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# Research Article
# Catfish (*Clarias gariepinus*): A Potential Alternative Raw Material for Surimi Production

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## Abstract

**Background and Objective:** The properties of surimi gel are influenced by the fish species used to generate the gel, their chemical compositions and the endogenous enzymatic activity in fish muscle. The aim of this research was to investigate the potential of *Clarias gariepinus* (local name "lele dumbo") as an alternative raw material for surimi production based on proximate composition, amino acid profile, protein composition and transglutaminase (TGase) and protease activity. **Materials and Methods:** This study used lele dumbo muscle as the raw material. The chemical properties of proximate composition, amino acid profile, protein composition and endogenous enzyme activity of the sample were determined using standard methods with three replicates. The results were presented as the means±standard deviation. **Results:** The moisture, ash, crude protein and crude fat content of fish muscle were 73.01±0.05, 0.78±0.02, 16.08±0.03 and 2.03±0.05%, respectively. The lele dumbo muscle has glutamine and lysine residues that can support TGase activity. Myofibrillar proteins were found as the major protein compounds in the fish muscle (16.57±0.03 mgN $g^{-1}$ muscle) and the sarcoplasmic and stromal protein contents were lower, 4.38±0.03 and 0.71±0.05 mgN $g^{-1}$ muscle, respectively. The TGase activity of the fish muscle was 0.18 U $mL^{-1}$ (ΔAbs. = 0.21), which was higher than the protease activity (ΔAbs. = 0.10). **Conclusion:** Lele dumbo is a potential alternative raw material for surimi production.

Key words: Lele dumbo, catfish, alternative raw material, surimi, TGase

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Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Catfish is one of the aquaculture species grown in Indonesia. According to the Indonesian Ministry of Marine Affairs and Fisheries[1], cultured catfish production reached 543.461 t in 2013, which is 23.17% higher than the previous year. Catfish (*Clarias gariepinus*), which has the local name "lele dumbo", is the most widely cultivated species. One of the advantages of lele dumbo is that it grows faster than the other species and can even reach a weight of 200-300 g at an age of 3 months[2]. Lele dumbo have been widely used for fresh consumption and partly exported in the form of fillets. Because of the current high production, in addition to an increasing value, lele dumbo can be used as an alternative raw material, especially for surimi production and for diversification of the fishery business in Indonesia.

Surimi is a refined myofibrillar protein that is produced by washing minced meat repeatedly with cold water (±4°C), mixing with cryoprotectants and freezing. The marine white fish species used in Indonesia for surimi production are primarily the threadfin bream (*Nemipterus japonicus*), bigeye snapper (*Priacanthus tayenus*), goatfish (*Upeneus sulphureus*), lizardfish (*Saurida tumbil*), white croaker (*Genyonemus lineatus)* and silver biddy (*Gerres oyena*)[3]. However, the main problem facing the surimi industry in Indonesia is the supply of raw materials[4]. The Government of Indonesia, through the Ministry of Marine Affairs and Fisheries, issued a regulation, No. 72/MEN-KP/II/2016, regarding restrictions on the use of fishing gear in Regional Fisheries Management in order to avoid over-exploitation of fishery resources in Indonesian waters. The regulation causes a limited supply of raw materials for the surimi industry, so it is necessary to find other fish sources, such as lele dumbo, as alternative surimi raw materials.

Surimi gel characteristics are highly dependent on the fish species and are influenced by the muscle composition, especially the myofibrillar versus sarcoplasmic protein and/or stroma protein concentrations, the endogenous transglutaminase activity and the proteolytic enzyme activity[5-7]. The myofibrillar protein is the largest proportion of the muscle used in surimi production and is responsible for the gel formation during heating[8]. Furthermore, endogenous transglutaminase and protease may affect surimi gel formation by cross linking or degrading myofibrillar proteins, respectively. Endogenous transglutaminase (TGase, EC 2.3.2.13) catalyzes the cross linking reactions of muscle proteins, especially myosin heavy chain (MHC), forming a covalent non-disulfide ε-(γ-glutamyl) lysine bond, which increases the elasticity of the gel[5,9]. Conversely, endogenous proteases have the opposite effect; they inhibit optimal gel formation due to proteolytic degradation of the myofibrillar proteins[10].

