

Minerals, Amino Acids and Fatty Acids Profile of Two Different Species of Catfish Epidermal Mucus

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ABSTRACT The minerals, amino acids (AAs) and fatty acids (FAs) profile of the epidermal mucus of *Clarias gariepinus* (African catfish) and *Clarias* sp. (local catfish) were determined. Minerals were identified by using atomic absorption spectrophotometer (AAS) and inductive coupled plasma mass spectrometry (ICPMS), amino acids by high liquid performance chromatography (HPLC) and fatty acids by gas chromatographic with flame ionisation detector (GC-FID). The levels of macroelements (K, Na, Mg, Ca and P) in the epidermal mucus of *Clarias* sp. were higher than in the *C.gariepinus* while the concentrations of trace elements (Cu, Zn, and Fe) of both catfish species are lower than the toxic levels described by FAO/WHO. The high level of AAs total content was found in *Clarias* sp. epidermal mucus (83.72 mg g⁻¹ fresh weight) mainly essential amino acids (EAA), where the EAA/total AA ratio (50.75 ± 0.423 mg g⁻¹ Fresh Weight) were comparable to FAO/WHO requirements. The epidermal mucus of *Clarias* sp. contained high amounts of polyunsaturated fatty acids (PUFAs) as compared to saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) while SFAs were found higher in *C.gariepinus*. This study suggested that local catfish, *Clarias* sp. despite of cultured (African) catfish, *C.gariepinus*, could be potentially used as ingredients to improve nutritive value and texture of functional foods for human consumption.

KEYWORDS: Catfish, epidermal mucus, minerals, amino acids and fatty acids

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Full Article

INTRODUCTION

One of the most popular freshwater fish for human consumption throughout the world is the catfish, including our country Malaysia. *Clarias gariepinus* (African catfish) is a lean and highly nutritious fish that is rich in vitamins, proteins and minerals, has little or no saturated fat and is low in carbohydrates (Ersoy *et al.*, 2009). Catfish is also rich in lipids and a good source of unsaturated fatty acid, including n-3 fatty acid that has a positive impact on human health (Hwang *et al.*, 2004). The lipid content, fatty acid and other chemical composition in catfish are affected by various factors such as their environment conditions, size of fish, species, tissue, diet (Hwang *et al.*, 2004), water temperature, seasons, habitat and water salinity (Thammapat *et al.*, 2010). However, the study was limited to the fish fillet, and no on other parts of the fish such as the epidermal mucus.

Mucus, on the other hand, is the final protective barrier between fish and the environment. In most conditions, it is regarded as useless and discarded during fish processing (Mat Jais *et al.*, 1998). The coating of mucus that makes fish so slippery is produced by epidermal mucous cells and is believed to serve primarily for protection (Shephard, 1993 & 1994). Two lines of evidence support this view: (a) removal of mucus renders fish susceptible to attack by bacteria, fungi and parasites and (b) the mucus coating tends to be thicker on naked or sparsely scaled fishes than on those that are heavily scaled, although there are exceptions among the more active fishes, such as tunas (Lewis, 1970). The presence of amino acids and lipids in fish mucus has been largely overlooked, although they are found in mucus from other sources, such as that of the human cervix and submaxillary gland, canine gastric juice and snail epithelium. In 1970, Lewis reported that epidermal mucus from a catfish contained 98.07 % of lipid mixture where other hydrocarbons, squalene, sterol and wax

esters, triglycerides, cholesterol, diglycerides, monoglycerides, free fatty acids and phospholipids were also identified.

The chemical composition of food materials has an important role in human health in supply of essential nutrients for maintaining prosperous health (Chalamaiah *et al.*, 2012). Chemical composition of catfish epidermal mucus is also important in nutritive perspective of human health. Furthermore, evaluating the functional properties of epidermal mucus of catfish requires a clear idea on their biochemical composition, which can provide a platform for identification of the molecules responsible for the different biological activities. Therefore, this study was carried out to determine the minerals, amino acids and fatty acids profiles of *C.gariepinus* and *Clarias* sp. epidermal mucus that are found in Sabah, Malaysia.

