Properties of gelatins from some selected *Pangasius* skins

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Abstract

The objective of this study was to characterize the gelatin from the skin of some members of Panasiidae family including striped catfish (*Pangasius hypophthalmus*), black ear catfish (*Pangasius larnaudii*) and diamond green fish (*Pangasius larnaudii* x *Pangasius bocoarti).* Proximate compositions and physicochemical properties of fish skin gelatins were evaluated. The highest yield (18.15 % dry basis) for gelatin extraction was obtained from the skin of diamond green fish. The major component of gelatins from various fish species were 89.09–91.24% dry basis of protein. Fat contents were in the range of 0.77–2.73% dry basis depended on fish species (p<0.05). The pH values of gelatins were 5.04-5.06. In comparison with commercial gelatin, the significant difference of gel strength, color and turbidity were found (p<0.05). The highest gel strength (205.54 g) was observed for gelatin extracted from skin of striped catfish while the lowest turbidity and highest lightness was found for commercial gelatin. In addition, black ear catfish gelatin had higher foaming expansion. The results indicated that *Pangasius* skins could be used as a potential gelatin source for the industrial application.

Keywords: Gelatin, Fish skin, Striped catfish, *Pangasuis*, Physicochemical properties

1. Introduction

Gelatin is a gelling protein and is obtained from colloagenous raw materials. Gelatin is normally produced from skin and bone of bovine and skin of porcine (Hinterwaldner, 1977; Ward and Court, 1977). It is widely found applications in food industry such a deserts, bakery products, candies, jellied meat and dairy products. Pharmaceutical industry uses gelatin as one of the important ingredients in the manufacturing of capsules, cosmetics, tablet coating, and emulsions. It also applies in photography and some specialized industries (Djany *et al*., 2001).

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Due to the outbreak of mad cow disease or Bovin Spongiform Encephalopathy (BSE) as well as the religious constraints, the numerous studies have investigated the use of alternative gelatin sources, especially skin and bone from seafood processing wastes. Skin gelatins from several fish species has been isolated and characterized using Alaska Pollock skin (Zhou and Regenstein, 2004), grass carp skin (Kasankala *et al*., 2007), channel catfish skin (Liu *et al*., 2008; Yang *et al*., 2007), freshwater fish (catfish, *Pangasius* catfish, snackehead and red tilapia) (See *et al*., 2010), Red tilapia, Walking catfish and Striped catfish (Jamilah *et al*., 2011) and Salmon skin (Díaz-Calderon *et al*., 2017). Recently, study on gelatin from crap skin (Tkaczewska *et al*., 2018), golden crap skin (Ali *et al*., 2018), tigger puffer skin (Pan, *et al*., 2018) and channel catfish (Duan *et al*., 2018) were studied.

Pangasius is in the family Pangasiidae. The production of *Pangasius*, though aquaculture, has become an important source of fish supply within the last few years. *Pangasius* is a fish of economic importance in Thailand. Production of catfishes including *Pangasius* in Thailand was about more than 3 million metric tonnes in 2018 (Tveteras, 2018). *Pangasius* is also in high demand in the international market and the demand is steadily increasing in Vietnam and the United States. *Pangasius* breaks into top 10 seafood list of all U.S seafood consumption, according to the National Fisheries Institute in 2016 (National Fisheries Institute, 2016). The ensuing increase of fish consumption means that more waste is observed. In the fish processing industry which in the production process for fillets contains about 60-70% of fish waste, such as skin scale and bone. (Tahergorabi, 2016). Fish skin, which is one of major byproduct of the fish-processing industry, could serve as valuable source of gelatin.

