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Physicochemical characteristics of gummy added with sutchi catfish *(Pangasius hypophthalmus)* gelatin

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Abstract

Gelatin from sutchi catfish *(Pangasius hypophthalmus)* skin was extracted and applied in the preparation of gummy in order to determine the suitability of sutchi catfish gelatin in gummy production. The skin was subjected to pre-treatment in the following sequence; 0.8M NaCl, 0.19 N NaOH followed by 0.12 N acetic acid prior to 12 hours extraction in distilled water at 50ºC. The physicochemical characteristics of sutchi catfish gelatin was analysed and compared with the commercial bovine gelatin. Gummy added with sutchi catfish gelatin was also compared with gummy added with commercial gelatin. Analysis comprises of yield, gel strength, setting point and setting time, amino acid composition, texture profile analysis and sensory evaluation. The extraction resulted in 14.47% yield of gelatin. Sutchi catfish gelatin showed higher gel strength value (360.86 g) compared to the commercial gelatin (217.37 g) which is in accordance with proline content. Texture profile analysis showed that gummies prepared using sutchi catfish gelatin had significantly higher ($p<0.05$) gumminess value while amino acid compositions such as proline, glycine and lysine were also higher. Sensory analysis indicated that both gummies were equally acceptable. Therefore, sutchi catfish gelatin can be successfully used as a future gelling agent in food.

Introduction

Sutchi catfish *(Pangasius hyphophthalmus)* belongs to the Pangasiidae family which is also known as the Thai pangas due to its origin from the Mekong river of Vietnam to the Chao Phraya River of Thailand and then spreads to other countries like Malaysia, Indonesia, Bangladesh and China (Rohul *et al*., 2005). As a benthopelagic species, sutchi catfish typically lives in water with pH ranging from 6.5 to 7.5 and temperature between 22 to 26°C (Ruiz-de-Cenzano *et al*., 2013). Female fish requires at least three years to reach maturity and grow to over 3 kg in weight while male fish requires at least two years to mature (Phu and Hein, 2003). The fish is mostly marketed as frozen, thawed fillets or steak (Department of Agriculture, 2008; Lam *et al*., 2009). The flesh varies from creamy white to orange with mild flavor and medium firm texture (Department of Agriculture, 2008).

Gelatin is a protein obtained by hydrolyzing the collagen contained in bones and skin (Gomez-Guillen *et al*., 2009). Fish skin serves as a suitable raw material for gelatin extraction because it contains high amount of collagen, for example, Japanese sea bass contains 51.4% collagen, chub mackerel contains 49.8% collagen and bullhead shark contains 50.1% collagen (Nagai and Suzuki, 2000). Skins and bones from various cold-water fish such as cod, hake, Alaska Pollock and salmon have been used for gelatin extraction (Gomez-Guillen *et al*., 2002; Zhou and Regenstein, 2005; Kołodziejska *et al*., 2008). The fresh water fish or warm water fish are also being used as gelatin source such as tuna, catfish, tilapia, Nile perch, shark and megrim fish (Muyonga *et al*., 2004; Jamilah *et al*., 2011; Peck and Mashitah, 2013). Gelatin is a multifunctional ingredient used in foods, pharmaceuticals, cosmetics, and photographic films as a gelling agent, stabilizer, thickener, emulsifier, and film former (Boran and Regenstein, 2010). Gelatin can also be successfully used as a new binder in place of poly (ethylene oxide) (PEO) in the fabrication of the sulfur cathode in lithium–sulfur batteries (Sun *et al*., 2008). In gummy production, gelatin is added as gelling agent to give a gummy texture characterized by suitable hardness and transparency (Marfil *et al*., 2012; Hartel and Hartel, 2014). Several studies have been done regarding the preparation of gummy using a range of gelatin/:high-methoxyl (HM) pectin and acid modified corn starch (DeMars and Ziegler, 2001; Marfil *et al*., 2012). Increases in sucrose/glucose syrup ratio and citric acid content resulted in a slight reduction of viscosity, hardness and chewiness of gummy jelly product (Meesang *et al*.,

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2003). According to previous studies, catfish gelatins exhibited higher gel strength and protein content compared to other fish (See *et al*., 2010; Jamilah *et al*., 2011; Mahmoodani *et al*., 2014). Gelatin with good gel strength is necessary for gummy production. Therefore, in this study, gummies were prepared using sutchi catfish skin gelatin and commercial gelatin from bovine source and the characteristics of the gummy were compared.