METHODOLOGY

Mucus Collection

In this study, two types of catfish species (*Clarias gariepinus* and *Clarias* sp.) were used. Healthy and adult (both sexes) African catfish (*Clarias gariepinus*), was obtained from fish pond in Hatchery Universiti Malaysia Sabah (UMS), Sabah, Malaysia while the local catfish (*Clarias* sp.), was obtained (wild catch) from Bongawan River, Sabah, Malaysia. Both catfish species were identified and confirmed by Professor Dr. Abdul Hamid Ahmad, the expert from Institute For Tropical Biology and Conversation, Universiti Malaysia Sabah (UMS), Sabah, Malaysia. All fish were allowed to settle down in two different tanks in the laboratory at least for 3 days before experimentation (Mat Jais *et al.*, 1998). Mucus was collected as described by Mat Jais *et al.*, 1998 and Nagashima *et al.*, 2004 with slight modification. Live whole fish were cleaned by washing them with distilled water to remove any apparent dirt. Fish were then weighed and placed into an enclosed clean plastic bag (12" X 18", ventral side of the body facing downward), one fish per bag. An estimated amount of distilled water (v / v fish / distilled water 1 : 1) was added and transferred into a freezer (- 20 °C) for 2 hours to induce a hypothermic stress condition where stressed fish will naturally secrete copious amounts of mucus (Mat Jais *et al.*, 1998). The mucus was collected by carefully scrapped from the dorsal body using a clean plastic spatula. Ventral skin mucus was not collected to avoid blood, urino-genital, intestinal and sperm contamination (Nagashima *et al.*, 2004; Ebran *et al.*, 1999; Hellio *et al.*, 2002; Su, 2011; Jurado *et al.*, 2015). The mucus was centrifuged at 12, 000 x g for 10 minutes to get the bottom gel-like layer and the upper watery (supernatant) layer was discarded. The mucus harvest was immediately frozen at - 20 °C to prevent any bacterial contamination. The mucus samples obtained from individual fish of each species were pooled to yield one mucus sample per fish species.

Mineral analysis

To remove carbon, the epidermal mucus of *C.gariepinus* and *Clarias* sp. (1 g) was ignited and incinerated in a muffle furnace at 550°C for 24 hours. The ash was wetted with distilled water, and dissolved with 1 mL of nitric acid (HNO₃). The mixture was shaken and filtered using filter paper. The filtrate was used for mineral determination (Yaich *et al.*, 2011). The macro minerals: sodium (Na), potassium (K), magnesium (Mg), and calcium (Ca) and trace minerals: iron (Fe), zinc (Zn), and copper (Cu) were determined using a Hitachi Z-5000 atomic absorption spectrophotometer (AAS) and also an air-acetylene burner was used. Selenium (Se) was determined using a Perkin Elmer ELAN 9000 inductive coupled plasma mass spectrometry (ICP-MS). The concentrations of the elements were determined from calibration curves of the standard elements. The results were expressed in mg 100 g⁻¹ fresh weight (FW) basis and all measurements were performed in triplicate.

Amino Acid Determination

The content of amino acids (AAs) in *C.gariepinus* and *Clarias* sp. epidermal mucus were determined according to Benjama and Masniyom (2011) with some slight modification. The epidermal mucus of both catfish species (0.20 g) was weighed in screw cap test tubes to which 5 mL of 6 N hydrochloride acid (HCl) was added. The test tubes were tightly capped and placed in an oven at 110°C for 24 hours to allow complete hydrolysis. The hydrolysates were then cooled down to room temperature. The cooled hydrolysates were quantitatively transferred into 100 mL volumetric flask, with 4 mL of internal standard; 2.5 mM of DL-2-aminobutyric was added. The solution was then brought to 100 mL with ultrapure water. Approximately 100 mL of the sample solution was filtered through filter paper before filtering again through syringe filter. Next, 10 µL filtered solution was collected into a microcentrifuge tube for derivatization process. 100 µL of AccQ Fluor reagent was added and the volume of 5 µL of samples were injected into the high performance liquid chromatography (HPLC) (50 pmole of AABA = 5.156 ng AABA). The HPLC (Waters Corporation e2695, Milford, MA, USA) was equipped with degasser, autosampler, and fluorescence detector was used to analyse AAs in epidermal mucus of both catfish species. The mobile phase used was: (A) AccQ Tag Eluent A (100 mL diluted with 1000 mL of ultrapure water) and (B) HPLC grade acetonitrile (60%). The mobile phase was filtered through 0.45 µm cellulose membrane filters before it was used. All separation was carried out on an AccQ Tag column (3.9 x 150 mm) (Waters Corporation, Milford, MA, USA), with the column temperature set at 36°C, flow rate 1.0 mL/minute. The fluorescence detector was operated with a 250 nm excitation and a 395 nm emission wavelength and the run time for the analysis was 50 min. The quantity of each AA was determined from the peak area of known quantity of AA standard mixture and peak area of individual AA in sample that contained internal standard. The amount of each AA in epidermal mucus of both catfish species was expressed in percentage (w/w) basis. All the measurements of AAs were performed in triplicate.