Although gelatin from striped catfish (*Pangasius hypophthalmus*) has been studied (See *et al*., 2010; Jamilah *et al*., 2011) but information regarding the gelatin from other fish species of *Pangasius* has been rarely reported. Therefore, the objectives of this study was to determine the physicochemical properties of gelatin extracted from skin of some *Pangasius* including striped catfish (*Pangasius hypophthalmus*), black ear catfish (*Pangasius larnaudii*) and diamond green fish (*Pangasius larnaudii* x *Pangasius bocoart).*

2. Materials and Methods

2.1 Fish skin preparation

Fish skin was obtained from a local fish market at Warinchamrap in Ubon Ratchathani province, Thailand. Fish skin is by-product from fish fillet process. Each freshwater fish used as a raw material was harvested from the culture pond. Fish with 50–55 cm in length and 1.2–1.5 kg in weight was subjected to process. Fish skin was transported to the laboratory under iced condition within 30 min. Residual meat was removed manually and washed with tap water. Cleaned samples were then cut into small pieces (0.5×0.5 cm^2) and placed in polyethylene bags (30 g/bag) and stored at – 20°C until used but not longer than 3 months.

All the chemicals used were of analytical grade. Commercial gelatin from cold water fish skin was purchased from Sigma-Aldrich Co. LLC.

2.2 Extraction of gelatin

Cleaned skin was soaked in 0.2 mole/L NaOH with a sample/solvent ratio of 1:10 (w/v) at 5–8°C for 30 min to remove non collagenous protein (repeated three times). Sample was washed with tap water until the neutral pH of wash water was obtained. The sample was then defatted with 10 % butyl alcohol with a solid/solvent ratio of 1:10 (w/v) at 5–8°C for 30 min 1 h. Defatted skin was washed with cold water, followed soaking in 0.05 M acetic acid at with a solid/solvent ratio of 1:10 (w/v) for 3 h at room temperature. After swelling process, the residue was rinsed using tap water until pH of wash water became neutral. The swollen skin was extracted with distilled water at 60°C for 12 h. The dark pigmented thin layer was removed with two layers of cheesecloth and air-dried in a convention oven at 65°C for 7 h until the final moisture content was less than 10%.

2.3 Gelatin Yield

The yield of gelatins was calculated on both weight and nitrogen basis according to method of Jamilah *et al*. (2011) with slightly modification as follows:

%Yield (wet wt basis) = (dry wt of gelatin/wet wt of skin) × 100

% Yield (dry wt basis) = (dry wt of gelatin/wet wt of skin-moisture content) × 100

% Yield (nitrogen basis) = dry wt of gelatin/nitrogen content of skin) × 100

2.4 Proximate analyses

Moisture, protein, fat and ash content were determined according to the method of AOAC (2000) number 950.46, 984.13, 960.39 and 920.153, respectively.

2.5 Turbidity

Gelatin solution (66.7g/L, Silva *et al*, 2014) was poured into 1 cm cuvette at room temperature. The turbidity of gelatin solution was measured using spectrophotometer at 620 nm against distilled water.

2.6 Viscosity

The viscosity of the gelatin (6.67% concentration at 60°C for 30 min,) was performed using a Brookfield Synchrolectic viscometer with spindle No. 2 and speed of 100 rpm. Data obtained were expressed as centipoises (cp).

2.7 Gel strength

A gelatin solution of 6.67% (w/v) (Kittiphattanabawon *et al*., 2010) was prepared with distilled water. The sample was stirred at room temperature for 30 min then heated at 65°C for 20 min until completely dispersed and then kept at 4–8°C for 16–18 h. The gel value was determined using texture analyzer (LLOYD model LRSK series) equipped with 1.27 cm diameter flat-faced cylindrical plunger with load cell of 50 N and cross-head speed 0.5 cm/sec. The dimensions of the gel sample were 2.5 cm diameter and 2.5 cm height using stainless steel cylindrical mold. The plunger had penetrated 4 mm into the gels. Gel strength was expressed as maximum force.

2.7 Texture profile analysis (TPA)

TPA was measured using the Texture Analyzer (TA.XT Plus). Gelatin gel samples were formed by using the same samples as used for the gel strength experiment. After gel maturation, the gels were removed from the glass bottles. The cylindrical gelatin samples were 25 mm in diameter and 25 mm in height. The gels were compressed by an aluminum probe (40 mm diameter plate) until the deformation reached 30% at a speed of 1.0 mm/s. Ten measurements were made for each sample in the same lot. Textural parameters including hardness, springiness, cohesiveness, gumminess, chewiness and adhesiveness were obtained.