Materials and Methods

Materials

Sutchi catfish *(Pangasius hypophthalmus)*, 6 months age with average size of 600 to 1000 g were obtained from a local freshwater fish supplier in Klang, Selangor. Commercial gelatin was obtained from Halagel Distributor Sdn. Bhd. Shah Alam, Selangor. All chemicals used are of analytical grade.

Gelatin extraction

Gelatin was extracted according to Normah *et al.* (2013). Prior to extraction all the steps were performed in cold solution (5°C) in order to minimized protein degradation or spoilage. Sutchi catfish was initially filleted and the skin was removed manually by using a sharp filleting knife. Any adhering tissues on the skin were scrapped off. The skin was cut into pieces of 2 to 3 cm and cleaned under running tap water. This was followed by soaking for 10 min in cold (5°C) 0.8 M NaCl (1:6 w/v) and rinsing under running tap water. Subsequently, the skin was soaked for 40 min in 0.19 N cold (5°C) NaOH (1:3 w/v) to remove non-collageneous protein, rinsed under tap water and soaked in 0.12 N cold acetic acid solutions (5°C) at the ratio of 1:3 w/v for 40 min. The skin was rinsed again until a basic pH 7 was achieved. Gelatin was extracted in distilled water at the ratio of 1:3 w/v skin to distilled water at 50°C for 12 hours in a shaking water bath (Water Bath Shaking-1086, Germany) followed by filtration through Whatman filter paper No. 42. The filtrate was freeze-dried and ground.

Gelatin yield

The ratio of dried gelatin weight to the total of fish skin on wet basis was used to calculate gelatin yield according to Sanaei *et al.* (2013).

$$\text{Yield of gelatin (\%)} = \frac{\text{weight of dried gelatin (g)}}{\text{weight of the skin (g)}} \times 100$$

Preparation of gummy

Gummy was prepared by mixing 60 ml water, 10 ml freshly squeezed lemon juice, 26 ml glucose syrup and 5 g of extracted sutchi catfish gelatin or commercial gelatin (Gomez, 2013). The mixture was then slowly heated in water bath set at 90°C to dissolve the gelatin and the syrup. The gummy was then poured into a mould and left to solidify for two days at 7°C.

Determination of gel strength

Gel strength was determined according to Mohtar *et al.* (2011). Dry gelatin powder at 6.67% (w/v) was prepared in bloom jar (internal diameter 59 mm, height 85 mm, 150 ml capacity) by dissolving 7.5 g of gelatin in 105 ml distilled water at 60°C. The mixture was cooled at room temperature for 15 minutes and kept at 7°C for 18 hours for maturation. The penetration test was performed by using TA.XT2 Texture Analyser (Stable Micro Systems, Surrey UK). Load cell of 5 kg and 0.5 cm diameter bottom plunger were used. The maximum force (g) was determined until the probe penetrated into the gel to a depth of 4 mm. This test was repeated for gummy added with sutchi catfish gelatin and gummy added with commercial gelatin.

Setting point and setting time

The setting point and setting time were determined for sutchi catfish gelatin and commercial gelatin according to Muyonga *et al.* (2004). Gelatin at 10% (w/v) was placed in warm water bath at 40°C until the gelatin completely dissolved. Thirty milliliter of the gelatin solution was transferred into a test tube (12mm x 75 mm) and then placed in a beaker containing warm water (40ºC). The temperature was reduced every 2°C interval by the addition of cold water. An aluminum needle (diameter 0.1 cm and length 8.5 cm) was inserted into the gelatin solution and the setting point was determined when gelatin solution no longer dripped from the tip of the rod.