Fatty Acid Determination

The fatty acids (FAs) of catfish epidermal mucus were determined using gas chromatographic with flame ionisation detector (GC-FID) for quantification of their methyl esters (FAMES) (Yaich *et al.*, 2011). For the extraction of oil from epidermal mucus of *C.gariepinus* and *Clarias* sp., 1 g of samples was extracted with 90 mL of petroleum ether in a Soxtec system to obtain the oil as well as to determine its fat content. Hexane (1 mL) was added to 0.1 mL of oil samples, and then 1 mL of sodium methoxide solution was added to the oil solution. The mixture was stirred vigorously using a vortex mixer for 10 seconds and later was allowed to stand for 10 min to separate the clear solution of FAMES from the cloudy aqueous layer. The upper layer of the FAMES was collected and was analysed by Hewlett-Packard 5890 series II plus GC-FID fitted with a capillary DB 23 Supelco column (30 m 0.25 mm 0.25 µm, Sigma-Aldrich, St. Louis, MO, USA). The injection volume was 1 µL, the temperature column was programmed from 30 to 250 °C with the increase of 1 °C min⁻¹, and the injection and detector temperatures were set at 250 °C and 260 °C respectively with the split ratio of 1:100, and the carrier gas was hydrogen with a flow rate of 1.3 mL min⁻¹. Identification and quantification of FAMES were accomplished by comparing the retention times of the peaks with those of pure FAME standard (Sigma, USA) analysed under the same conditions. The results were expressed as a percentage of individual FAs in the lipid fraction. All measurements were performed in triplicate.

Statistical analysis

Data collected in this study were analyzed using IBM SPSS version 24.0. One way analysis of variance (ANOVA) and Tukey post-hoc analysis test was used to compare differences in the means of the mineral, amino acid contents and fatty acid profile of *C.gariepinus* and *Clarias* sp. epidermal

mucus. A significant difference was considered at the level of $p < 0.05$. All the samples and analysis were measured in three replicates and presented as mean \pm standard deviation.

RESULT AND DISCUSSION

Table 1 gives the mineral content (macro and trace elements) in the epidermal mucus of *C.gariepinus* and *Clarias* sp.. Statistical analyses of results by ANOVA showed significant differences among the levels of K, Na, Mg, Ca, P and Fe and there were no significant differences among the levels of Zn and Cu in the epidermal mucus of the catfish studied. The levels of macroelements K, Na, Mg, Ca and P in the epidermal mucus of *Clarias* sp. were higher than in the *C.gariepinus* epidermal mucus. Among the main elements, the highest level was potassium (*C.gariepinus*: 0.276 ± 0.002 ; *Clarias* sp.: 0.470 ± 0.005), respectively. Low concentrations of Na and high of K were observed and makes epidermal mucus of both catfish species a good meal for human health, especially in the case of cardiovascular disease prevention, the Na/K ratio in food should be less than 1 (Njinkoue et al., 2016) as shown in Table 1. Both Ca/P ratio for both catfish species epidermal mucus gave similar values and it was less than 1. The Ca/P ratio in food should be about 1 (Njinkoue et al., 2016). Mg, Ca and P were also the major minerals found in crude catfish viscera oil (Sathivel et al., 2003). The concentrations of Cu, Zn, and Fe (trace elements) in epidermal mucus of both catfish species are lower than the toxic levels described by FAO/WHO (2001). The microelements are not only toxic but also essential for human nutrition (Francisca et al., 2013).

Table 1. The mineral content in the epidermal mucus of *Clarias gariepinus* and *Clarias* sp.

Mineral (mg 100 g ⁻¹)	<i>C.gariepinus</i>	<i>Clarias</i> sp.
K	0.276 ± 0.002^i	0.470 ± 0.005^k
Na	0.156 ± 0.001^g	0.249 ± 0.004^h
Mg	0.024 ± 0.000^d	0.038 ± 0.000^f
Ca	0.018 ± 0.000^c	0.028 ± 0.000^e
Zn	0.001 ± 0.000^a	0.002 ± 0.000^a
Fe	0.002 ± 0.000^a	0.010 ± 0.000^b
Cu	0.001 ± 0.000^a	0.002 ± 0.000^a
P	0.272 ± 0.002^i	0.386 ± 0.004^j
Na/K (ratio)	0.565 ± 0.500	0.529 ± 0.800
Ca/P (ratio)	0.066 ± 0.000	0.072 ± 0.000

* All values are mean \pm standard deviation of triplicate experiments.