2.8 Foaming properties

Foam expansion (FE) and foam stability (FS) of gelatin samples were determined. Ten milliliters of the gelatin solution of 6.67% (w/v) was added in 50 ml cylinder with cover. The sample was shaken severely 20 times to incorporate air. Then the whipped sample was immediately stood for 15 min. Foam ability was calculated as follows.

$$FE(\%) = (VT/V0)/V0 \times 100$$

$$FS(\%) = (Vt/V0)/V0 \times 100$$

Where VT is total volume after whipping; V0 is the original volume before whipping and Vt is total volume after leaving at room temperature for 15 min.

2.8 Sensory attribute

Gelatin powder and gelatin gel were photographed. Their sensory attributes including color and odor were described.

2.9 Statistical analysis

The collected data was analyzed using the analysis of variance (ANOVA). Duncan's multiple range tests to determine the significant difference between means. The significance level was 95%.

3. Results and Discussion

3.1 Gelatin Yield

The yield of gelatin based on wet, dry, and nitrogen basis is shown in Table 1. The extractability of gelatin among the three species was observed. The highest yield was obtained from diamond green fish followed by striped catfish. The yield of gelatin extracted from black ear catfish was lowest ($p<0.05$). The different yield of gelatin extracted from fish skins was literature reported. The gelatin yield (dry weight basis) of striped catfish (13.41%) was lower than that reported by Jamilah *et al*. (2011) but was higher than those reported by See *et al*. (2010). The variation of gelatin content depends on the differences in proximate compositions, the collagen content and amount of soluble components in the skins which these properties is varied with the species and the age of the fish as well as the variation the extraction method (Songchotikunpan *et al.,* 2008). In this study, three different species of *Pangasius* influenced the gelatin formation yield. It was noted that the gelatin yield based on nitrogen basis was related to that based on dry basis.

Table 1 Yield of gelatin from three selected *Pangasius*

Pangasius	**Yield (%)**		
	Wet basis	**Dry basis**	**Nitrogen basis**
Striped catfish (*Pangasius hypophthalmus*)	13.41±3.86^{b}	14.69±4.22^{b}	34.50±7.44^{b}
black ear catfish (*Pangasius larnaudii*)	12.25±1.11^{b}	13.37±1.20^{b}	33.19±2.90^{b}
diamond green fish (*Pangasius larnaudii* x *Pangasius bocoarti)*	17.64±3.37^{a}	18.15±3.55^{a}	45.43±5.92^{a}

Note: Values with the different superscripts within each column indicate significantly difference ($p<0.05$). Values are means ±SD from triplicates determination.

3.2 Proximate composition

Table 2 shows the proximate compositions of three selected *Pangasius* skins and gelatins. The skin of black ear catfish has lowest protein content ($p<0.05$). Protein was considered as the major component of gelatins extracted from three fish skins. This result was related to its gelatin yield based on nitrogen basis. The protein contents of fish gelatins were found to be approximately 88.86–91.24%. Moreover, protein content of striped catfish (89.09%) was higher than those reported by See *et al*. (2010) (80.02%) and Jamilah *et al*. (2011)