The setting time was determined at 4ºC. Aluminum needle (diameter, 0.1 cm and length, 8.5 cm) was manually inserted into the gelatin solution at the interval of 15 s. The setting time was recorded when the needle could not detach from the gelatin.

Analysis of amino acid composition

Amino acid composition of the gelatin and gummy were analysed according to the methods of Sanaei *et al.* (2013). Samples were hydrolyzed for 16 hours in 15 ml of 6N HCL at 110°C. The samples were then dissolved in deionized water and filtered. About 20 µL sample was injected into the High Performance Liquid Chromatography (HPLC) equipped with a Water 410 Scanning Fluorescence and AccQ Tag column (3.9 × 150 mm). Sixty percent acetonitrile was used as the mobile phase with flow rate of 1 ml/min.

Texture profile analysis (TPA)

TPA of gelatin and gummy were measured using TA.XT2 Texture Analyser (Stable Micro Systems, Surrey UK). Gelatin solution (6.67% w/v) was placed in a Bloom jar (internal diameter 59mm, height 85mm, capacity 150ml) and then heated at 60°C for 30 min to completely dissolve the gelatin (Yang *et al*., 2007). The gelatin solution was cooled at room temperature for 30 min before chilling in refrigerator at 7°C for 18 hours for gel maturation. The cylindrical gelatin gel was shaped by cutting into 35 mm diameter and 25 mm height. The aluminium probe (100 mm diameter plate) was used until the deformation reach 30% at a speed of 1.0 mm/s. The pause between the first and second compression were 3 s. The measurement was in triplicate for all samples. From the force-time curve of texture profile, the hardness, springiness, cohesiveness, adhesiveness, gumminess and chewiness were obtained. Gummies were cut into a similar shape and dimension as sutchi catfish gel prior to analysis using the texture analyser.

Sensory evaluation

Sensory evaluation was designed to measure the degree of liking of samples by 9 point hedonic scale. Category scale ranging from 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much and 9 = like extremely was used. Panelists indicated their degree of liking for each sample by choosing the appropriate category. Panelists were given samples of gummy made using sutchi catfish gelatin and commercial gelatin to evaluate their degree of liking in terms of color, odour, taste and appearance. They were also instructed to rinse their mouth with plain water in between tasting.

Statistical analysis

The test was conducted in triplicate and reported as means ± standard deviation. Statistics was performed with the analysis of variance (ANOVA) procedure of SAS (Release 9.1, SAS Institute Inc., Cary, NC, USA, 2004) software. Significant differences at $p < 0.05$ were used to determine the differences between means.

Results and Discussion

Yield

The yield of sutchi catfish skin gelatin expressed as percentage wet basis was 14.47%. The reported yield of gelatin from the skin of other fish were 5.67% grey triggerfish *(Balistes capriscus)*, 17.135% rainbow trout *(Onchorhynchus mykiss)*, 12.92% red tilapia, 13.06% walking catfish and 11.17% striped catfish (Shahiri Tabarestani *et al*., 2010; Jamilah *et al.*, 2011; Jellouli *et al*., 2011). Gelatin yield depends on the collagen content, composition of the skin and extraction conditions including solvent, temperature and extraction time (Jongjareonrak *et al*., 2010; Jellouli *et al*., 2011). Neutral and acid pretreatment contributed to higher gelatin yield while soaking in saturated lime solution [$Ca(OH)_2$] at the concentration of 27 g L^{-1}, 20°C for 14 days helps to condition the skin prior to gelatin extraction which leads to better recovery of the gelatin (Zhou and Regenstein, 2005; Jamilah *et al.*, 2011). Yield was higher at longer pretreatment time and when acetic acid concentration was increased from 0.01 to 0.18 N (Shahiri Tabarestani *et al*., 2010). In this study, sutchi catfish skin was pretreated in 0.12 N acetic acid and the yield was reasonably high.