Studies on the potential of particular fish species as raw materials for surimi production have been widely reported; muscle characterization has been reported from two species of bigeye snapper[11], frigate mackerel (*Auxis thazard*) and catfish (*Clarias macrocephalus*)[6] and TGase characterization has been reported for tropical tilapia (*Oreochromis niloticus*)[12], bigeye snapper (*Priacanthus hamrur*), Indian oil sardine (*Sardinella longiceps*), tilapia (*Oreochromis mossambicus*) and the common carp (*Cyprinus carpio*)[5]. The muscle composition of fish and the endogenous enzyme activity, especially that of TGase and proteases are different for various species and may affect surimi gel formation[13,14]. However, information about the chemical composition of lele dumbo muscle is still very limited, so this type of fish has not been developed as a raw material for surimi production yet. Studies conducted by Osibona *et al.*[15] and Ibhadon *et al.*[16] have highlighted the fatty acid composition and the amino acid profile of *Clarias gariepinus* muscle. The aim of this study was to examine the potential of lele dumbo as an alternative raw material for surimi production based on the proximate composition, amino acid profile, protein composition, TGase and protease activities.

## MATERIALS AND METHODS

**Materials:** Live lele dumbo (*Clarias gariepinus*), with an average weight of 150-200 g, was purchased from a fish farm in Boyolali, Central Java, Indonesia. Immediately after harvest, the fish samples were transported to the laboratory. The fish were killed, washed with cold water (4°C), gutted, filleted, skinned and immediately used for further analysis.

**Chemicals and reagents:** CBZ-glutaminyl-glycine, hydroxylamine, glutathione, L-glutamic acid γ-monohydroxamate, trichloroacetic acid (TCA), 2-mercaptoethanol, trizma base (tris(hydroxymethyl) amino methane), casein hammarsten bovine, Folin and Ciocalteu's phenol reagent and L-tyrosine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents and chemicals were of analytical grade.

**Proximate analysis:** Moisture, ash, protein and fat contents of fish muscle were determined according to the AOAC method[17].

**Amino acid analysis:** Amino acids were analyzed using the AOAC method[17]. Minced fish were defatted using chloroform/methanol. The defatted sample was hydrolyzed using 6 N HCl, heated at a constant temperature of 110°C under an $N_2$ atmosphere for 24 h. The sample was filtered using a Spartan-HPLC syringe filter, 13 mm, 0.45 μm and then diluted with water at a ratio of 1:20 (v/v). The sample was derivatized with O-phthalaldehyde (OPA) and 9-Fluorenylmethyl chloroformate (FMOC). The HPLC conditions were eluent A: composition of $NaH_2PO_4$ + $H_2O$, eluent B: Composition of water+methanol+acetonitrile, a temperature of 40°C and a flow rate of 2 mL $min^{-1}$ for 30 min. The excitation wavelength was 340 nm and the emission wavelength was 450 nm.