(81.6%) for the same fish species. Protein content of fish was varied depending on the species, sex, age, season and feeding (Silva *et al.,* 2014). The highest protein content of gelatin from diamond green fish skin was related to the highest yield of gelatin extracted from that skin based on both dry basis and nitrogen basis. The protein content of the collagenous material represented the maximum possible yield of gelatin expected from them (Muyonga *et al*., 2004) although it is an indirect indication. The composition of hydroxyproline in the collagenous material has been suggested as a better indicator to determine the yield of gelatin extracted from fish skin (Nalinanon *et al.*, 2008). Fat content of *Pangasius* skins was approximately 27.11–43.73%. The fat content of black ear catfish skin was highest. This would cause the highest content of lipid to be found for its gelatin (2.32%). See *et al*. (2010) reported that the fat content of gelatin from *Pangasius* catfish skin was 2.63 %. Fat content of fish skins was reduced from 27.11–43.73% to 0.77–2.32%, since during gelatin preparation; a process of degreasing was done by using butyl alcohol. Although the degreasing process reduced fat approximately 20 times for gelatins when compared to fat content of fish skins, but one cycle of degreasing process may not be enough to get rid of fat from skin of *Pangasius* which is considered as fatty fish. Ash contents of skins from three selected *Pangasius* were lower than 1%. The ash content of gelatins varied from 0.28 to 0.59%. The ash content of a high quality gelatin should be lower than 0.5% (Ockerman and Hansen, 1988). Comparisons with commercial gelatin from cold water fish skin, gelatin extracted from three fish skins contained higher protein content. Gelatin from diamond green fish skin recorded the lower fat content but higher ash content than commercial gelatin.

Table 2 Proximate compositions of three selected *Pangasius***.**

Samples	**Proximate compositions (% dry basis)**					
	Fish skin			**Gelatin**		
	Protein	**Fat**	**Ash**	**Protein**	**Fat**	**Ash**
Striped catfish (*Pangasius hypophthalmus*)	67.02±1.58[a]	34.30±1.37[b]	0.89±0.15[a]	89.09±0.93[ab]	1.73±0.34[ab]	0.28±0.07[b]
Black ear catfish (*Pangasius larnaudii*)	59.90±1.85[b]	43.73±0.53[a]	0.28±0.02[b]	88.86±0.99[ab]	2.32±0.30[a]	0.33±0.11[b]
Diamond green fish (*Pangasius larnaudii* × *Pangasius bocoarti)*	66.32±1.15[a]	27.11±1.65[c]	0.55±0.07[b]	91.24±1.13[a]	0.77±0.23[b]	0.59±0.18[a]
Commercial gelatin				86.71±2.11[b]	1.67±0.01[ab]	0.46±0.12[ab]

Note: Values with the different superscripts within each column are significantly different (p<0.05).
Values are means ±SD from triplicates determination.

3.3 Physicochemical properties

All physicochemical properties of gelatins from three selected *Pangasius* skins were tabulated in Table 3. Gel strength of fish skins was in the range of 165.9–205.5 g. Commercial gelatin from cold water fish skin showed lower gel strength than that form *Pangasius* skins. The gel strength of three selected *Pagasius* gelatins was comparable to black tilapia gelatin (181 g) and red tilapid gelatin (128 g) (Jamilah and Harvinder, 2002). See *et al*. (2010) reported that a bloom strength of 324.53 g for *Pangasius* catfish. This was probably associated with the gel forming ability of gelatin which depended on the chain length of protein molecules. The shorter chain length gelatin molecules would result in the weaker gel network (Nalinanon *et al.,* 2008). The gel strength of commercial gelatins is in the range of 50–300 g bloom. The gelatins are separated as: high-bloom, 200–300 g; medium-bloom, 100–200 g; and low-bloom, 50-100 g (Haug and Draget, 2009). The gel strength of gelatin from three selected *Pangasius* skins was in range of the medium-boom.

Highest viscosity was observed for gelatin extracted from striped catfish. It was noticed that the gelatin extracted from diamond green fish had higher yield and protein content but lower viscosity and gel strength was found. As mentioned, the chain length of protein molecules would be dependent. Viscosities of fish skins were in the range of 9.31–11.12 cP. Jamilah and Harvinder (2002) reported fish skin gelatin had a viscosity of 7.70, 6.28 and 8.21 cP for red tilapia, walking catfish and striped catfish, respectively. Whereas the viscosity of *Pangasius* catfish skin gelatin of 3.82 cP was reported by See *et al*. (2010). The viscosity of gelatin is partially controlled by molecular weight and molecular size distribution of gelatin subunits such as degradation extent, concentration and temperature (Mahjoorain *et al*. 2013). Since the viscosities for commercial gelatin have been reported to be from 2.0 to 7.0 cP for most gelatins and up to 13.0 cP for specialized ones (Johnston-Banks, 1990; Rafieian *et al*, 2015), the viscosity of gelatin from three selected *Pangasius* skins could be considered to be in the high-range.