* abc Different superscripts indicate significant ($P < 0.05$) differences between samples.

The amino acid (AA) content of *C.gariepinus* and *Clarias* sp. epidermal mucus is presented in Table 2. The total content of AAs in *C.gariepinus* epidermal mucus was 3.711 ± 0.079 % w/w (37.11 mg g⁻¹ Fresh Weight) while *Clarias* sp. epidermal mucus was 8.372 ± 0.085 % w/w (83.72 mg g⁻¹ Fresh Weight). This value was comparable to its corresponding crude protein content (*C.gariepinus*: 6.34 ± 1.69 %; *Clarias* sp.: 7.92 ± 0.63 %), indicating that the amount of non-protein nitrogenous materials in this catfish epidermal mucus was insignificant. Nine essential amino acids (EAAs) including threonine, arginine, tyrosine, valine, methionine, lysine, isoleucine, leucine, phenylalanine and seven non-EAAs (NEAAs) including aspartic acid, serine, glutamic acid, glycine, histidine, alanine, and proline were present in epidermal mucus of *C.gariepinus* and *Clarias* sp., except for tryptophan and cysteine which were eliminated after acid hydrolysis of the protein samples (not shown in the result). The EAAs of *C.gariepinus* epidermal mucus ranged from 0.107 ± 0.004 to 0.317 ± 0.009 % w/w,

while *Clarias* sp. epidermal mucus ranged from 0.054 ± 0.002 to 0.749 ± 0.004 % w/w. The EAA/total AA ratio suggests that more than 50 % of the AAs were EAAs. The result also indicates a good ratio of EAAs to NEAAs (*C.gariepinus* : 1.114 ± 0.013 ; *Clarias* sp.: 1.030 ± 0.007).

Table 2. The amino acids content in the epidermal mucus of *Clarias gariepinus* and *Clarias* sp.

Amino Acids (% w/w)	<i>C.gariepinus</i>	<i>Clarias</i> sp.
Aspartic acid (Asp)	0.325 ± 0.003^{hi}	0.751 ± 0.007^n
Serine (Ser)	0.236 ± 0.003^f	0.600 ± 0.014^m
Glutamic acid (Glu)	0.535 ± 0.003^l	1.225 ± 0.006^o
Glycine (Gly)	0.231 ± 0.006^f	0.609 ± 0.007^m
Histidine (His)	0.058 ± 0.007^b	0.156 ± 0.003^{de}
Alanine (Ala)	0.231 ± 0.002^f	0.496 ± 0.008^l
Proline (Pro)	0.136 ± 0.007^{cde}	$0.286 \pm 0.004^g^h$
Thereonine (Thr)	0.224 ± 0.011^f	0.525 ± 0.003^l
Arginine (Arg)	0.317 ± 0.009^{hi}	0.748 ± 0.004^n
Thyrosine (Tyr)	0.107 ± 0.004^c	$0.283 \pm 0.002^g^h$
Valine (Val)	0.174 ± 0.003^e	0.430 ± 0.005^k
Methionine (Met)	0.261 ± 0.003^{fg}	0.054 ± 0.002^b
Lysine (Lys)	0.306 ± 0.008^h	0.749 ± 0.004^n
Isoleucine (Ile)	0.159 ± 0.005^{de}	0.357 ± 0.003^{ij}
Leucine (Leu)	0.286 ± 0.002^{gh}	0.732 ± 0.009^n
Phenylalanine (Phe)	0.122 ± 0.001^{cd}	0.371 ± 0.004^j
Total AAs	3.711 ± 0.079	8.372 ± 0.085
Total EAAs	1.956 ± 0.046	4.249 ± 0.036
Total non-EAAs	1.755 ± 0.033	4.123 ± 0.049
EAAs/Total AAs	0.527 ± 0.058	0.507 ± 0.042
EAAs/Non-EAAs	1.114 ± 0.013	1.030 ± 0.007

* All values are mean \pm standard deviation of triplicate experiments.

* ^{abc}Different superscripts indicate significant ($P < 0.05$) differences between samples.

