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Extraction and characterization of gelatin from different marine fish species in Malaysia

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Abstract: Gelatins from the skin of four local marine fish, namely "kerapu" (*Epinephelus sexfasciatus),* "jenahak" (*Lutjianus argentimaculatus),* "kembung" (*Rastrelliger kanagurta),* and "kerisi" (*Pristipomodes typus)* have been successfully extracted by acid extraction. Results characterization showed that the fish gelatins were comparable to the fish gelatins from other fish species previously reported. They appeared snowy white in color with crystal-like and light texture. The gelatine extracted from "kerapu" had the strongest fishy odor, followed by the gelatines derived from "jenahak", "kembung" and "kerisi". In terms of bloom strength, the gelatin extracted from "kerapu" was found to be the strongest one compared to others, with the bloom value of more than 2000 g. The gelatins developed in this study contained almost all essential amino acids, with glycine being the most predominant one.

Key words: Fish gelatin, amino acids, bloom strength, Malaysian waters, halal

Introduction

Gelatin is one of the most widely used food ingredients. Its applications in food industries are very broad including enhancing the elasticity, consistency and stability of food products. Gelatin is also used as a stabilizer, particularly in dairy products (Gimenez, Gormez-Guillen and Montero, 2005) and as a fat substitute that can be used to reduce the energy content of food without negative effects on the taste (Riaz and Chaudry, 2004). Besides for the food industry, gelatin is also useful in medicine, pharmaceutical and photographic industries.

Gelatin is a valuable protein derived from animal by-products obtained through partial hydrolysis of collagen originated from cartilages, bones, tendons and skins of animals. It is a translucent brittle solid substance, colourless or slightly yellow, nearly tasteless and odourless (Sakr, 1997). Today, gelatin is usually available in granular powder form, although in Europe countries, sheet gelatin is still available (Sakr, 1997). Most commercial gelatin is currently sourced from beef bone, hide, pigskin and, more recently, pig bone. It was reported that 41% of the gelatin produced in the world is sourced from pig skin, 28.5% from bovine hides and 29.5% from bovine bones (Hayatudin, 2005). In recent times, the concern and fear of BSE or "mad cow disease" has affected the gelatin market and

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has shifted the market towards porcine gelatin.

At present, the fish gelatin production is very low, yielding about 1% of the annual world gelatin production of 270,000 metric tonnes (Jamilah and Harvinder, 2002). Factors such as the outbreak of BSE and increasing demand for non-mammalian gelatin for *halal* and *kosher* food markets have revived the interest in gelatin from fish raw materials (Jamilah and Harvinder, 2002). Recently, several studies of gelatins from the skin of various fish species have been published (Jamilah and Harvinder, 2002).

It is well established that temperature characteristics of collagen and gelatin from fish skin reflect the fish habitat temperature (Andreva, 1971). The composition of amino acids is of particular importance regarding both gelatin gel strength and melting point. Due to the rigidity of their R-groups in the amino acids provide rigidity to triple helix structures both in intact collagen and gelatin gels. Apparently, high contents of hydrophobic amino acids have a similar effect, although less prominent (Badii and Howell, 2005).

Malaysia is a tropical country and produces warm water fish species. Many studies have indicated that collagen from warm water fish species contains more amino acids than collagen from cold water fish (Gudmundsson, 2002). However, the contents of both hydrophobic and hydroxylated amino acids, as well as other properties such as molecular weight distribution and gelatin viscosity, seem to be species specific, and to reveal such properties, gelatin from each actual fish species must be studied.

The aim of the present work was to study the extraction and some physico-chemical characteristics of gelatin from the skin of various marine fin fish species found off Langkawi island coastal area, a famous tourist resort in Malaysia.

Materials and Methods

Raw materials and chemicals

Four different species of marine local fishes caught off Langkawi Island, Malaysia were used as the samples in this study. They were "kerapu" (*Epinephelus sexfasciatus),* "jenahak" (*Lutjianus argentimaculatus),* "kembung" (*Rastrelliger kanagurta),* and "kerisi" (*Pristipomodes typus).* The samples were washed with clean sea water at the point of collection, separated by species, and packed in polyethylene plastic bags inside a cooler and transferred to the laboratory. After reaching the laboratory, the samples were stored at -27^{o}C. Sodium hydroxide, sulphuric acid, and citric acid were purchased from local suppliers. All the chemicals used were of analytical grade.