Turbidity value of gelatins extracted from three selected *Pangasius* skins is shown in Table 3. Gelatin extracted from striped catfish skin showed lower turbidity than that obtained from either black ear catfish or diamond green fish. In addition, turbidity value of *Pangasius* gelatins was much lower than commercial gelatin. Therefore the efficiency of the filtration process during gelatin extraction should be considered to control the degree of turbidity of gelatin solution.

The pH was 5.04-5.06 in *Pangasius* and commercial gelatins. The pH of gelatin from black tilapia and red tilapia were 3.91 and 3.05 (Jamilah and Harvinder, 2002). The pH of glass carp (4.4) gelatin was higher value than rohu (4.1) and common carp (4.1) gelatin (Ninan *et al*., 2014). The pH value of gelatin was varied from the amino acid composition. The pH of three selected Pangasius gelatins were in range of Type B gelatin.

Color characteristics of gelatins were different among fish species (Table 3). Yellowish was observed for *Pangasius* gelatins while commercial gelatin was light yellowish. Lightness (L) values of three gelatins were lower than that of commercial skin as the visual observations on the gelatins were noticed. The extraction temperature and time used in this experiment could be influenced the color value of gelatin. Increase in yellowness (b*) of gelatin from clown featherback skin when the extraction temperature and time increased (Kittiphattanabawon *et al*., 2016). The L* value of striped catfish obtained in our study (69.13) was similar to that reported by Jamilah *et al* (2011) (68.69) for the same fish species. The color of gelatin depends on the nature of raw material and the extraction method. The lighter color of gelatin gel from seabass skin was observed for gelatin gel extracted at 45 C compared to those extracted at 55 C.(Sinthusamran et al., 2014). Gelatin gel of *Pangasius* skin was extracted at 65 C. Non-enzymatic browning reaction would be taken place. Fish odor was detectable in all gelatins. Fishy odor is correlated to the muddy odor and flavor associated with the fish species and also related to the feed of the fish (Jamilah and Harvinder, 2002)**.** The pigment and fishy odor would be removed during pretreatment process and the extraction conditions such as temperature and time used would also be considered during gelatin extraction process.

Texture profile of gelatins shown in Table 4. The significant difference of texture profile was found. Gelatin from black ear catfish had higher hardness, cohesiveness, gumminess and chewiness. Hardness of *Pangasius* gelatins was higher than commercial gelatin which was related to the result of gel strength. Wangtueai and Noomhorm (2009) reported that hardness, gumminess and chewiness of gelatin from lizardfish scales were lower than bovine gelatin but there were insignificantly different in springiness and cohesiveness. Jamilah *et al*. (2011) reported that the total amino acid concentration of striped catfish was highest, followed by walking catfish and red tilapia. The highest glycine and proline contents were also found in the striped catfish. A higher amino acid content of proline and hydroxyproline in warm-water fish gelatin (22-25%) and mammalian gelatin (30%) has been previously shown as one of the major cause for its higher gel properties than cold-water fish gelatin commercial (17%) (Muyonga et al., 2004). Springiness and adhesiveness of all samples were not significantly different, indicated that the elasticity of *Pangasius* gelatins were comparable to commercial gelatin. Similarly to several researches, Sow and Yang (2015) and Tkaczewska *et al*. (2018) did not detect any difference in springiness between fish gelatin samples. Gelatin from black ear catfish was harder and chewier but that from striped catfish was more gumminess. In addition, it was noticed that the higher gel strength but lower hardness were observed for gelatin from striped catfish when compared to gelatin from black ear catfish. The difference of gelatin properties might be depended on protein content and amino profile of fish skins. Higher hydroxyprotein content in cobia (*Rachycentron canadum*) skin contributed to higher rheological properties (Silva *et al.* 2014). The gelatin from brownbanded bamboo shark skin with a higher content of proline exhibited higher gel strength than that from blacktip shark containing lower content of proline (Kittiphattanabawon *et al.* 2010)