* % w/w : percentage weight per weight

It was discovered that, lysine, arginine, leucine were found to be the highest EAA in epidermal mucus of *Clarias* sp. representing 17.62, 17.45, 17.22 % of total EAAs, respectively. Arginine was the highest EAA found in epidermal mucus of *C.gariepinus*, representing 16.20 % of total EAAs. Arginine is also termed as 'conditionally EAA' that can usually be synthesized by the human body under normal condition. This EAA found in epidermal mucus of catfish where the highest was found in *Clarias* sp., will be important to certain conditions such as during growth, illness, and metabolic stress, as well as during the first month of a new born (Saini et al., 2013; Chan et al., 2014). The second highest EAA presence in epidermal mucus of *C.gariepinus* was lysine (15.64 % of total EAAs) while in epidermal mucus of *Clarias* sp. was thereonine (12.35 % of total EAAs). The third highest EAA presence in epidermal mucus of *C.gariepinus* and *Clarias* sp.. were: leucine > methionine > thereonine > valine > isoleucine > phenylalanine > thyrosine; and valine > phenylalanine > isoleucine > thyrosine > methionine, respectively. For non-EAAs, epidermal mucus of *Clarias* sp. showed high amount of glutamic acid (14.63 % of the total AAs,) and followed by epidermal mucus

of *C.gariepinus* (14.41 % of the total AAs). The next highest non-EAAs in epidermal mucus of *C.gariepinus* and *Clarias* sp. were: aspartic acid > serine > glycine and alanine > proline > histidine and aspartic acid > glycine > serine > alanine > proline > histidine, respectively.

The fatty acids profile of *C.gariepinus* and *Clarias* sp.. epidermal mucus are presented in Table 3. *C.gariepinus* epidermal mucus had higher proportions of saturated fatty acids (SFAs) > polyunsaturated fatty acids PUFAs > monounsaturated fatty acids (MUFAs) while *Clarias* sp. had higher proportions of PUFAs > SFAs > MUFAs. This showed that *C.gariepinus* epidermal mucus has a high composition of saturated FAs, representing almost 70% of total FAs. The main SFAs present in *C.gariepinus* epidermal mucus are arachidic acid (C20:0), and stearic acid (C18:0) and both of these FAs represent 50 % of the total SFA. Similar findings were reported in other epidermal mucus of *Channa striatus*, the sum of C16:0 and C18:0 represent 50 % of total SFA (Mat Jais et al., 1998). The PUFAs in *Clarias* sp. epidermal mucus range from 11.27 ± 0.015 to 17.17 ± 0.029 % with the sum of PUFAs of 71.64 ± 1.97 %. This value suggested that more than half of the total FAs detected were of PUFAs in epidermal mucus of *Clarias* sp.. Docosahexaenoic (C22:6 ω 3) (DHA) representing 23.96 % of the total PUFAs indicates the main PUFA detected is DHA followed by EPA (C20:5 ω 3) representing 19.58 % of the total PUFAs. As discussed earlier, the wild catfish epidermal mucus (*Clarias* sp.) contains high amounts of PUFAs as compared to SFAs and MUFAs. Omega-3 PUFAs help prevent the growth of atherosclerotic plaque that affects blood clotting, blood pressure and improves the immune function, while ω -6 PUFAs decrease low density lipoprotein cholesterol (LDL-C) and may also decrease high density lipoprotein cholesterol (HDL-C) which adversely induces heart disease risk (Chan et al., 2017). Therefore, it is important to maintain a balanced consumption of ω -6 and ω -3 in diet based on a ratio of ω -6/ ω -3 < 10 recommended by the WHO (Matanjun et al., 2009). In the current study, the ω -3 PUFAs have a higher value of 44 % of the total PUFAs as compared to ω -6 PUFAs having only 35 %. This in turn leads to a low ω -6/ ω -3 ratio which is within the WHO standard. The low ω -6/ ω -3 ratio in *Clarias* sp. epidermal mucus might be due to the presence of a high concentration of DHA. This suggests that epidermal mucus of *Clarias* sp. might be a potential food or supplement source to improve the ω -3 deficiency, especially DHA as compared to others epidermal mucus of catfish species. Previous study had shown the beneficial effects of DHA on immune functions (Tomobe et al., 2000). Hence, epidermal mucus of *Clarias* sp. may be used to compliment diet containing sufficient EPA but low in DHA.

