PROGRESS REPORT

Analysis of Amino Acid And Fatty Acid for *Monopterus alba* Body and Waste

For:

Pelasari Aquamarine Sdn Bhd
No 9 & 11 Blok C1,
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1.0 Introduction

This report was prepared for Pelasari Aquamarine Sdn. Bhd. to provide results of the analysis on amino acid and fatty acid of the eel in two parts which are the body and waste (head and tail).

2.0 Methodology

a) The applied test method for determining and profiling amino acid is acid hydrolysis for the raw eel body and waste with reference to Waters Accq Tag using High-performance liquid chromatography (HPLC).

b) The applied test method for determining and profiling fatty acid is based on in-house method no. STP/Chem/A02 with reference to AOAC 18th Edition 905.02 and 989.05.

3.0 Result and discussion

The results are discussed in two parts: amino acids and fatty acids.

Part 1: Amino acid

Amino acids are substrates and regulators of protein metabolism and highly safe for humans. Amino acid content of the eel in both parts, body and waste, are presented in Table 1 according to types of amino acid. The content was reported in g/100g of the sample.

Table 1. Amino acid contents of eel's body and waste (head+tail) in g/100g

Type of amino acid	Amino acid contents (g /100 g)	
	Body	Waste
Hydroxyproline	0.49	0.75
Aspartic acid	**1.55**	**1.28**
Serine	0.86	0.85
Glutamic acid	**2.44**	**1.71**
Glycine	**1.10**	**1.53**
Histidine	0.51	0.53
Arginine	**1.45**	**1.31**
Threonine	**1.22**	**1.08**
Alanine	0.97	0.99
Proline	0.76	0.91
Thyrosine	0.59	0.93
Valine	0.68	0.70
Methionine	0.55	0.25

Lysine	0.96	0.83
Isoleusine	0.76	0.65
Leusine	**1.42**	**1.29**
Phenylalanine	0.64	0.62
Total	**16.94**	**16.22**

- The highest amino acid contents in both parts, body and waste, is **glutamic acid (Glu**) which is 2.44g/100g for body sample and 1.71g/100g for waste sample. Other dominant amino acid are aspartic acid, glycine, arginine, threonine and leusine. These amino acids constitute >1g/100 g samples (>1%).
- Other amino acids are found to be less than 1% in 100 g sample.
- Table 2 summarized the major amino acids found in eel's body and waste with their common physiological effect when integrated in skin care products.

Table 2. The physiological benefit of amino acids when integrated in skin care products (topical application)

Amino acids	pH balance	Retain moisture (hydration)	Skin firmness (Anti-aging)	Skin repair and wound healing	Antioxidant	References
Glutamic acid	√	√		√		Burlando & Cornara (2013)
Aspartic acid		√	√			Burlando & Cornara (2013)
Glycine		√	√	√		Marrubini et al. (2008)
Arginine		√	√	√	√	Saini et al. (2013)
Threonine			√	√		Marrubini et al. (2008)
Leusine*			√**	√		Hines and Florence (2015)

* May improve wrinkles when used in conjunction with glycine and proline

- The widely used amino acid from Table 2 in market and have been proven to help improve the condition of skin are: glutamic acid, glycine, arginine and leusine.

- Below are the description for the physiological effect listed in Table 2.
    - pH balance: Amino acid helps to maintain the skin at its ideal pH of 5.5 and prevent sensitivity, wrinkles, inflammation or acne.
    - Hydration: Amino acid are able to bind with water molecules and protect the skin’s moisture barrier.
    - Skin firmness: Amino acid stimulate the collagen production since they are crucial part for creating collagen and therefor reduce sagged and wrinkled skin.
    - Skin repair and wound healing: amino acid play important roles in cell metabolism and the structural elements of skin. Since the acids can help increase the rate of turnover (eliminating dead cells and allowing new cells to grow in their place), acne scarring may also disappear at a faster rate than it would on its own. Hence, they have potential to reduce the depth of stretch marks.
    - Antioxidant: The amino acid protect skin from free-radical damage and reduce signs of ageing by scavenging the radicals for example from sun radiation.

<u>Part 2: Fatty acids</u>

Fatty acids is important component of lipids (fat-soluble components of living cells).

- The total content of fatty acid in the eel’s body was found to be 0.11g/100g sample and majorly consisted of saturated fat, followed by monounsaturated fat and polyunsaturated fat.
- Meanwhile, the total content of fatty acid in the eel’s waste was found to be higher than the body, which is 0.17g/100g sample and majorly consisted of saturated fat, followed by monounsaturated fat and polyunsaturated fat.

- The fatty acid composition was determined as the fatty acids methyl esters (FAME) and are presented in Table 3 as percentage (%) in fat.