Table 3 Physicochemical properties of gelatin from three selected *Pangasius* skins

Properties	Striped catfish (*Pangasius hypophthalmus*)	Black ear catfish (*Pangasius larnaudii*)	Diamond green fish (*Pangasius larnaudii* × *Pangasius bocoarti)*	Commercial gelatin
Gel strength (g)	205.54±9.07 ^{a}	180.68±3.34 ^{b}	165.94±4.07 ^{c}	145.68±10.15 ^{d}
Viscosity (cP)	11.12 ±0.18^{a}	10.15±0.03^{b}	9.31±0.11^{c}	9.20±0.24^{c}
Turbidity (OD_{620nm})	0.93±0.16^{c}	1.40±0.19^{a}	1.02±0.15^{b}	0.091±0.003^{c}
pH @ 25 ^{o}C^{ns}	5.05±0.12	5.04±0.03	5.06±0.06	5.06±0.01
Color value L*	69.13±0.26^{c}	68.59±0.15^{c}	70.69±0.12^{b}	74.90±1.62^{a}
C	22.29±0.23^{a}	22.06±0.18^{a}	18.48±0.09^{b}	18.23±2.3^{b}
h	85.65±0.20^{c}	89.06±0.16^{b}	88.82±0.21^{b}	95.52±2.19^{a}
Sensory attributes				
Color (powder)	Yellowish	Yellowish	Yellowish	Light yellowish and Shiny
				
Odor (powder)	Detectable fishy odor	Detectable fishy odor	Detectable fishy odor	Slightly fishy odor
Color after re-dissolving				
Odor after re-dissolving	Detectable fishy odor	Detectable fishy odor	Detectable fishy odor	Slightly fishy odor

Note: Results are presented as mean ± standard deviation. Values within a row with different superscripts are significantly different (p<0.05).

Table 4 Texture profile of three selected *Pangasius* and porcine skin gelatin

Texture Profile	Samples			
	Striped catfish (*Pangasius hypophthalmus*)	Black ear catfish (*Pangasius larnaudii*)	Diamond green fish (*Pangasius larnaudii* × *Pangasius bocoarti)*	Commercial gelatin
Hardness (N)	0.86±0.43[b]	1.17±0.18[a]	0.61±0.07[c]	0.59±0.14[c]
Cohesiveness	0.47±0.06[b]	0.68±0.40[a]	0.49±0.07[b]	0.46±0.05[b]
Springiness(mm)[ns]	5.69±0.06	5.95±0.28	5.73±0.36	5.71±0.11
Gumminess (kgf)	0.42±0.01[b]	0.09±0.08[a]	0.03±0.00[b]	0.02±0.00[b]
Chewiness (kgf.mm)	0.24±0.07[b]	0.58±0.55[a]	0.18±0.04[b]	0.16±0.04[b]
Adhesiveness (kgf.mm) [ns]	0.03±0.01	0.03±0.02	0.15±0.01	0.02±0.00

Note: Results are presented as mean ± standard deviation of n = 10. Values within a row with different superscripts are significantly different (p<0.05).

3.4 Foaming properties

Foaming expansion and stability of *Pangasius* gelatins are shown in Figure 1. Gelatin from black ear catfish exhibited highest foam ability than both that from others fish and commercial gelatins. The difference in foaming ability is related to film forming ability at the interface which depends on the different sizes of protein, the hydrophobic areas on the peptide chain and degree of protein unfolding (Damodaran, 1997). Foaming stability was significantly different between samples. This result was not related to the protein contents (Table 2) and the viscosity values of gelatins (Table 3). The stability of foam depends on various parameters, such as the rate of attaining equilibrium surface tension, bulk and surface viscosities, steric stabilization, and electrical repulsion between the two sides of the foam lamella (Lui *et al*, 2003). The various values of foaming expansion and stability of *Pangasuis* gelatins possibly result from the differences in the intrinsic properties, compositions and conformations of proteins between different fish species.