The FA compositions of the dietary fats, particularly of some individual FA, are of great importance in human nutrition and health concern. Low intake of saturated fat and increased PUFAs to SFAs ratio are associated with a lower risk of human coronary heart diseases (Kumar et al., 2011). Thus, the PUFA /SFA ratio is one of the parameters used to assess the nutritional quality of the lipid fraction of foods. In the present study, the PUFA/SFA ratio of *Clarias* sp. epidermal mucus was 1.23, which is within the nutritional guidelines that recommended a PUFA/SFA ratio above 0.4 (Kumar et al., 2011), except for *C.gariepinus* epidermal mucus where the PUFA/SFA ratio was below 0.4. Other than PUFA/SFA ratio, the AI and TI are also related to nutritional factors linked with coronary diseases, which are also used to assess the FA nutritional quality. The AI indicates the relationship between the sum of the main saturated FAs and that of the main class of unsaturated FAs, while TI shows the relationship between the pro-thrombogenic and the anti-thrombogenic of FAs as thrombosis is a central event in atherosclerosis (Ghaeni et al., 2013). Therefore, lower AI and TI maintain better nutritional quality of the FAs. The AI and TI epidermal mucus of *C.gariepinus* and

Table 3. Fatty acids profiles in the epidermal mucus of *Clarias gariepinus* and *Clarias* sp. (in % of total lipid).

Structure	Fatty Acid Methyl Ester	<i>Clarias gariepinus</i>	<i>Clarias</i> sp.
C 4	Butyric	ND	ND
C 6	Caproic	ND	ND
C 8	Caprylic	ND	ND
C 10	Capric	ND	ND
C 11	Undecanoic	ND	ND
C 12	Lauric	6.740 ± 0.005 ^a	ND
C 13	Tridecanoic	ND	ND
C 14	Myristic	7.730 ± 0.015 ^c	ND
C 14:1	Myristoleic	ND	ND
C 15	Pentadecanoic	ND	ND
C 15:1	Cis-10-Pentadecenoic	ND	8.720 ± 0.068 ^d
C16	Palmitic	8.960 ± 0.006 ^d	8.970 ± 0.009 ^d
C 16:1	Palmitoleic	ND	ND
C 17	Heptadecanoic	ND	9.870 ± 0.008 ^e
C 17:1	Cis-10-Heptadecanoic	ND	ND
C18	Stearic	10.690 ± 0.007 ^f	10.710 ± 0.027 ^f
C 18:1 ω 9	Elaidic (Trans)	ND	ND
C 18:1 ω 9c	Oleic (Cis)	10.870 ± 0.003 ^f	10.900 ± 0.021 ^f
C 18:2 ω 6	Linolelaidic (Trans)	ND	ND
C 18:2 ω 6	Linoleic (Cis)	11.260 ± 0.016 ^g	11.270 ± 0.015 ^g
C 18:3 ω 6	γ -Linolenic	ND	ND
C 18:3 ω 3	α -Linolenic	ND	ND
C 20	Arachidic	12.510 ± 0.006 ^h	12.530 ± 0.012 ^h
C 20:1 ω 9	Cis-11-Eicosenoic	ND	ND
C 20:2	Cis-11,14-Eicosadienoic	ND	ND
C 20:3 ω 6	Cis-8,11,14-Eicosatrienoic	ND	13.600 ± 0.015 ⁱ
C 20:3 ω 3	Cis-11,14,17-Eicosatrienoic	ND	ND
C 21: 0	Henicosanoic	ND	ND
C 20:4 ω 6	Arachidonic	ND	ND
C 20: 5 ω 3	Cis-5,8,11,14,17-eicosapentaenoic	ND	14.030 ± 0.012 ^j
C 22	Behenic	ND	ND
C 22:1 ω 9	Erucic	ND	ND
C 22:2	Cis-13,16-Docosadienoic	ND	15.550 ± 0.020 ^k
C 23:0	Tricosanoic	ND	16.040 ± 0.032 ^l
C 22:6 ω 3	Cis-4,7,10,13,16,19-Docosahexaenoic	ND	17.170 ± 0.029 ^m
C 24:0	Lignoceric	ND	ND
C 24:1	Nervonic	ND	ND
Σ SFAs		46.650 ± 1.720	58.140 ± 0.580
Σ MUFAs		10.870 ± 0.020	19.620 ± 0.900
Σ PUFAs		11.260 ± 0.980	71.640 ± 2.500
Σ PUFAs/ Σ SFAs		0.240 ± 0.001	1.230 ± 0.040
ω -6/ ω -3		0	0.790 ± 0.008
AI ^I		0.170 ± 0.003	0.002 ± 0.001
TI ^{II}		0.690 ± 0.007	0.003 ± 0.001

* All values are mean ± standard deviation of triplicate experiments.

* abc Different superscripts indicate significant ($P < 0.05$) differences between samples.