**Determination of protein composition:** Fish muscle was fractionated according to the method of Hashimoto *et al.*[18]. Minced fish was homogenized with ten volumes of phosphate buffer, pH 7.5, using an Ultra Turrax homogenizer (Ultra-Turrax, T 50 basic, Werke Staufen, Germany). The resulting homogenate was centrifuged at 2800 xg for 15 min at 4°C using a Sorvall Biofuge Primo R centrifuge (Germany). The supernatant was separated and the precipitate was added to ten volumes of the same buffer, homogenized and centrifuged again. Both of these supernatants were combined and added to trichloroacetic acid to concentration of 5%. The resulting precipitate was separated by filtration and used as the fraction of sarcoplasmic protein. The filtrate was used as the fraction of non-protein nitrogen compound. Meanwhile, precipitates of the sarcoplasmic extraction were homogenized with ten volumes of phosphate buffer, pH 7.5, containing 0.45 M KCl and then centrifuged at 2800 xg for 15 min at 4°C. The procedure was repeated twice. Both supernatants were combined and used as the fraction of myofibrillar protein. The obtained precipitate was mixed with 0.1 N NaOH and stirred for 12 h at 4°C, then centrifuged at 2800 xg for 15 min at 4°C. The resulting supernatant was used as the alkali-soluble protein fraction. Finally, the remaining precipitates were used as the fraction of the stromal protein. Each fraction was analyzed for its nitrogen content using the Kjeldahl method[17].

**Preparation of the crude transglutaminase (TGase) extract:** The crude extract was prepared as described by Binsi and Shamasundar[5] with a slight modification. Minced fish was homogenized with four volumes of extraction buffer (10 mM Tris-HCl, pH 7.5, which contained 10 mM NaCl, 2 mM 2-Mercaptoethanol and 0.01% Triton X-100) at 8000 rpm for 10 min using an Ultra Turrax homogenizer (T 50 Basic, Werke Staufen, Germany). The homogenate was centrifuged at 10000 xg and 4°C for 30 min (Eppendorf Centrifuge 5417 R, Hamburg, Germany). The resulting supernatant was used as the crude TGase extract.

**Determination of TGase activity:** The TGase activity was measured by the method of Folk and Cole[19] (slightly modified) using hydroxamate as a standard product. The enzyme reaction solution was a mixture of 1000 mM Tris-acetate buffer (200 mM CBZ-glutaminyl-glycine, 200 mM hydroxylamine, 20 mM glutathione and 100 mM $CaCl_2$), pH 6.0 and the TGase crude extract. The enzymatic reaction was carried out by incubating the mixture at 37°C for 10 min in a water bath shaker and terminated by the addition of 12% TCA. The precipitate was removed by centrifugation at 5000 xg at 4°C for 10 min. The resulting hydroxamate product gives a color when treated with 5% $FeCl_3$ prepared in 0.1 N HCl. The absorbance at 525 nm of the resulting solution was measured using a UV-VIS spectrophotometer (Genesys 10S, China). L-glutamic acid γ-monohydroxamate was used as a standard. One unit of TGase was described as the amount of enzyme that catalyzed the formation of 1.0 μmole of hydroxamate in 1 min from CBZ-glutaminyl-glycine and hydroxylamine at pH 6.0 and 37°C.

**Preparation of the crude protease extract:** The protease crude extract was prepared according to the Murthy *et al.*[20] method with a slight modification. Minced fish was homogenized with 10 mM sodium acetate buffer, pH 7.5 and 5 mM calcium acetate at 8000 rpm for 10 min using an Ultra Turrax homogenizer (T 50 Basic, Werke Staufen, Germany). The resulting homogenate was centrifuged at 10000 rpm and 4°C for 30 min (Eppendorf Centrifuge 5417 R, Hamburg, Germany). The supernatant was used as the crude protease extract.

**Determination of protease activity:** Protease activity was analyzed using casein hammarsten as a substrate according to the method of An *et al.*[21] with a slight modification. The reaction mixture containing 5.0 mL of 6.5 mg mL casein in McIlvaine's buffer (0.2 M phosphate and 0.1 M citrate), pH 5.5, was pre-incubated at 55°C for 5 min. The enzymatic reaction was started by the addition of the crude enzyme (1.0 mL) into the reaction mixture and incubated at 55°C for 1 h in the water bath shaker. The reaction was stopped by the addition of 5.0 mL of 110 mM cold TCA. The reaction mixture was centrifuged at 5000 xg at 4°C for 10 min. The supernatant (2.0 mL) was collected and added to 5.0 mL of $Na_2CO_3$ and 1.0 mL of Folin's reagent, then incubated for 30 min. A sample blank (as a control treatment) was prepared in the same way but the crude enzyme was preheated in boiling water for 10 min before being added to the substrate mixture. The

absorbance was measured at 660 nm using a UV-VIS spectrophotometer (Genesys 10S, China). The protease activity is expressed as the absorbance difference between the enzyme sample and the blank ($\Delta A_{660\ nm}$).