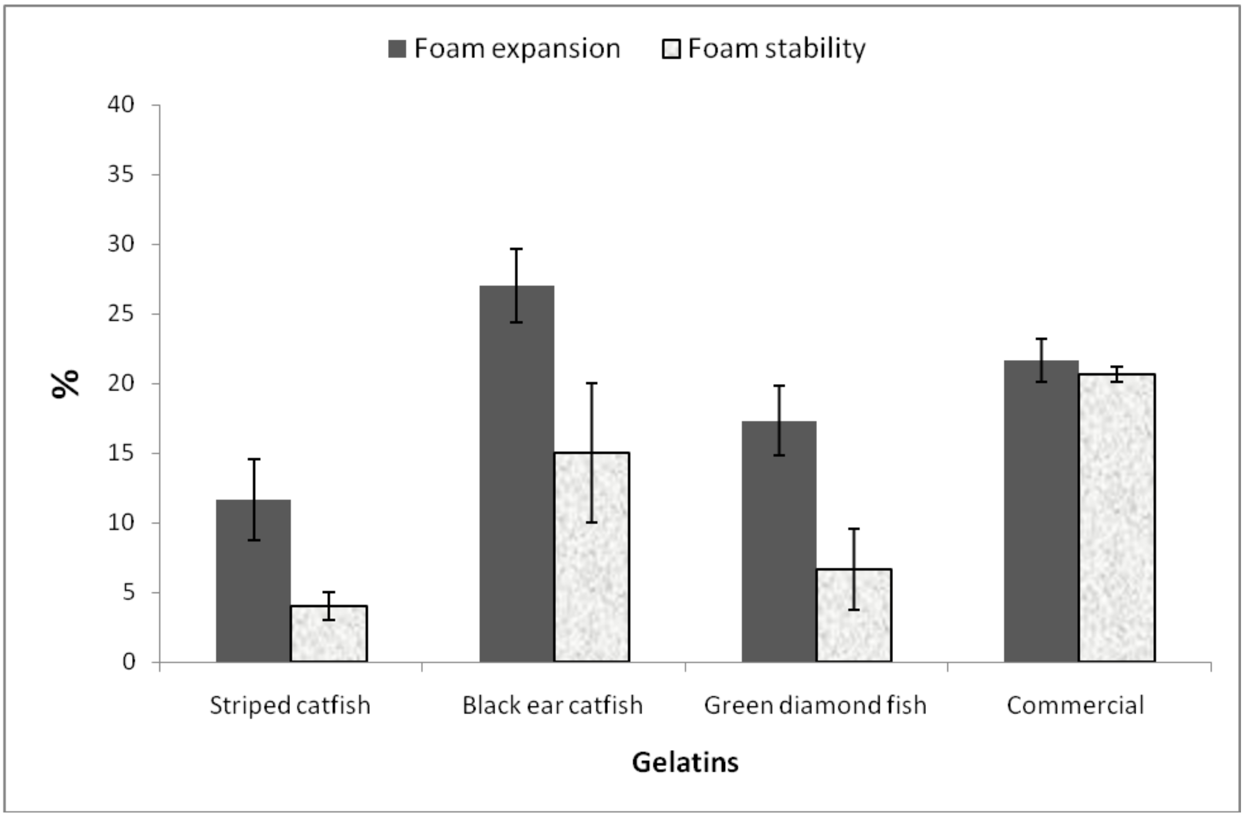

Figure 1 Foam expansion and foam stability of three selected *Pangasuis* and commercial gelatins.

4. Conclusion

The research findings indicated that the gelatins extracted from three selected *Pagasuius*, including striped catfish (*Pangasius hypophthalmus*), black ear catfish (*Pangasius larnaudii*) and diamond green fish (*Pangasius larnaudii* x *Pangasius bocoart),* exhibited a number of interesting features that indicated their potential for future applications in food which have dealt with gel strength, viscosity, and foaming properties.

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