* I AI: atherogenic index = $(C12:0 + C14:0 + C16:0) / (\omega$ -3PUFAs + ω -6PUFAs + MUFAs)

* II TI: thrombogenic index = $(C14:0 + C16:0 + C18:0) / (0.5 \omega$ -6PUFAs + 3PUFAs + ω -3PUFAs) / ω -6PUFAs)

* Not detected (ND); Omega (ω); Carbon (C); Fatty acids (FAs); Saturated fatty acids (SFAs); Monounsaturated fatty acids (MUFAs); Polyunsaturated fatty acids (PUFAs).

Clarias sp., in the current study were 0.17 ± 0.003 and 0.69 ± 0.007 ; 0.02 ± 0.001 and 0.003 ± 0.001 respectively. In addition, this TI value was also lower than products such as lamb (1.58), bovine meat (1.08), lean pork (1.37) and milk based products (2.1) (Chan *et al.*, 2017). In view of this, the addition of epidermal mucus of catfish especially the wild catfish to meat products, may not only be useful for technological reasons (gel forming) but also could be of a more satisfactory strategy for the development of healthier lipid formulation (Kumar *et al.*, 2011). For an example, the meat system with the addition of seaweeds (that had lower AI and TI index : 0.03 to 0.04) (Chan *et al.*, 2017) can caused an increased in ω -3 PUFAs and decrease in the ω -6/ ω -3 PUFAs ratio, as well as the TI index (López-López *et al.*, 2009).

CONCLUSION

In conclusion, different species of catfish will give different amount of chemical composition. This study discovered that epidermal mucus of *Clarias* sp. contained high levels of macroelements (minerals), amino acids and high amounts of PUFAs as compared to SFAs and MUFAs which can contribute positively to human nutritional requirements and consumptions. The nutritional compositions found in both catfish species from this study can be recommended for human consumption, health and become an important functional ingredient in the food industry.

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REFERENCES

- [1] Benjama, O. & Masniyom, P. (2011). Nutritional composition and physicochemical properties of two green seaweeds (*Ulva pertusa* and *U.intestinalis*) from the Pattani Bay in Southern Thailand. Songklanakar. *Journal of Science and Technology*, **33**(5), 575-583.
- [2] Chan, P. T., Matanjun, P., Yasir, S. M. & Tan, T. S. (2014). Antioxidant and hypolipidaemic properties of red seaweed, *Gracilaria changii*. *Journal of Applied Phycology*, **26**, 987-997.
- [3] Chan, P.T. & Matanjun, P. (2017). Chemical composition and physicochemical properties of tropical red seaweed, *Gracilaria changii*. *Food Chemistry*, 302-310.
- [4] Chalamaiah, M., Dinesh Kumar, B., Hemalatha, R. & Jyothirmayi, T. (2012). Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: A review. *Food Chemistry*, 3020-3038.
- [5] Ebran, N., Julien, S., Orange, N., Saglio, P., Lemaitre, C. & Molle, G. (1999). Pore-forming properties and antibacterial activity of proteins extracted from epidermal mucus of fish. *Comparative Biochemistry and Physiology Part A*, **122**, 181-189.
- [6] Ersoy, B. & Ozeren, A. (2009). The effect of cooking methods on mineral and vitamin contents of African catfish. *Food Chemistry*, **115**, 419-422.
- [7] FAO (2001). Human vitamin and mineral requirements, Report of a Joint FAO/WHO Expert Consultation Bangkok, Thailand, Food and Nutrition Division-FAO, Rome.
- [8] Francisca, G., Ogamune, R., Odulate, D. & Arowolo, T. (2013). Seasonal variation in heavy metal content of tongue sole, *Cynoglossus brownii* and croaker, *Pseudotolithus typus* from Lagos and Delta states, Nigeria. *Br. J. Appl. Sci. Technol.* **3**(4), 1548-1557.
- [9] Ghaeni, M., Ghahfarokhi, K. N. & Zaheri, L. (2013). Fatty Acids Profile, Atherogenic (IA) and Thrombogenic (IT) Health Lipid Indices in *Leiognathusbindus* and *Upeneussulphureus*. *Marine Science Research & Development*, **3**(4), 3-5.