Table 3. Fatty acid contents of eel's body and waste (head+tail) in percentage of total fat (%)

Fatty acid methyl ester	Percentage in fat (%)	
	Body	Waste
SATURATED FAT		
Butryic	0.00	0.00
Caproic	0.00	0.00
Caprylic	0.95	2.05
Capric	0.85	1.67
Undecanoic	0.00	0.00
Lauric	**8.37**	**14.83**
Tridecanoic	0.00	0.00
Myristic	5.09	**8.47**
Pentadecanoic	**8.62**	3.73
Palmitic	**14.80**	**39.86**
Heptadecanoic	0.00	0.00
Stearic	4.29	**8.31**
Arachidic	**8.96**	0.00
Henicosanoic	0.00	0.00
Behenic	0.00	0.00
Tricosanoic	0.00	0.00
Lignoceric	5.18	0.00
Total	57.10	78.92
MONOUNSATURATED FAT		
Myristoleic	0.00	0.00
Cis-10-Pentadecenoic	1.64	1.19
Palmitoleic	0.00	0.00
Cis-10-Heptadeconoic	5.08	2.08
Elaidic (Trans)	0.00	0.00
Oleic	**8.56**	**14.94**
Cis-11-Eicosenoic	0.00	0.00
Erucis	0.00	0.00
Nervonic*	**9.13**	0.00
Total	24.40	18.21
POLYUNSATURATED FAT		
Linolelaidic (trans)	0.00	0.00
Linoeleic (Cis)	0.00	0.00
y-Linoleic	0.00	0.00
a-Linoleic	**6.81**	**2.88**
Cis-11,14-Eicosadienoic	0.00	0.00
Cis-8,11,14- Eicosatrienoic	0.00	0.00

Cis-8,11,17- Eicosantrienoic	0.00	0.00
Arachidonic	0.00	0.00
Cis-5,8,11,14,17- Eicosapentaenoic	**11.71**	0.00
Cis-13,16-Docosahexaenoic	0.00	0.00
Cis-4,7,10,13,16,19-Docosahexaenoic	0.00	0.00
Total	18.51	2.88

* Nervonic acid may is too large in molecular volume to pass the barrier of the skin.

- In eel's body, the dominant fatty acids are listed as below:
    - Saturated fat (>8%): palmitic, arachidic, pentadecanoic and lauric acid.
    - Monounsaturated fat (>8%): oleic and nervonic
    - Polyunsaturated fat (>6%): Cis-5,8,11,14,17- Eicosapentaenoic and a-Linoleic
- In eel's waste, the dominant fatty acids are listed as below:
    - Saturated fat (>8%) : palmitic, lauric, myristic and stearic
    - Monounsaturated fat : oleic
    - Polyunsaturated fat: Only a-Linoleic was detected, however with low percentage of 2.88%.
- The commonly used fatty acid in skin care products which are dominant in eel's body and waste are summarized in Table 4 with their common physiological effect.

Table 4. The physiological benefit of fatty acids when integrated in skin care products (topical application)

Fatty acid	Dominant in body	Dominant in waste	Emollient Retain moisture	Antioxidant	Surfactant : cleansing	Surfactant: emulsifying	Anti-inflammatory	Skin repair and protection	Reference
Palmitic	√	√	√	√	√	√			Sajna et al. (2015)
Lauric	√	√			√		√		Nakatsuji et al. (2009)
Oleic	√	√			√			√	Lin et al. (2017)
a-Linoleic	√	√*	√	√			√		Lautenschläger (2003)
Myristic		√			√			√	Mank et al. (2016)
Stearic		√	√		√	√		√	

- Below are the description for the physiological effect listed in Table 4.

- Emollients: When applied to skin, the fatty acid form a hydrophobic layer and prevent the water loss from skin, eventually reduce the skin dryness and make the skin smooth and soft.
- Antioxidants: The fatty acid are used to alleviate oxidative damage associated with sun exposure. They were reported to inhibit collagen breakdown and eliminate aging skin symptoms like wrinkles.
- Anti-inflammatory: The fatty acid has strong bactericidal properties of that can be used to effectively and naturally treat inflammation. Particularly, linoleic acid produces prostaglandins, which stimulate circulation via vasodilatation and strengthen the body's natural defenses, suppressing inflammation.
- Surfactant cleansing: Fatty acid which act as surfactant that clean skin by helping water to mix with oil and dirt so that they can be rinsed away.
- Surfactant emulsifying: Fatty acid which act as surfactants that help to form emulsions by reducing the surface tension of the substances to be emulsified.
- Skin repair and protection: Fatty acids help maintain lipid balance, contributing to the skin's natural repair process and restore protective barrier.

4.0 Conclusion

Both body and waste of the eel contained more than 1g/100 g samples for glutamic acid, aspartic acid, glycine, arginine, threonine and leusine. These acids are beneficial for skins health such as pH balance, skin hydration, skin firmness (anti-aging), skin repair, wound healing and as antioxidant. Meanwhile, for the fatty acid, the dominant fatty acids in eel's body and waste are palmitic, lauric, oleic and a-Linoleic. The waste has shown further high fatty for myristic and stearic acids. All these fatty acids have been used in skin care products as ingredients since they have health benefits such as emollient, antioxidant, cleansing effect, emulsifying effect, anti-inflammatory, skin repair and protection.