Gelatin extraction

The extraction procedure was conducted according to Grossman and Bergman (1992), with slight modifications. The fish were thawed prior to the experiments. The accurately weighed fish were cleaned and washed with tap water followed by peeling the fish skin using a sharp scalpel. The fish skins were thoroughly rinsed in excess water to remove superfluous materials. The fish skins were soaked in 0.2% (w/v) sodium hydroxide for 40 minutes. After washing out sodium hydroxide, two successive acid incubations were performed, each for 40 min, first in a sulphuric (0.2%, v/v) and then in a citric acid solution (1.0%, w/v). The acid solutions were drained and then samples were washed with cold water once.

The final extraction of gelatin was performed in distilled water at 45^{o}C for 18 hours. Solubilized gelatin was separated from residual skin fragments by filtration

Table 1. Yield of gelatins from each species of fish*

Types of fish	% of yield over the weight of fish	% of yield over the weight of fish skin
"Kerapu"	3.68 ± 1.02	68.47 ± 4.25.
"Jenahak"	1.82 ± 0.42	55.21 ± 4.10
"Kembung"	2.04 ± 0.34	67.82 ± 6.20
"Kerisi"	1.71 ± 0.26	43.57 ± 5.93

*Results are average of three replicates ± SD

Table 2. The visual appearance and odor description of gelatin from different species of fish*

Properties	"Kerapu"	"Jenahak"	"Kembung"	"Kerisi"
Appearance	Snowy white, crystal-like and light textured	Snowy white, crystal-like and light textured	Snowy white, crystal-like and light textured	Snowy white, crystal-like and light textured
Odor description	Very strong fishy odor	Strong fishy odor	Less fishy odor	Least fishy odor

*Average of 20 students' reports through a sensory evaluation panel.

through a Whatman No. 4 filter paper, collected and kept at -80°C for at least 24 hours. Residual water in the gelatin extract was removed by a freeze dryer.

Physical and chemical analysis

Yield of gelatin extracts produced from each fish species was determined by comparing the weight of gelatine obtained to the weight of the fish used. Physical appearance and the odor of the gelatin extracts were determined through physical observations. The bloom strength of each type of fish gelatin was determined according to Arnesen and Gilberg (2002) using the TAXT2i version 2.3 (Texture Analyzer Stable Micro Systems, U.K.) with a p10 probe. The condition of the analysis was set as follows: pre-test speed = 2.0 mm/s; test speed = 1.0 mm/s; post-test speed = 2.0 mm/s; and distance = 50 %. Amino acid analysis was performed on hydrolyzed gelatin samples essentially as described by Pedersen, Gilberg, Steiro, and Olsen (2003).

Amino acid profile analysis

The amino acids compositions of the gelatins were determined on a Waters-PICO-TAG amino acid auto analyser high performance liquid chromatography (Model: Waters 501), equipped with the amino acid analyzing software. The column used was the Waters-Pico Tag (measuring 3.9×150 mm). Each sample was hydrolysed with 6 N hydrochloric acid at 110°C for 24 h.

Results and Discussion

Gelatin recovery and physical observation

The yield of gelatins obtained from different fish species in this study is presented in Table 1. The highest percentage of gelatin recovery was obtained from "kerapu" with 68.47% of its fish skin or 3.68% of total the fish weight. This was followed by "kembung" with a recovery of 67.82% of its skin or 2.04% of the total fish weight. Gelatin recovery from "jenahak" was 55.21% over the weight of the skin or 1.82% of the fish weight. "Kerisi" was found to produce the least gelatin extract,