Gel strength of gelatin

Gel strength expressed as Bloom value is one of the most important physical properties of a gelatin (Karim and Bhat, 2009). Gel strength which depends on the isoelectric point, pH, molecular weight distribution and amino acid content has been categorized as low (<150), medium (150-220) and high (220-300) (Nalinanon *et al*., 2008; Shyni *et al*., 2014). Gelatin with different gel strength is used for different applications in food manufacturing. As an example, Type B gelatin with the gel strength from 125 to 250 is commonly used for confectionary product while Type A gelatin with low gel strength of 70 to 90 is applied for fining of wine and juice (Wittich, 2005). Sutchi catfish skin gelatin had significantly ($p<0.05$) higher gel strength (360.86 g) compared to commercial gelatin (217.37 g) (Table 1).

Table 1. Gel strength (g), setting point (°C) and setting time (min) of sutchi catfish gelatin and commercial gelatin

Gelatin	Gel strength (g)	Setting point (°C)	Setting time at 4°C (min)
Sutchi catfish gelatin	360.86 ± 6.92^a	15.72 ± 2.32^b	3.71 ± 0.39^b
Commercial gelatin	217.37 ± 2.00^b	34.73 ± 1.50^a	6.79 ± 0.57^a

Values are means ±standard deviation.
Means within each column with different superscripts are significantly different at $p<0.05$

Table 2. Amino acid composition of sutchi catfish skin, sutchi catfish gelatin and commercial gelatin

	Amino acid composition (g/100g)		
Amino acid	Sutchi catfish skin	Sutchi catfish gelatin	Commercial gelatin
Asp	0.323	0.100	0.055
His	2.272	2.101	1.847
Arg	3.954	1.367	0.297
Gly	5.343	4.776	0.776
Thr	2.681	2.660	0.383
Ala	1.851	0.870	0.489
Pro	9.521	2.974	0.446
Cys	2.964	1.087	1.113
Tyr	4.415	3.447	0.725
Val	0.628	0.760	0.055
Met	3.055	1.175	0.906
Lys	3.223	2.266	0.441
Ile	3.087	1.009	2.636
Leu	2.828	2.965	0.158
Phe	4.152	1.628	0.460

Depending on their habitat, fish can be categorized as warm or cold water fish which contribute to the variation in gel strength (Trakul and Patcharin, 2013). Theoretically, the gelatin obtained from warm water fish is characterised by higher gel strength than gelatin extracted from cold water fish because the collagen obtained from warm water fish was more stable compared to the collagen from cold water fish (Gilsenan and Ross-Murphy, 2000). The gel strength of sutchi catfish gelatin was higher than cold water fish including salmon with gel strength of 108 g and Alaskan Pollock with gel strength of 98 g (Zhou *et al*., 2006; Arnesen and Gildberg, 2007). The gel strength of sutchi catfish gelatin were also comparable with other freshwater fish including yellowfin tuna skin (426 g), red tilapia (487 g), catfish (278 g) and Pangasius catfish (324 g) (See *et al*., 2010). In the production of commercial gelatin from the bone, usually the bone was treated with hydrochloric acid solution for 5 to 7 days to remove minerals followed by soaking in alkaline solution for 35 to 70 days (Wasswa *et al*., 2007). Such severe extraction conditions contribute to the hydrolysis of collagen that leads to decreasing of gel strength (Eysturskaro *et al*., 2009). The gelatin extracted from sutchi catfish skin exhibits gel strength which is more similar to mammalian gelatins than cold water fish gelatins. This probably due to the higher concentration of proline and hydroxyproline which are mostly present in both warm water fish and mammalian gelatins compared to cold water fish gelatins where the proline and hyroxyproline content were approximately 30% for mammalian gelatins, 22% to 25% for warm water fish gelatins and 17% for cold water fish gelatins (See *et al*., 2010).

Setting point and setting time

Sutchi catfish skin gelatin sets at significantly ($p<0.05$) lower temperature (15.72°C) than the commercial gelatin (34.73°C) (Table 1). Porcine and bovine gelatin have the setting point in the range of 20 to 25°C and 28 to 35°C, respectively, while for the warm water and cold water fish gelatin between 8 to 25ºC and 11 to 28°C, respectively (Karim and Bhat, 2009).