Generally, fish contains 2-25% fat, 15-30% protein and 50-80% moisture content. Analysis from previous work shows that the results were between the range (refer Progress Report *"Nutrient Contents of Asian swamp eel; Monopterus albus)*. Eel sample from Pelasari Aquamarine Sdn Bhd contains 78.1% and 75% of moisture, 18.2% and 18.3% of protein; 0.3% and 0.2% of fats for body and waste respectively.

There are 20 monomer amino acids that build protein by polypeptide bonding. The glycine is a major component of collagen. Glutamine is used by the inflammatory cells within the wound for proliferation and as a source of energy. Fibroblasts use glutamine for protein and nucleic acid synthesis. Arginine is essential for efficient wound repair and immune function. In comparison with other species of eel, it showed similar pattern in overall amino acid composition, although slight differences in the compositions of some amino acids were observed. In fact, the total amount of amino acid contains in *Monopterus Albus* are slightly higher; 16.94g and 16.22g in 100g extract for body and waste respectively. The comparison is summarized in Table 5.

Table 5. The comparison of amino acid contains

Type of amino acid	Amino acid contents (g /100 g)					
	Eel species					
	Monopterus Albus		Anguilla japonica	Anguilla rostrata	Anguilla bicolor pacifica	Anguilla bicolor pacifica
	Body	Waste	Reference: Jun Cheul Ahn et al. (2015)			
Hydroxyproline	0.49	0.75	-	-	-	-
Aspartic acid	**1.55**	**1.28**	1.52	1.62	1.66	1.61
Serine	0.86	0.85	0.66	0.69	0.71	0.66
Glutamic acid	**2.44**	**1.71**	2.23	2.39	2.50	2.44
Glycine	**1.10**	**1.53**	1.08	1.10	0.88	0.91
Histidine	0.51	0.53	0.63	0.65	0.66	0.64
Arginine	**1.45**	**1.31**	0.97	1.05	0.99	0.99
Threonine	**1.22**	**1.08**	0.71	0.76	0.78	0.75

Alanine	0.97	0.99	1.06	1.05	1.06	1.05
Proline	0.76	0.91	0.72	0.62	0.62	0.62
Thyrosine	0.59	0.93	0.52	0.51	0.57	0.54
Valine	0.68	0.70	0.69	0.77	0.74	0.72
Methionine	0.55	0.25	-	-	-	-
Lysine	0.96	0.83	1.34	1.52	1.48	1.44
Isoleusine	0.76	0.65	0.65	0.72	0.70	0.68
Leusine	**1.42**	**1.29**	1.19	1.27	1.30	1.26
Phenylalanine	0.64	0.62	0.63	0.67	0.69	0.66
Total	**16.94**	**16.22**	**14.6**	**15.41**	**15.34**	**14.97**

The comparison of the fatty acid contents in *Monopterus albus* extracts and other species extract could be seen in Table 6. It can be seen that eel sample from Pelasari Aquamarine Sdn Bhd is high in saturated fat which may raise low-density lipoprotein (LDL) cholesterol levels, raise risk for heart disease and type 2 diabetes. Anyhow, there are other advantage of saturated fat if taken in considerable amount and it is benefited for topical application. Fatty acids play a role in repairing damaged tissue, development of brain nerve, wound healing agent and antithrombotic. In wound healing, fatty acids and proteins work together in the formation of keratinocytes and fibroblasts. The fatty acid components of *Monopterus albus* extract are needed in the wound healing process. Linoleic acid in the *Monopterus Albus* oils indicated that the oil was a light-oil and can easily be penetrated into the human skin compared to saturated fatty acid. Due to numerous beneficial effects, linoleic acid found applications in many branches of industry, particularly in the cosmetic industry (Zielinska et al., 2004). Linoleic acids also were reported to support the healing process and speed up the regeneration of skin barrier. Eicosapentaenoic acid (**EPA**) is one of several omega-3 fatty acids. Omega-3 fatty acids are part of a healthy diet that helps lower risk of heart disease. The comparison with other species is tabulated in Table 6.