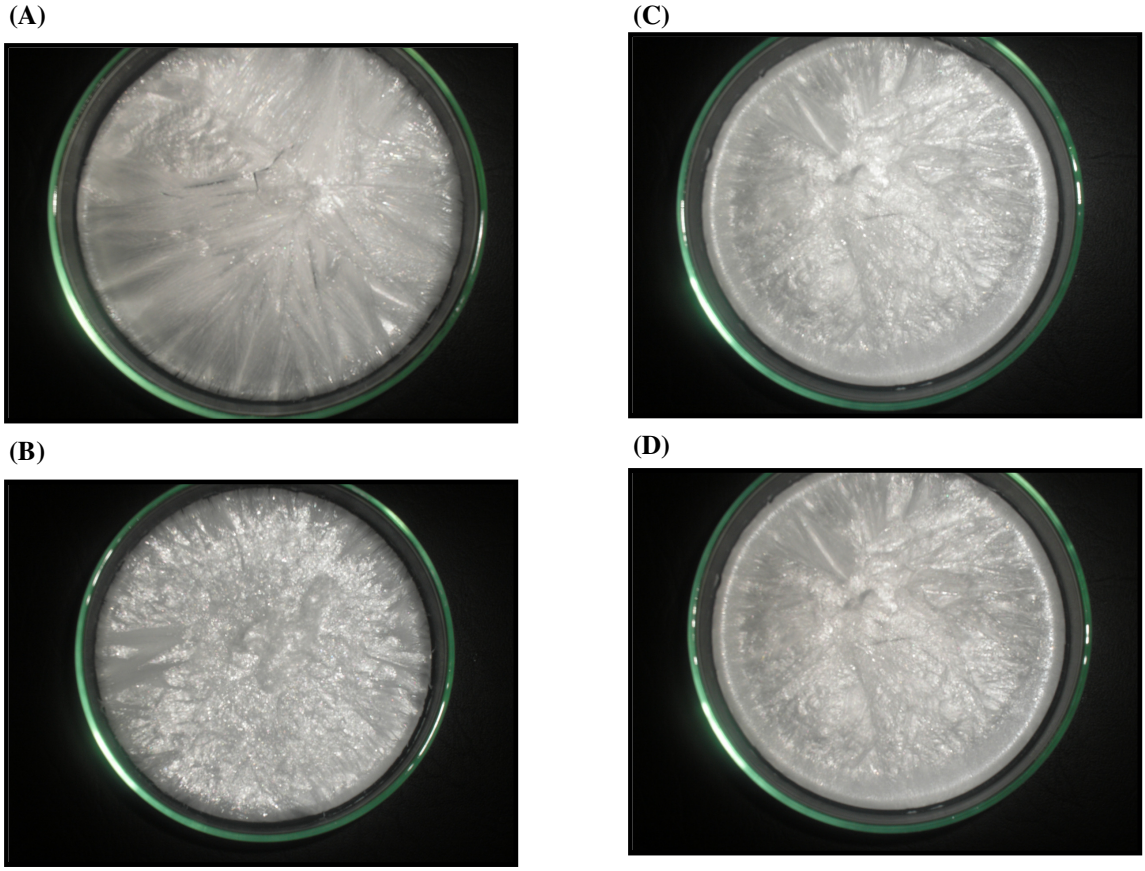

Figure 1. Gelatins extracted from different marine local fish in Malaysia (A= "Kerapu", B= "Jenahak", C= "Kembung", D= "Kerisi")

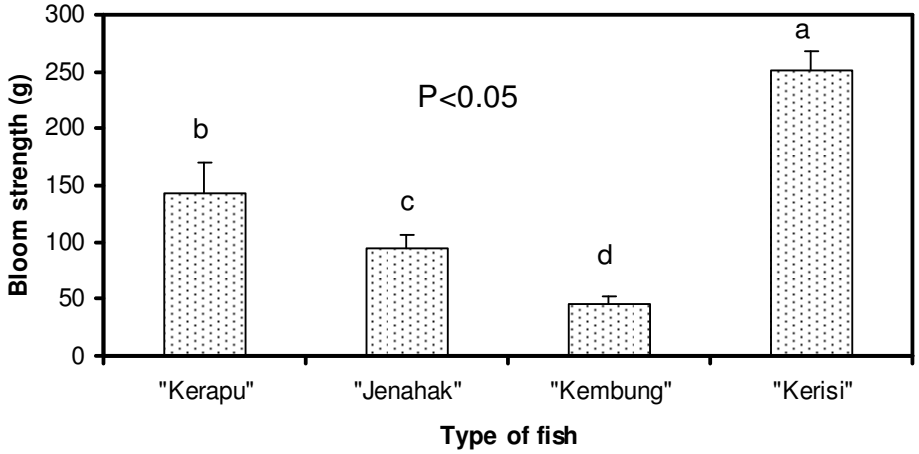

Figure 2. Bloom strength of gelatins extracted from different Malaysian salt-water fish (means of three replicates)

Table 3. Amino acid composition (mg/g) of gelatins extracted from four different fish species in Malaysia.