**Surimi gel preparation:** Lele dumbo surimi was prepared by the conventional washing process[22]. Surimi was mixed with 3% NaCl and adjusted to a moisture content of 80%. The raw paste was packed into a 3 cm diameter casing and pre-incubated at 35°C for 30 min prior to a final heating at 90°C for 20 min. The heated gels were cooled immediately in ice water for 30 min.

**Folding test of surimi gel:** The folding test was performed using gel slices (3 cm diameter, 0.3 cm thickness) according to Botta[23] method. The maximum score (FT = 5, grade AA) indicated no cracks observed when a surimi slice was folded into quarters. The minimum score (FT = 1, grade D) was assigned if the slice broke into fragments at the pressure of a finger.

**Statistical analysis:** All analyses were performed in triplicate. The results were presented as a means±standard deviation.

## RESULTS AND DISCUSSION

**Proximate muscle composition:** Table 1 shows the proximate muscle composition of lele dumbo. The moisture and crude protein content were higher than reported by Ibhadon *et al.*[16], who studied the same species, while the ash content was lower than that reported by Osibona *et al.*[15] and Ibhadon *et al.*[16] (1.20 and 1.19%, respectively). The crude protein of lele dumbo was slightly lower than that of the Alaska pollock (17.18%), the first fish to be used for surimi production on a large scale[24]. Sikorski[25] reported that protein content in the fish muscle ranged from 11-24% (wet weight). Differences in the protein content could be related to species, nutrition and the reproductive cycle. The crude fat value was similar with that obtained by Ibhadon *et al.*[16] but higher than that of the Alaska pollock in the previous study[24]. The species most suitable as surimi raw materials have white meat and a low fat content. Fish with high fat content can produce residual fat in the surimi that causes oxidation and increased protein denaturation during frozen storage[26]. However, lele dumbo is classified as a freshwater fish with white meat and is considered to be a lean fish because the fat content is less than 5%[27]. This study also showed that the surimi fat content made from lele dumbo was less than 1%.

Table 1: Proximate composition of *Clarias gariepinus*

| Composition | Values (% wet weight) |
|---|---|
| Moisture | 73.01±0.05* |
| Ash | 0.78±0.02 |
| Crude protein | 16.08±0.03 |
| Crude fat | 2.03±0.05 |

*Values are given as the Means±SD of the three replicates

Table 2: Amino acid composition of *Clarias gariepinus*

| Amino acid | Amino acid composition (g/100 g) |
|---|---|
| Aspartic acid (Asp)* | 0.71 |
| Serine (Ser) | 3.87 |
| Glutamic acid (Glu)** | 9.35 |
| Glycine (Gly) | 0.49 |
| Histidine (His) | 2.91 |
| Arginine (Arg) | 8.03 |
| Threonine (Thr) | 3.36 |
| Alanine (Ala) | 2.96 |
| Proline (Pro) | 1.52 |
| Cysteine (Cys) | 1.11 |
| Tyrosine (Tyr) | 1.64 |
| Valine (Val) | 4.58 |
| Methionine (Met) | 2.79 |
| Lysine (Lys) | 3.75 |
| Isoleucine (Ile) | 7.90 |
| Leucine (Leu) | 8.55 |
| Phenylalanine (Phe) | 4.14 |

*Aspartic acid+asparagine (Asn), **Glutamic acid+glutamine (Gln)

