and 3.01% for *C. striata*, respectively. Figure 5.5. reflects that *C. micropelthes* has highest albumin band density.

Based on the SDS-PAGE electrophoresis (Fig. 1) and the SDS-PAGE standard curve (Fig. 3), the migration distance data of freeze drying and *vacuum drying* products of *C. micropelthes*, the Rf value and the molecular weight are presented in Table 3 and Table 4.

Standard equation of the SDS PAGE was then used to estimate the molecular weight logaritm of each migration distance of any sample by putting x value as molecular weight and Rf value as migration distance.

Table 4 and Table 5 shows that *C. micropelthes* has higher number of migration in *freeze dry*ing powder than that in *vacuum drying* powder. The molecular weight is also higher in *freeze dry*ing powder (71.84 kDa) than that in *vacuum drying* product (67.79 kDa).

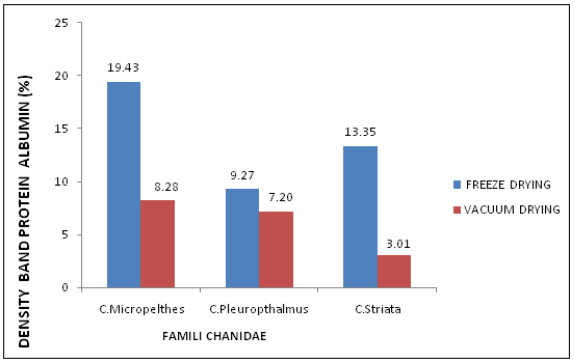


Fig. 2. Albumin Density Band (%) of Family Channidae.

The migration distance data of *freeze dry*ing and *vacuum drying* powder of *C. pleuropthalmus* for the SDS PAGE and its molecular weight are given in Table 5 and Table 6.

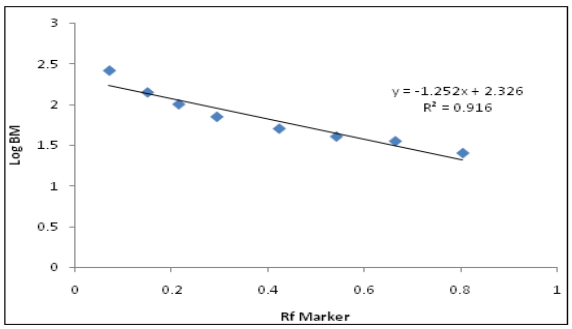


Fig. 3. Standard Curve of SDS-PAGE.

Based on Table 5 and Table 6, the migration distance of *C. pleuropthalmus* is higher in *freeze drying* powder than in *vacuum drying* powder. Moreover, the molecular weight is higher in *freeze drying* powder (74.50 kDa) than in *vacuum drying* powder (66.76 kDa).

Migration distance data of the freeze drying and *vacuum drying* powder of C. striata for SDS or molecular weight are presented in Table 7 and Table 8. *C. striata* has higher migration distance in freeze drying powder than that in *vacuum drying* powder. Higher molecular weight was recorded in the freeze

drying powder (9.98 kDa) than that in the *vacuum drying* powder (53.92 kDa) as well.

As a whole, the molecular weight of the three species selected is given in Table below.

Based on the Table 9, *vacuum drying* and freeze drying powder have molecularic weight range of 13.89-67.79 kDa and 13.72-71.83 kDa, respectively, for *C. micropelthes*, 14.81-66.75 kDa and 13.889-74.49 kDa, respectively, for *C. pleuropthalmus*, and 9.97-53.92 kDa and 12,81-98,98 kDa, respectively, for *C. striata*. Lower number of protein band in *vacuum drying* treatment than in freeze drying reflects that the treatments affect the protein density of the SDS PAGE. As a whole, protein profile of *vacuum drying* and freeze drying powder products has immugenic protein triggering the antibody formation.

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