	Type of Fish			
Amino acid*	**"Kerapu"**	**"Jenahak"**	**"Kembung"**	**"Kerisi"**
Aspartic acid (Asp)	Trace	Trace	Trace	Trace
Glutamic acid (Glu)	Trace	Trace	Trace	Trace
Serine (Ser)	35.24	26.82	26.29	30.76
Glycine (Gly)	206.70	190.31	174.00	202.24
Histidine (His)	Trace	Trace	Trace	Trace
Arginine (Arg)	75.92	70.92	65.62	73.09
Threonine (Thr)	25.21	21.63	21.21	19.32
Alanine (Ala)	88.38	85.31	75.43	90.58
Proline (Pro)	95.97	94.51	88.36	94.88
Tyrosine (Tyr)	3.97	4.78	Trace	5.42
Valine (Val)	16.07	15.21	15.68	14.92
Methionine (Met)	16.92	16.23	16.53	17.86
Cysteine (Cys)	Trace	6.37	Trace	Trace
Isoleucine (Ile)	7.06	6.15	8.48	6.10
Leucine (Leu)	18.87	20.73	20.51	21.33
Phenylalanine (Phe)	18.00	16.10	17.28	18.27
Lysine (Lys)	42.75	51.49	31.10	41.26

* Results obtained from duplicate readings

yielding only 43.57 % of the weight of its skin or 1.71% of the total fish weight. The difference in the gelatin recovery could be due to the difference in the characteristics of the skin and scale of the fish. For example, "kerapu" and "kembung" apparently have harder skin and scale, compared to the other two fish species. Gomez-Guellen *et al.* (2001) also revealed that different marine species had different structural and physical properties of gelatin. Hence, the use of fish skin for gelatin production has to take into account at least two different aspects. First, the wide diversity among the fish species, that present intrinsic differences in the collagen molecules present in their skin. Second, the higher susceptibility of the collagenous material from fish skin to degradation due to the lower content in intra- and interchain non-reducible crosslinks (Norland, 1990; Montero *et al.* 1990) in contrast to the more stable collagen from mammals (Yang and Wang, 2009; Gimenez *et al*., 2004)

Table 2 shows the visual appearance and the odor description for gelatins extracted from 4 different species of fish studied. All four types of gelatin had a snowy white appearance and light texture, as shown in Figure 1 (A-D). Gelatin extracted from the skin of "kerapu" possessed the strongest fishy odor, followed by the gelatins extracted from "jenahak" and "kembung". The gelatin extracted from the skin of "kerisi" on the other hand had the least fishy odour. The difference in terms of odor for each gelatin could be influenced by the living environment of the fish (Jamilah and Harvinder, 2002). The environmental factors that might influence the characteristics of fish gelatin included the depth of habitat where the fish live, types of materials, level of pollution, types of plankton living around, etc.

Gel strength

Bloom or gel strength is a measure of the hardness, stiffness, strength, firmness and compressibility of the gel at a particular temperature and is influenced by concentration and molecular weight (Ockerman and Hansen, 1988).

Results from the present study showed that each fish species produced gelatins with different gel strength (Figure 2). The gelatin derived from "kerisi" was found to be the strongest with a bloom strength value of 251.7 g. The value was five times higher than the gel strength value of the gelatin extracted from "kembung" (46.3 g). The gel strength values for "kerapu" and "jenahak" were 143.0 g and 94.6 g, respectively. The gel strength values of different fish species obtained in this study were comparable to the values of other Malaysian fish gelatins previously reported. Jamilah and Harvinder (2002) reported that gelatins from red and black tilapia reported by possessed the gel strengths of 128.1 g and 180.8 g, respectively. However, Grossman *et al*. (1992) reported gel strength of 263.00 g for the tilapia spp. which was higher than those reported by Jamilah and Harvinder (2002).

The variation in the reported gel strength could be explained by differences in molecular weight distribution rather than in amino acid composition without disregarding the existence of additional factors that may influence these parameters. It is well established that fish gelatin has a lower melting point than mammalian gelatins (Norland, 1990) and melting point of gelatins increase with increasing in molecular weight (Ward and Courts, 1977). It is also well established that hydrogen bonds between water molecules and free hydroxyl groups of amino acid in gelatin are essential for the gelatin's gel strength (Arnesen *et al*., 2002). The higher the hydroxyproline content, the higher the gel strength of the gelatin (Sarabia *et al*., 2000).

In general, gelatin extracts obtained in this study have good and relatively high gel strength values. The availability of gelatins with high Bloom values, together with the use of cross-linking agent, could provide a successful answer to those biomedical problems, where applications of gelatin are limited by its poor mechanical properties. On the other hand, gelatins with lower Bloom values could be more usefully employed in different applications, such as for the preparation of composites with stiffer materials. In this regards, the presence of gelatin is aimed to improve the handling and the fabrication as well as to modulate the mechanical properties of the composites (Bigi *et al*., 2004).