**Amino acid composition:** The amino acid composition of fish muscle defines the surimi gel properties. Table 2 shows that glutamic acid was the highest compared to the other amino acids. This content of glutamic acid was the total content of glutamine and glutamic acid, since during hydrolysis, glutamine is hydrolyzed into glutamic acid[28]. The presence of glutamine and lysine residues in lele dumbo muscle is supportive evidence that endogenous TGase activity produces myosin heavy chain (MHC) cross-linking. The specific substrates of TGase are glutamine and lysine[29]. TGase catalyzes the acyl transfer reaction where the protein-bound glutamine residues as an acyl-donor and the primary amino group includes the ε-amino of lysine group as the acyl-acceptor[30]. The formation of MHC cross linking will produce surimi with high gel elasticity[31].

**Protein composition:** The protein composition of the fish muscle is correlated with the surimi gel characteristics. Surimi is a myofibrillar protein stabilized by heat so it has good gelation property. The most suitable species for surimi raw materials are white meat fish with a higher proportion of myofibrillar protein than sarcoplasmic protein. Table 3 shows that lele dumbo muscles contained large amounts of myofibrillar protein (16.57 mgN $g^{-1}$ muscle, 72% of the total muscle protein). These results are consistent with a previous study, which found that the myofibrillar protein in fish muscle

ranged from 65-75% (w/w) of the total protein and played an important role in coagulation and gel formation[32]. An excessive surimi washing process dissolves a small part of the myofibrillar protein. Park *et al.*[26] reported that two washing cycles were sufficient to produce primary grade surimi. This means that the quantity and quality of the myofibrillar protein must be maintained during the surimi production. An important finding in this experiment was that the myofibrillar protein content between the muscle and surimi made from lele dumbo was not significantly different ($p>0.05$). The sarcoplasmic protein content was 4.38 mgN $g^{-1}$ muscle (Table 3), 19% of the total muscle protein. This result was consistent with a previous study reported by Venugopal *et al.*[32] that the sarcoplasmic protein in fish is between 15-35% (w/w) of the total protein. The content of non-protein nitrogen compounds was 2.27 mgN $g^{-1}$ muscle. This protein consisted of amino acids, dipeptides, nucleotides, trimethylamine and urea, which would be wasted along with the sarcoplasmic protein in the washing process. Meanwhile, the protein content of the stroma in lele dumbo muscle was quite low (0.71 mgN $g^{-1}$ muscle). Kristinsson *et al.*[33] reported that dark muscle species, such as sardines and mackerel, have more stroma protein than ordinary white muscle. Stroma protein was related to the high mechanical strength of dark muscle that is characteristic of fish that are fast swimmers. During the production of surimi, the stromal protein is removed from the mince that is washed in the refining process. Table 3 shows that the protein soluble in alkali was 1.46 mgN $g^{-1}$ muscle. Chaijan *et al.*[34] reported that alkaline soluble protein showed the accumulation of denatured myofibrillar and sarcoplasmic proteins in fish muscle. Regarding the muscle protein composition, especially the myofibrillar and sarcoplasmic protein concentrations, lele dumbo is suitable for surimi production with good gel characteristics. Lele dumbo surimi in this study showed very strong gel-forming ability, achieving the maximum score on the folding test (FT = 5, grade AA). The myofibrillar protein content of lele dumbo was higher than that reported for two species of bigeye snapper (44-45%), which are the marine white fish species commonly used for surimi production[11]. Conversely, the sarcoplasmic protein content was lower than that reported in previous studies. Benjakul *et al.*[11] showed that the sarcoplasmic protein content of *Priacanthus tayenus* and *Priacanthus macracanthus* were 36 and 30%, respectively. Haard *et al.*[35] reported that sarcoplasmic proteins have a negative effect on the gel strength of fish myofibril and cause poor gelation characteristics. The sarcoplasmic protein was soluble in water and mostly removed during the washing step of the surimi-making process.