The setting time was measured at 4°C where sucthi catfish gelatin had significantly shorter ($p<0.05$) setting time (3.71 min) compared to the commercial gelatin (6.79 min). Gelatin high gel strength demonstrated a negative correlation with the setting time (Kittiphattanabawon *et al*., 2010). Setting time and setting temperature of gelatin also depend on the age of the fish used for the extraction of gelatin (Muyonga *et al*., 2004). Gelling ability is also species dependent for example gelatins from flat-fish species (sole and megrim) presented the best gelling ability and the gels were more thermostable compared to cold-adapted fish such as cod and hake (Gomez-Guillen *et al*., 2002). In food production, gelatin with high gel strength required shorter gelling time and lesser amount of gelatin to obtain a firm gel (Schrieber and Gareis, 2007).

Amino acid composition

Fifteen amino acids were detected in sutchi catfish skin and sutchi catfish gelatin (Table 2). Sutchi catfish skin shows high amount of proline

Table 3. Texture profile analysis of sutchi catfish gelatin and commercial gelatin

	Sutchi catfish gelatin	Commercial gelatin
Hardness	1327.22±5.07[a]	1132.77±2.07[b]
Adhesiveness	-1.03±0.01[a]	- 0.56±0.12[b]
Cohesiveness	0.92±0.00[a]	0.91±0.01[a]
Springiness	4.07±0.12[a]	3.95±0.05[a]
Chewiness	965.55±0.40[a]	872.83±4.18[b]
Gumminess	1001.72±0.79[a]	985.00±0.97[b]

Values are means ±standard deviation
Means within each row with different superscripts are significantly different at $p<0.05$

(9.521 g/100 g) followed by tyrosine (4.415 g/100 g), phenylalanine (4.152 g/100 g), arginine (3.954 g/100g) and lysine (3.223 g/100 g). Proline and lysine content in sutchi catfish gelatin was higher than those in the commercial gelatin. In shark cartilage gelatin, the amount of proline was also reported as slightly higher than the analytical grade gelatin and food additive grade gelatin which are from porcine skin recorded as 11.97%, 11.36% and 11.01%, respectively (Cho *et al.*, 2004). The imino acids (proline and hydroxyproline) content are important in stabilizing gelatin gel network (Haug *et al*., 2004). However, hydroxyproline has been suggested to play a more significant role (Cho *et al*., 2004). In addition, lysine also stabilizes gelatin structure by forming cross-linking structures between chains (Cho *et al*., 2004). Previous study showed that high content of proline, hydroxyproline and alanine contribute to higher viscoelastic properties of gelatin (Gomez-Guillen *et al*., 2002).

Texture profile analysis

Texture profile analysis was used to measure the compression test on sutchi catfish gelatin and commercial gelatin. Hardness is known as the force that required compressing a substance between two molar teeth (Alina, 2002). Hardness increased as concentration of gel increased, for example, 10% fish skin gel showed significant differences from those obtained from 20% and 30% gels (Shafiur Rahman and Al-Mahrouqi, 2009). It has also been reported that gel hardness was higher in the skin than the bone gelatins from fish (Muyonga *et al*., 2004). Hardness, adhesiveness, chewiness and gumminess of sutchi catfish gelatin gel was significantly higher ($p<0.05$) than gel from commercial gelatin as shown in Table 3. In terms of cohesiveness and springiness, there was no significant different ($p>0.05$). The hardness value which is higher in sutchi catfish gelatin than

Table 4. Amino acid composition of gummy added with sutchi catfish gelatin and gummy added with commercial gelatin

	Amino acid composition (g/100g)	
Amino acid	Sutchi catfish gummy	Commercial gelatin gummy
Asp	0.098	0.053
His	2.057	1.743
Arg	1.371	0.266
Gly	3.675	0.113
Thr	2.711	0.397
Ala	0.773	0.454
Pro	2.562	0.381
Cys	1.099	1.013
Tyr	3.211	0.672
Val	0.057	0.035
Met	0.992	0.886
Lys	1.232	0.437
Ile	0.995	0.977
Leu	2.775	0.115
Phe	1.563	0.448