Amino acid composition

Collagens are major structural proteins of most connective tissues, such as skin, bone and tendons, where they provide structural integrity to the tissues (Bigi *et al*., 2004). The peculiarity of the amino acid sequence accounts for the characteristic coiled coil structure of the collagen molecule, where three distinct polypeptide chains, each of which is coiled into a left-handed helix are thrown into a right-handed superhelix stabilized through interchain hydrogen bonds and covalent crosslink (Brodsky and Ramshaw, 1997). Thermal denaturation or physical and chemical degradation of collagen involves the breaking of the triple-helix structure into random coils to give gelatin (Bigi *et al*., 2004). At temperature of about 40^{o}C, gelatin aqueous solutions are in the sol state and form physical thermoreversible gels on cooling. During gelling, the chains undergo a conformational disorder; order transition and partly regenerate the collagen triple-helix structure (Pezron *et al*., 1991).

In general, the composition of amino acids obtained in this study was comparable to that of gelatins extracted from other fish species and mammals as previously reported, with glycine (Gly) being the most predominant one (Jamilah and Harvinder, 2002). During acid hydrolysis of gelatin, some of the glutamine and asparagine will be converted to the acidic forms; i.e. glutamic acid and aspartic acid, respectively, and the concomitant release of ammonia.

From Table 3, it is obviously shown that glycine (Gly) content in each type of gelatin is far higher than any other amino acids. Of all four types of gelatin, the highest glycine content was found in the gelatin extracted from the skin of "kerapu" (206.70 mg/g), followed by the gelatins from "kerisi" (202.24 mg/g), "jenahak" (190.31 mg/g) and "kembung" (174.00 mg/g). However, glycine content in each type of gelatin in this study was slightly lower than that of red tilapia (*Oreochromis nilotica*) and black tilapia (*Oreochromis mossambicus*) as reported by Jamilah and Harvinder (2002), or cod (*Gadus morhuua*) and hake (*Merluccius merluccius*, K.) as reported by Gormez-Guillen *et al*. (2002).

Table 3 also shows that proline (Pro) was another major amino acid present in the gelatin extracted from the four different Malaysian marine fish species. The proline contents obtained in this study ranged from 88.36 mg/g ("kembung") to 95.97 mg/g ("kerapu"). This finding was much higher than the proline contents of the gelatins extracted from other Malaysian fish previously reported. Jamilah and Harvinder (2002) reported that the proline contents of the gelatins from red and black tilapia was very low and nearly not detectable.

Johnston-Banks (1990) reported that the imino acids proline and hydroxyproline impart considerable rigidity to the collagen structure. Relatively, limited imino acid content should result in a less sterically hindered helix and may affect the dynamic properties of the gelatins. Although proline is important, hydroxyproline is believed to play a singular role in the stabilization of the triple-stranded collagen helix due to its hydrogen bonding ability through its –OH group (Gormez-Guillen *et al*., 2002).

Other major amino acids found in this study included alanine (Ala), ranging from 75.43-90.58 mg/g, arginine (Arg) (65.62-75.92 mg/g), lysine (Lys) (31.10-51.49 mg/g), and serine (Ser) (26.29-35.24 mg/g). However, some amino acids were present only in trace amounts (less than 0.1 mg/g), such as aspartic acid (Asp), glutamic acid (Glu) and histidine (His). Except for the gelatine from "jenahak", cysteine (Cys) was also found to be trace in all samples.

According to Stainsby (1987) and Johnston-Banks (1990), amino acid composition plays important role in the physical properties of gelatin. However, the physical properties of the gelatin depends not only on the amino acid composition, but also on the relative content of β- or γ-components and higher molecular weight aggregates, as well as on the presence of lower molecular weight protein fragments. Thus, in addition to the source or species, gelatin properties will also strongly depend on the preservation of raw materials.

Conclusion

The gelatins extracted from the skin of "kerapu", "jenahak", "kembung" and "kerisi" exhibited different physico-chemical characteristics. Gelatin extracted from "kerapu" had the highest percentage of yield (68.47%) while "kerisi" gave the lowest percentage of yield (43.57%). All four types of gelatin obtained in this study had good gel strength, ranging from 46.3 g to 251.7 g. Amino acid composition of the gelatins produced in this study was

comparable to that of the gelatins from other Malaysian fish species previously reported.

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