Table 6. The comparison of fatty acid contains

Fatty acid methyl ester	Percentage in fat (%)					
	Monopterus Albus		Anguilla japonica	Anguilla rostrata	Anguilla bicolor pacifica	Anguilla bicolor pacifica
	Body	Waste	Reference: Jun Cheul Ahn et al. (2015)			
SATURATED FAT						
Butryic	-	-	-	-	-	-
Caproic	-	-	-	-	-	-
Caprylic	0.95	2.05	-	-	-	-
Capric	0.85	1.67	-	-	-	-
Undecanoic	-	-	-	-	-	-
Lauric	**8.37**	**14.83**	-	-	-	-
Tridecanoic	-	-	-	-	-	-
Myristic	5.09	**8.47**	3.7	3.9	3.6	3.5
Pentadecanoic	**8.62**	3.73	-	-	-	-
Palmitic	**14.80**	**39.86**	22.4	23.7	21.0	22.4
Palmitoleic	-	-	7.1	10.1	5.4	5.0
Heptadecanoic	-	-	-	-	-	-
Stearic	4.29	**8.31**	4.8	3.7	5.5	3.9
Arachidic	**8.96**	-	-	-	-	-
Henicosanoic	-	-	-	-	-	-
Behenic	-	-	-	-	-	-
Tricosanoic	-	-	-	-	-	-
Lignoceric	5.18	-	-	-	-	-
Total	57.10	78.92	30.9	50.3	30.1	29.8
MONOUNSATURATED FAT						
Myristoleic	-	-	-	-	-	-
Cis-10-Pentadecenoic	1.64	1.19	-	-	-	-
Palmitoleic	-	-	-	-	-	-
Cis-10-Heptadeconoic	5.08	2.08	7.1	10.1	5.4	5.0
Elaidic (Trans)	0.00	0.00	-	-	-	-
Oleic	**8.56**	**14.94**	46.3	44.1	45.5	46.1
Cis-11-Eicosenoic	-	-	2.4	4.0	1.5	2.1
Erucis	-	-	-	-	-	-
Nervonic*	**9.13**	-	-	-	-	-
Total	24.40	18.21	55.8	41.5	52.4	53.3
POLYUNSATURATED FAT						
Linolelaidic (trans)	-	-	-	-	-	-
Linoelenic (Cis)	-	-	0.9	0.2	0.8	0.5
y-Linoleic	-	-	0.3	-	0.2	0.2
a-Linoleic (ALA)	**6.81**	**2.88**	-	-	-	-

Cis-11,14-Eicosadienoic	-	-	-	-	-	-
Cis-8,11,14- Eicosatrienoic	-	-	-	-	-	-
Cis-8,11,17- Eicosantrienoic	-	-	-	-	-	-
Arachidonic	-	-	1.0	0.4	1.6	1.5
Cis-5,8,11,14,17-Eicosapentaenoic (EPA)	**11.71**	-	-	-	-	-
Cis-13,16-Docosahexaenoic	-	-	-	-	-	-
Docosatetraenoic acid	-	-	0.3	0.3	0.4	0.4
Cis-4,7,10,13,16,19-Docosahexaenoic (DHA)	-	-	6.9	6.9	9.7	9.1
Total	18.51	2.88	13.3	8.3	17.6	17.0

5.0 Recommendations

Eels have traditionally been caught, bred for trade and consumed live, fresh, chilled, frozen or smoked. Here are the ways in which it can be processed namely fish oil, fish protein hydrolysate and gelatine. Other products are including smoking, canning and the preparation of jellied eels.

Fish Oil

Fish oil is an industrial product of great nutritional value due to its content of long chain omega-3 polyunsaturated fatty acids (PUFA), such as docosahexaenoic acid (DHA), docosapentaenoic acid (DPA) and eicosapentaenoic acid (EPA), which are currently highly valued for their prophylactic and therapeutic properties in nutritional and health fields. Based on the analysis, sample from Pelasari Aquamarine Sdn Bhd have good sources of value fatty acids and amino acids and comparable with other species of eel. The extraction of fish oil by wet pressing is the most commonly used method for production on an industrial scale, and is basically carried out in four stages: fish cooking, pressing, decantation and centrifugation (FAO, 1986). Another conventional procedure is the extraction using solvents, applied generally for analytical purposes but not for industrial production, because of the disadvantages of using substances with restrictions in the food industry (Rubio et al., 2010). It also produces waste byproducts, which cause environmental problems.

Enzymatic Hydrolysis

Enzymatic hydrolysis was developed to produce fish oil using commercial proteases. Enzymatic hydrolysis is carried out at low temperature with no waste by-product produced. It is used to extract oil for production of biodiesel, and/or omega-3 fatty acids. Several enzymes (alcalase, neutrase, protamex and flourzyme) can be used to extract fish oil. The enzymatic hydrolysis of fish proteins is also the principle method for converting under-utilized fish into valuable products for the pharmaceutical and health food industries. However, this technique normally at laboratory scale, not as in full industrial-scale operation, probably due to the high costs of the enzyme.

Gelatin

Fish gelatin can be obtained from the skin and bones of fish. Gelatin manufacturing practices involves in 3 stages:

- Pre-treatment of the raw material
- Extraction of the gelatin
- Purification and drying

- Acidic treatment suitable for the less covalently crosslinked collagens found in pig and skin meanwhile alkaline treatment suitable for more complex collagen found in bovine (Karim et al., 2009)
- Typically involves a mild chemical pre-treatment of the raw material and mild temp. conditions

Ointment preparation for wound healing

The eel extract ointment can be made by homogenizing the eel extract (*Monopterus albus*) with certain concentration and formulating the extract with a PGA ointment base ingredient (100 gm) and vaseline album (100 gm).

The process is summarized in Table 7.