Table 3: Protein composition of *Clarias gariepinus*

| Nitrogenous compositions | Value (mg N $g^{-1}$ muscle) |
|---|---|
| Myofibrillar protein | 16.57±0.03* |
| Sarcoplasmic protein | 4.38±0.03 |
| Non-protein nitrogen | 2.27±0.12 |
| Stromal protein | 0.71±0.05 |
| Alkaline-soluble protein | 1.46±0.19 |

*Values are given as the Means±SD of the three replicates

Table 4: Endogenous enzymes activity of *Clarias gariepinus*

| Endogenous enzymes | ΔAbs. | Enzyme activity (U $mL^{-1}$) |
|---|---|---|
| Transglutaminase (TGase) | 0.21± 0.01 | 0.18± 0.00* |
| Protease | 0.10± 0.01 | ND |

*Values are given as the Means±SD of the three replicates, ND: Not detected, absorbance value was low and could not be converted to U $mL^{-1}$

**Activity of endogenous enzymes:** The thermal gelation of surimi involves endogenous enzymes in fish muscle, especially TGase and protease enzymes. Table 4 shows the activity of these two enzymes in lele dumbo muscle. The presence of the enzymes in the raw material of surimi should be monitored, since they have the opposing effects to reduce the surimi gel strength. Table 4 shows that the TGase activity of lele dumbo muscle was 0.18 U $mL^{-1}$. Worratao and Yongsawatdigul[36] reported that different fish species have different endogenous enzyme content, which might be affected by habitat, food and physiological condition. There is no standard value for TGase activity that should be present in surimi raw materials. In this study, the most interesting finding was that the presence of endogenous TGase in lele dumbo muscle could significantly increase surimi gel strength. This finding was demonstrated when a TGase inhibitor (iodoacetic acid) was added to the surimi paste and surimi gel strength decreased significantly ($p<0.05$). TGase is an endogenous enzyme that catalyzes the cross linking of proteins during surimi production. The cross linking of MHC results in the formation of non-disulfide covalent bonds ε-(γ-glutamyl)-lysine both intra and inter-molecularly[5]. Yongsawatdigul *et al.*[37] reported that the non-disulfide covalent bonds were stronger than hydrogen bonds and hydrophobic interactions. This phenomenon, called "suwari", is one of the important processes that occurs in the surimi paste during incubation at temperatures between 25-40°C. As the temperature is increased over 50-60°C, the 3-dimensional structure of the surimi gel is partially disrupted by endogenous protease. This phenomenon is called "modori" and results in the hydrolysis of MHC[38]. The results showed that the protease activity of lele dumbo muscle was very low (ΔAbs 0.10.) (Table 4), lower than that of the Tilapia muscle (ΔAbs.0.27)[20]. This protease activity was very low (absorbance value was low and could not be converted to U $mL^{-1}$), therefore, it is possible that the modori phenomenon during

the formation of the surimi gel is minimized. The presence of enzymes in surimi feed stocks should be monitored, since they have opposing effects in reducing the surimi gel strength.

## CONCLUSION

The chemical composition and the natural content of endogenous enzymes may vary in fish muscle and are affected by the species, fish habitat, food and physiological condition. This study found that lele dumbo has a high protein content, low fat content, high concentrations of glutamine and lysine residues, a high myofibrillar protein content relative to sarcoplasmic protein and TGase activity that was higher than the protease activity. These features strongly supported the formation of surimi gel, thus, it was concluded that lele dumbo is a potential alternative feedstock for surimi production.

## SIGNIFICANCE STATEMENT

This study shows that *Clarias gariepinus* ("lele dumbo") could be an alternative raw material for surimi production, based on proximate composition, amino acid profile, protein composition and TGase and protease activity. For manufacturers of surimi, lele dumbo can be used as an alternative raw material, especially when the harvest of fish is abundant. The potential findings of lele dumbo from Central Java Indonesia as a surimi raw material have not been published yet and research on optimizing production conditions of surimi from lele dumbo is ongoing.

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