Analysis of the gummy

Amino acid composition

Sutchi catfish skin gelatin gummy contains 3.675 g/ 100 g glycine, 2.562 g / 100 g proline and 1.232 g/100 g lysine compared to the commercial gelatin gummy which contains 0.113 g/100 g glycine, 0.381g/ 100g proline and 0.437 g/100 g lysine (Table 4). Gelatin containing high amount of proline contribute to a better viscoelastic properties while low amount of glycine and proline affects the collagen stability which contribute to the reduced in denaturation temperature (Liu *et al*., 2008). Furthermore, lysine stabilizes gelatin structure (Cho *et al*., 2004). The higher glycine, proline and lysine content in sutchi catfish skin gelatin gummy probably contribute to better properties of the gummies such as gumminess and chewiness.

Texture profile analysis

Sutchi catfish gelatin gummy has significantly

Table 5. Texture profile and sensory analysis of gummy added with sutchi catfish gelatin and gummy added with commercial gelatin

Texture attributes	Sutchi catfish gummy	Commercial gelatin gummy
Hardness	912.90±1.01[a]	897.88±7.68[b]
Adhesiveness	-2.66±0.05[a]	-0.87±0.18[b]
Cohesiveness	0.86±0.02[a]	0.88±0.01[a]
Springiness	2.68±0.03[a]	2.67±0.04[a]
Chewiness	800.74±1.64[a]	763.78±3.48[b]
Gumminess	867.97±2.34[a]	818.60±2.44[b]

Sensory attributes	Sutchi catfish gummy	Commercial gelatin gummy
Appearance	6.30±0.84[a]	6.43±1.17[a]
Color	6.53±1.01[a]	5.63±1.03[b]
Odor	4.93±0.64[b]	6.20±0.89[a]
Texture	7.23±0.82[a]	6.80±1.00[a]
Overall acceptability	6.50±0.97[a]	6.43±0.77[a]

Values are means ±standard deviation.
Means within each row with different superscripts are significantly different at $p<0.05$

higher ($p<0.05$) values of hardness, adhesiveness, chewiness and gumminess than commercial gelatin gummy (Table 5). There was no significant different ($p>0.05$) in cohesiveness and springiness between sutchi catfish gelatin gummy and commercial gelatin gummy. The higher value of hardness and gumminess in sutchi catfish gummy suggested that sutchi catfish gelatin can be used as a replacement for bovine gelatin in gummy production.

Sensory analysis

There was no significant different ($p>0.05$) between sutchi catfish gelatin gummy and commercial gelatin gummy in terms of appearance, texture and overall acceptability (Table 5). This indicates that gummy from sutchi catfish gelatin are able to attract panelists. The color of gummy from sutchi catfish gelatin was significantly ($p<0.05$) more acceptable compared to those prepared using the commercial gelatin due to the more transparent gummy obtained from sutchi catfish skin gelatin. However, in terms of odor, sutchi catfish gelatin gummy was less acceptable probably due to the fishy odor. In general, sensory evaluation results showed that sutchi catfish skin gelatin was suitable to be used in the food products because it has similar properties as commercial gelatins which are from the mammalian source.

Conclusion

Extraction at 50°C for 12 hrs resulted in reasonably good yield of sutchi catfish gelatin. The gelatin exhibited higher gel strength compared to the commercial gelatin which is from bovine source. The higher gel strength value and shorter setting time was in accordance with proline content which was higher compared to those in the commercial gelatin. Shorter setting time of sutchi catfish gelatin accelerates the gelling process. Texture profile analysis showed that gummy prepared using sutchi catfish gelatin were more gummy than those prepared with commercial gelatin while sensory analysis indicated that sutchi catfish gummy and commercial gummy are equally acceptable. The high content of certain amino acids such as proline, glycine and lysine as well as high gel strength value of sutchi catfish gelatin contributed to the gummy and chewy characteristics of sutchi catfish gummy. Therefore, sutchi catfish gelatin can be used as gelling agent in food and can serve as alternative to mammalian gelatin.

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