Table 7. Processing of eel

Products	**Fish Oils**	**Fish Protein hydrolysate**	**Gelatin**
Process	Reflux/ Steaming	Enzymatic hydrolysis (chemical (acid or alkali treatment) or by biochemical methods.	1. Extract using Acetic acid 2. Extract using Pepsin
Product	Fresh fish oil Meat extract - For ointment preparation - Encapsulated fish oil	Fresh fish oil Protein hydrolysate Omega 3 fatty acid - Protein powder - Encapsulated fish oil	- Collagen is one of the materials that make up cartilage, bone, and skin. - Gelatin might help for arthritis and other joint conditions. - Gelatin is used as a stabiliser (yoghurt), thickener (jam), and texturizer and emulsifier (oil-in-water emulsions). - Gelatin is used as a foaming, emulsifying, and wetting agent in food, pharmaceutical, medical, and technical applications due to its surface-active properties. - Gelatin was among the fi rst commercial raw materials suitable as a contact preservative for meat and meat products. - To encapsulate nutritional supplements as well as medications
References	1. Heru et al. (2018) 2. Viski et al. (2020)	1. He et al. (2013) 2. Cheng et al. (2019) 3. Priatni et al. (2020)	Veeruraj et al. (2013)
Problem	Fish and fish tissues possess relatively high autolytic activities and high polyunsaturated fatty acid content which are prone to both lipolysis and oxidation.	Normally at laboratory scale, not as in full industrial-scale operation, probably due to the high costs of the enzyme.	High tech process involved; pre-treament, extraction, purification and drying. In gelatin manufacture, two methods are usually used: the acid and the alkaline processes.
Suggestion	1. Company to identify the product concepts and targeted market including potential buyer and proceeds with lab scale research. 2. Company to survey any joint venture for trial run before set-up a proper plant.		

6.0 References

Burlando, B., & Cornara, L. (2013). Honey in dermatology and skin care: a review. Journal of Cosmetic Dermatology, 12(4), 306-313.

Febriyenti, A. Almahdy and Dwi Mulyani (2019). Amino Acids and Fatty Acids Profiles of Eel (Monopterus albus) Water Extractions. Rasayan Journal of Chemistry, Vol. 12 | No. 3 |1591 – 1594

Heru Sasongko, Bayu Anggoro Saputro, Renita Wahyu Nur Hidayati, Raka Sukmabayu W.S, and Tiara Annisa Sekarjat. (2018). The Effect of Hydrocarbon Ointment Containing Eel fish (Anguilla bicolor bicolor) and Snakehead Fish (Channa striata) Oil for Wound Healing in Wistar Rats. AIP Conference Proceedings 2019, 050006 (2018); https://doi.org/10.1063/1.5061899

Hines, M., & Florence, T. (2015). U.S. Patent Application No. 14/637,009.

I-Chun Cheng, Jin-Xian Liao, Jhih-Ying Ciou, Li-Tung Huang, Yu-Wei Chen and Chih-Yao Hou (2019). Characterization of Protein Hydrolysates from Eel (Anguilla marmorata) and Their Application in Herbal Eel Extracts. Catalysts 2020, 10, 205.

Jun Cheul Ahn, Won-Seog Chong, Jin Ho Na, Hyoeng Bok Yun, Kyung Jae Shin, Kyeong Woo Lee and Jun Taek Park (2015). An Evaluation of Major Nutrients of Four Farmed Freshwater Eel Species (Anguilla japonica, A. rostrata, A. bicolor pacifica and A. marmorata). Kor J Fish Aquat Sci 48(1),044-050.

Lautenschläger, H. (2003). Essential fatty acids—cosmetic from inside and outside. In Beauty Forum (Vol. 4, pp. 54-56).dical analysis, 47(4-5), 716-722.

Lin, T. K., Zhong, L., & Santiago, J. L. (2018). Anti-inflammatory and skin barrier repair effects of topical application of some plant oils. International journal of molecular sciences, 19(1), 70.

Mank, V., & Polonska, T. (2016). Use of natural oils as bioactive ingredients of cosmetic products. Ukrainian food journal, (5, Issue 2), 281-289.

Marrubini, G., Caccialanza, G., & Massolini, G. (2008). Determination of glycine and threonine in topical dermatological preparations. Journal of pharmaceutical and biome

Nakatsuji, T., Kao, M. C., Fang, J. Y., Zouboulis, C. C., Zhang, L., Gallo, R. L., & Huang, C. M. (2009). Antimicrobial property of lauric acid against Propionibacterium acnes: its therapeutic potential for inflammatory acne vulgaris. Journal of Investigative Dermatology, 129(10), 2480-2488.

Saini, R. & Zanwar, A. A. (2013) Arginine Derived Nitric Oxide: Key to Healthy Skin, Bioactive Dietary Factors and Plant Extracts in Dermatology (pp. 73-82)

Sajna, K. V., Gottumukkala, L. D., Sukumaran, R. K., & Pandey, A. (2015). White biotechnology in cosmetics. In Industrial Biorefineries & White Biotechnology (pp. 607-652). Elsevier.