- [10] Hellio, C., Pons, A.M, Beaupoil, C., Bourgoignon, N. & Gal, Y.L. (2002). Antibacterial, antifungal and cytotoxic activities of extracts from fish epidermis and epidermal mucus. *International Journal of Antimicrobial Agents*, **20**, 214-219.
- [11] Hwang, K.T., Kim, J.E., Kang, S.G., Jung, S.T., Park, H.J. and Weller. C.L. (2004). Fatty acid composition and oxidation of lipids in Korean catfish. *J. Am. Oil Chem. Soc.*, **81**(2), 123–127.
- [12] Jurado, J., Fuentes-Almagro, C.A., Guardiola, F.A., Cuesta, M.A., Esteban, A. and Prieto-Álamo, M.J. (2015). Proteomic profile of the skin mucus of farmed gilthead seabream (*Sparus aurata*). *Journal of Proteomics*, **120**: 21-34
- [13] Lewis, R.W. (1970). Fish cutaneous mucus: a new source of skin surface lipid. *Short Communication*, September 14.
- [14] López-López, I., Bastida, S., Ruiz-Capillas, C., Bravo, L., Larrea, M. T., Sánchez-Muniz, F. & Jiménez-Colmenero, F. (2009). Composition and antioxidant capacity of low-salt meat emulsion model systems containing edible seaweeds. *Meat Science*, **83**, 492–498.
- [15] Kumar, M., Kumari, P., Trivedi, N., Shukla, M. K., Gupta, V., Reddy, C. R. K. & Jha, B. (2011). Minerals, PUFAs and antioxidant properties of some tropical seaweeds from Saurashtra coast of India. *Journal of Applied Phycology*, **23**, 797–810.
- [16] Matanjun, P., Mohamed, S., Mustapha, N. M. & Muhammad, K. (2009). Nutrient content of tropical edible seaweeds, *Eucheuma cottonii*, *Caulerpa lentillifera* and *Sargassum polycystum*. *Journal of Applied Phycology*, **21**(1), 75–80.
- [17] Mat Jais, A.M., Matori, M. F., Kittakoop, P. & Sowanborirux, K. (1998). Fatty Acid Compositions in Mucus and Roe of Haruan, *Channa Striatus*, for Wound Healing. *Gen. Pharmac.*, **30** (4), 561-563.
- [18] Nagashima, Y., Takeda, M., Ohta, I., Shimakura, K. and Shiomi, K. (2004). Purification and properties of proteinaceous trypsin inhibitors in the skin mucus of pufferfish *Takifugu pardalis*. *Comparative Biochemistry and Physiology, Part B*, (**138**), 103-110.
- [19] Njinkoue, J.M, Gouado, I., Tchoumboungang, F., Yanga Ngueguim, J.H., Ndinteh, D.T., Fomogne-Fodjo, C.Y. & Schweigert, F.J. (2016). Proximate composition, mineral content and fatty acid profile of two marine fishes from Cameroonian coast: *Pseudolithus typus* (Bleeker, 1863) and *Pseudolithus elongatus* (Bowdich, 1825) . *NFS Journal*, **24**, 27-31.
- [20] Saini, R., Badole, S. L. and Zanwar, A. A. (2013). Arginine derived nitric oxide: key to health skin. In R. R. Watson & S. Zibadi (Eds.), *Bioactive dietary factors and plant extracts in dermatology*. Totowa, NJ: Humana Press, pp. 73–82.
- [21] Sathivel, S., Prinyawiwatkul, W., M.King, J, C.Grimm, C. and Llyod, S. (2003). Oil production from Catfish Viscera. *JAOCS*, **80**(4), 377-382.
- [22] Shephard, K.L. (1993). Mucus on the epidermis of fish and its influence on drug delivery. *Advanced Drug Delivery Review*, **11**, 403-417.
- [23] Shephard, K. L. (1994). Functions of fish mucus. *Reviews in Fish Biology & Fisheries*, **4**, 401-429.
- [24] Su, Y. 2011. Isolation and identification of pelteobagrin, a novel antimicrobial peptide from the skin mucus of yellow catfish (*Pelteobagrus fulvidraco*). *Comparative Biochemistry and Physiology, Part B*, **158**, 149-154.
- [25] Thammapat, P., Raviyan, P. & Siriamornpun, S. (2010). Proximate and fatty acids composition of the muscles and viscer of Asian catfish (*Pangasius bocourti*). *Food Chemistry*, **122**, 223-227.
- [26] Tomobe, Y. I., Morizawa, K., Tsuchida, M., Hibino, H., Nakano, Y. & Tanaka, Y. (2000). Dietary docosahexaenoic acid suppresses inflammation and immunoresponses in contact hypersensitivity reaction in mice. *Lipids*, **35**(1), 61–69.
- [27] Yaich, H., Garna, H., Besbes, S., Paquot, M., Blecker, C. & Attia, H. (2011). Chemical composition and functional properties of *Ulva lactuca* seaweed collected in Tunisia. *Food Chemistry*, **128**, 895–901.