Shan He, Chris Franco and Wei Zhang (2013). Functions, applications and production of protein hydrolysates from fish processing co-products (FPCP). Food Research International, Vol 50, Issue 1, 289-297.

Sri Priatni, Kezia Harimadi, Efendi Buana, Wawan Kosasih and Rohmatussolihat Rohmatussolihat (2020). Production and Characterization of Spray-Dried Swamp Eel (Monopterus albus) Protein Hydrolysate Prepared by Papain. Sains Malaysiana 49(3) (2020): 545-552

Viski Fitri Hendrawan, Fairuz Ikhsan, Ani Setianingrum, Tiara Widyaputri (2020). The potency of eel meat (Monopterus albus) extract on tumor necrosis factor alpha (TNF-α) expression and epidermal thickness incision wounds on rats (Rattus norvegicus). Journal of Advanced Veterinary and Animal Research Vol 7, No. 1,77–82.

Zielinska, A. and Nowak, I. (2014). Fatty acids in vegetable oils and their importance in cosmetic industry. Chemik, 68(2), 103-110.

PROGRESS REPORT

Proximate Analysis, Vitamin E & A for *Monopterus albus* Powder

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1.0 **Introduction**

This report was prepared for Pelasari Aquamarine Sdn. Bhd. to:

- provide proximate results for *Monopterus albus* pure powder namely on total ash, moisture, total fat, protein, carbohydrate and energy while compare with the previous *Monopterus albus* body and waste samples
- provide results of the analysis on Vitamin E, Vitamin C and fatty acid for the *Monopterus albus* pure powder

2.0 **Methodology**

The following tests were carried out for the analyses:

a) Proximate analysis for the following nutritional contents:

- Protein
- Total fat
- Total carbohydrate
- Ash
- Moisture
- Energy

b) Vitamin E based on high-performance liquid chromatography (HPLC) technique

c) Vitamin C based on titration technique

d) Fatty acid profiling based on high-performance liquid chromatography (HPLC) technique

3.0 Result and discussion

The results are discussed in three parts: proximate composition, vitamins and fatty acids.

Part 1: Proximate composition

Proximate composition is an important criterion to determine the nutritional values and quality of the *Monopterus albus* pure powder as these may have changed throughout the processing of the livestock into the powder. Based on Table 2 below, the *Monopterus albus* pure powder was found to be very rich source of **protein** with the content of 76.5g/100g. This is expected since the raw livestock *Monopterus albus* was previously found to contain already high protein both in the body and waste parts (> 18g/100g). In addition, the water losses occurring during the powder processing resulted in a higher protein content in the pure powder compared to that of the fresh fish. Hence, the pure powder is a suitable ingredients for the production of high-protein food which is often required by active individuals, approximately 84-119 grams for active men and 66-94 grams for active women daily based on WHO recommendation (2011). (Average sedentary men requires about 56 grams and 46 grams for women daily).

The **total fat** content is only 2.3g/100g, and therefore, the pure powder can be classified as having low-fat content. This result also agreed with the previous findings that showed both body and waste parts have low fat content (0.2 – 0.3 g/100g). A slightly higher fat content was found in the pure powder sample due to water loss during the processing.

The **moisture content** of the pure powder is only 12.2g/100g (12.2%). This is expected due to water loss during the powder processing. Such low moisture content reduces the rate of quality degradation and thus, the powder can be stored for a longer time (preservation). Nevertheless, in order to guarantee the safety of powder from the microbial growth, it is recommended that the moisture content and water activity of the powder is kept below 10%

and 0.60–0.65, respectively (Mercer, 2008). With this recommendation, it is suggested the water activity of the powder should also be investigated in the future.

Ash content of the pure powder was found to be 9.0g/100g (9%) which is common for the processed food. The ash content is likely due to minerals commonly occur in *Monopterus albus* such as magnesium, iron, calcium and zinc. The minerals are directly absorbed from the water through their gills and skin, allowing them to compensate to some extent for mineral deficiencies in their diet (Wijayanti and Susilo, 2017).

As for **energy**, the pure powder was found to be high-calorie with energy values of 327 kcal/100g or 1368 kJ. This was due to high protein content in the powder. It is however important to note, that the protein's primary role when digested in our body is to build and repair tissues, including muscles, bones, and skin. Yet, the protein slows down the absorption of glucose in our blood and help to prevent energy-crashing and ensure that we have sustained energy.

The powder was found to have **no carbohydrate** content and this is expected as the powder was extracted from fish which is commonly low in carbohydrates.

Table 2: Comparison of proximate contents between *Monopterus albus* pure powder with previous body and waste samples

Parameter, Unit	**Pure powder**	**Body**	**Waste (head+tail)**
Protein, g/100g	76.5	18.2	18.3
Total fat, g/100g	2.3	0.3	0.2
Moisture, g/100g	12.2	78.1	75.6
Ash, g/100g	9.0	2.8	4.5
Energy, kcal/100g	327 (1368 kJ)	78 (328 kJ)	81 (340 kJ)
Total carbohydrate, g /100g	0.0	0.6	1.4

Part 2: Vitamin C and E

Table 3 shows the Vitamin C and E contents for the *Monopterus albus* pure powder, juice and capsule sample. The results showed that the pure powder has low Vitamin C contents when since the *Monopterus albus* itself contains low Vitamin C. The capsule sample is known to contain *Cantella asiatica* (pennywort) and *Labisia pumila* (*Kacip Fatimah*) which contain high Vitamin C. Meanwhile, the juice is known to contain *Hibiscus sabdariffa* (Roselle) which is also high in Vitamin C. On the other hand, the pure powder shows high Vitamin E content since the *Monopterus albus* itself was reported as one of aquatic biotas that is rich in Vitamin E (Wijayanti & Susilo, 2017). Hence, the pure powder can be a reliable ingredient source for Vitamin E that act as an antioxidant to scavenge "free radicals" that can damage cells.

Table 3. Vitamins C and E contents in *Monopterus albus* pure powder, juice and capsule samples (mg/100g)

Types of vitamins	**Pure powder**	**Juice**	**Capsule**
Vitamin C (mg/100g)	0.09	6.40	9.84
Vitamin E (mg/100g)	5.74	0.014	0.17

*The recommended daily allowance (RDA) for adult is 75 to 90 mg daily of Vitamin C for adults and 15 mg daily of Vitamin E for adults.

Part 3: Fatty acids

The total content of fatty acid in the *Monopterus albus* pure powder was found to be **3.11g/100g** sample. The pure powder showed major content of saturated fat (44.02%), followed by monounsaturated fat (38.65%) and polyunsaturated fat (17.33%). The low total fat content is advantageous as the general recommendation total fat intake is about 55-77g/day which is normally can be fulfilled by our daily diet.

Saturated fats have chain of carbon atoms that holds as many hydrogen atoms as possible and therefore it is saturated with hydrogens. A diet rich in saturated fats can drive up total cholesterol, which prompts blockages to form in arteries in the heart and elsewhere in the body.

Monounsaturated fats have a single carbon-to-carbon double bond. The result is that it has two fewer hydrogen atoms than a saturated fat and a bend at the double bond. This structure keeps monounsaturated fats liquid at room temperature and this fat help to lower the risk for cardiovascular disease. It is yet, recommended that monounsaturated fats are consumed along with polyunsaturated fats to replace saturated and trans fats. **Polyunsaturated** fats are essential fats and are required for normal body functions but our body cannot produce them. They have two or more double bonds in its carbon chain. There are two main types of polyunsaturated fats: omega-3 fatty acids and omega-6 fatty acids. Both types offer health benefits and are used to build cell membranes and the covering of nerves. They are needed for blood clotting, muscle movement, and inflammation. Eating polyunsaturated fats in place of saturated fats or highly refined carbohydrates reduces harmful LDL cholesterol and improves the cholesterol profile.

In details, the fatty acid profiles for the *Monopterus albus* pure powder, juice and capsule sample are shown in Table 4 as percentage (%) in the total fat. The pure powder showed high percentage in the following fatty acids:

- **Saturated fat: palmitic (24.58%) and stearic acid (9.14%)**

Consumption of palmitic acid raises concern as it may raises the level of total cholesterol and LDL (bad) cholesterol more than other saturated fats. There is convincing evidence that high consumption of palmitic acid may increase the risk of cardiovascular disease. Therefore, the consumption of palmitic acid should be minimized. Meanwhile, stearic acid is considered a healthy saturated fat and does not raise the risk of heart disease. In fact, parts of our body are able to convert stearic acid to oleic acid, a healthy unsaturated fat. Although the percentage of palmitic and stearic acid are high in its total fat, the actual values which 765 mg/100g and 284 mg/100g are still low as the total fat content is already low.

- **Monounsaturated fat: oleic acid (27.55%)**

Oleic acid is known to reduce bad cholesterol and therefore, provides protection against heart disease and blood-pressure. Other health benefits include role as powerful antioxidants that can reduce inflammation and cell damage caused by free radical (anticancer), help in fighting Alzheimer disease, reduce Type 2 diabetes risk, treat rheumatoid arthritis and has antibacterial properties. The actual value of oleic acid in *Monopterus albus* pure powder is 857 mg/100g and considered moderate.

- **Overall unsaturated fat which is composed of omega-3 and omega-6 fatty acids (17.33%)**

A balance intake of omega-3 and omega 6 fatty acids provides protection against fatal heart disease and other coronary events. Omega-3 acid is known to have anti-inflammatory effects plays role in the prevention of diabetes and certain types of cancer. Meanwhile omega-6 acid is known to reduce the risk of heart disease, lowering total cholesterol level and reduce cancer risk. Advantageously, the *Monopterus albus* pure powder contains both this omega-3 and 6 fatty acids about 539 mg/100g in moderate amount.

Table 4. Fatty acid contents of *Monopterus albus* pure powder, juice and capsule in percentage of total fat (%)

Fatty acid methyl ester	Percentage in fat (%)		
	Pure powder (in 3.11g)	Juice (in 0.18 g)	Capsule (in 1.76g)
SATURATED FAT			
Butryic	0.05	0.00	0.00
Caproic	0.04	0.27	0.00
Caprylic	0.06	3.69	0.06
Capric	0.09	3.11	0.06
Undecanoic	0.02	0.05	0.00
Lauric	0.06	38.16	0.88
Tridecanoic	0.09	0.06	0.05
Myristic	1.94	12.15	1.52
Pentadecanoic	1.13	0.12	0.77
Palmitic	**24.58**	11.85	22.36
Heptadecanoic	1.52	0.15	1.16
Stearic	**9.14**	22.43	9.04
Arachidic	0.32	0.20	0.38
Henicosanoic	0.18	0.13	0.27
Behenic	0.68	0.00	0.00
Tricosanoic	3.57	0.37	4.79
Lignoceric	0.00	0.43	0.36
Total	38.65	93.13	41.69
MONOUNSATURATED FAT			
Myristoleic	0.04	0.06	0.81
Cis-10-Pentadecanoic	1.12	0.10	0.87
Palmitoleic	4.04	0.62	2.48
Cis-10-Heptadecanoic	0.81	0.00	0.75
Oleic	**27.55**	3.52	27.13
Cis-11-Eicosenoic	3.62	0.00	2.82
Erucic	0.69	0.00	0.00
Nervonic	0.78	0.00	0.00
Total	38.65	4.29	34.85
POLYUNSATURATED FAT (Omega-3 and Omega-6 fatty acids)			
Linoeleic (Cis)	9.34	1.53	11.75

y-Linoleic (GLA)	0.57	0.00	0.72
Arachidonic (AA)	0.36	0.00	0.25
Cis-11,14-Eicosadienoic	0.76	0.00	0.93
a-Linoleic (ALA)	0.91	0.62	1.13
Cis-8,11,14- Eicosatrienoic	1.03	0.00	1.06
Cis-8,11,17- Eicosatrienoic	2.29	0.27	4.82
Cis-5,8,11,14,17- Eicosapentaenoic (EPA)	0.41	0.17	0.51
Cis-13,16-Docosahexaenoic (DHA)	0.00	0.00	0.00
Cis-4,7,10,13,16,19-Docosahexaenoic (DHA)	1.67	0.00	2.33
Total	17.33	2.59	23.48

4.0 Conclusion

Overall, the results show that the *Monopterus albus* pure powder is a rich source of protein that have the main health function to build and repair tissues, including muscles, bones, and skin as well as to restore energy when digested. The powder was also found to contain high amount of Vitamin E, whereby the primary role is to act as an antioxidant and scavenging loose electron or so-called “free radicals” that can damage cells. The powder has low fat content which is advantageous as a well-balanced diet should only contain low fat amount with more consumption on healthy fat such as monounsaturated and polyunsaturated fat. In this study, the powders was found to have high amount of oleic acid and total omega-3 and omega-6 acid. Nevertheless, the processing of the powder can be improved in order to achieve lower moisture content and water activity as to provide more stability and prolonged shelf-life to the powder. Improvement of processes may also give additional nutritional values.

5.0 References

1. Wijayanti, I. and Susilo, E. S. (2018). Proximate content of wild and cultured eel (*Anguilla bicolor*) in different part of body. *IOP Conf. Series: Erath and Enviromental Science* 116.
2. Wijayanti, I. and Susilo, E. S. (2017). Nutritional content of wild and cultured eel (*Anguilla bicolor*) from Southern Coast of Central Java. *Ilmu Kelautan* 23 (1): 17-44.
3. Kefas, M, Michael, K.G. Abubakar, K. A., Edward, A. and Wahide, J. A. (2014). Proximate and mineral contents of flesh and body parts of Oreochromis Niloticus and Synodontis Clarias in Mubi, Nigeria. *Global of J. Biology science* 8:7-13.
4. Mercer, D. G. (2008). Solar drying in developing countries: possibilities and pitfalls. Using Food Science and Technology to Improve Nutrition and Promote National Development (Edited by Robertson, GL and Lupien, J. R). International Union of Food Science and Technology. [www. iufost. org/publications/books/documents/Mercer. pdf.] site visited on, 11(05), 2012.
5. Puwastien P, Judprasong K, Kettwan E, Vasanachitt K, Nakngamanong Y, Bhattacharjee L (1999). Proximate composition of raw and cooked Thai Freshwater and Marine Fish. Journal of Food Composition and Analysis, 12, 9.
6. WHO Technical Report Series 935. Protein and Amino Acid Requirements in Human Nutrition: report of a joint FAO/WHO/UNU expert consultation. Report of a Joint WHO/FAO/UNU Expert Consultation. 2011.