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Effect of Pre-Treatment on Physical Properties and Sensory Attributes of Gelatin Extracted from Sutchi Catfish *(Pangasius sutchi)* Skin

Normah Ismail* and Wan Najma Farhaten Wan A Latiff

Department of Food Technology, Faculty of Applied Sciences,
Universiti Teknologi MARA, 40450 Shah Alam, Selangor Darul Ehsan, Malaysia

**Corresponding author e-mail: norismel@uitm.edu.my*

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ABSTRACT

This study was conducted to evaluate the effect of pre-treatment on the fishy flavour and odour removal of gelatine extracted from the skin of sutchi catfish (Pangasius sutchi). Pre-treatment of the skin involved soaking at 4°C in distilled water (GC), lime followed by tamarind (GLT) or salt followed by activated carbon (GSC) prior to extraction in warm distilled water (50°C) for 12 hours. Yield, physical properties and sensory were determined. Results showed that GLT produced highest yield (19.72%) compared to GSC (15.01%) and GC (15.81%). Although, GLT exhibited lowest gel strength (282.29g), viscoelasticity (14.1°C) and setting point (10.46°C) compared to other pre-treatments, fishy flavour and odour of the gelatine were almost absent with the score of 1.68 and 1.74, respectively. These values were below those of reference which are 1.87 (fishy flavour) and 2.71 (fishy odour) denoting from 'absent to weak'. Since fishy flavour and odour were almost absent, soaking sutchi catfish skin in lime followed by tamarind could be a good method for achieving the desired sensory attributes of the freshwater fish by the reduction of the gelatine off flavour.

Keywords: *gelatine, sutchi catfish, fishy, tamarind, lime*

INTRODUCTION

Sutchi catfish is a freshwater fish usually found in countries like South Africa, India, Burma, Indonesia, Malaysia, Cambodia and Thailand [1]. It had been studied for the production of protein hydrolysate, fatty acid content, gelatine and storage stability [2, 3, 4, 5]. Study on the sensory properties of gelatines from striped catfish, walking catfish and red tilapia revealed that soaking in saturated lime solution [$Ca(OH)_2$] at 27 gL^{-1}, 20°C for 14 days resulted in detectable fishy odour in striped catfish gelatine while walking catfish and red tilapia emitted a slight fishy odour and barely detectable fishy odour, respectively [6]. In addition, passing Nile perch (Lates niloticus) gelatine through a column of activated carbon removed the fishy odour [7]. Positive correlation between fishy odour and time has also been shown when the skins were stored at an extended period of time in ice prior to extraction [8].

Commercialisation of gelatine from fish is a problem when consumers cannot tolerate fishy odour. The chemicals that cause the fishy odour include 2-methylisoborneol, geosmin and trimethylamine [9, 10]. Efforts have been made to reduce the fishy odour problems [11, 7]. Methods such as air scrubbing, high temperature combustion, photocatalysts, chemical inactivation and biological oxidation involve high operational costs [12, 11]. Thus, simpler methods to remove the fishy odour with low operational costs and more practical such as soaking in natural sources; lime and tamarind need to be discovered. This study was designed to determine the effect of pre-treatment in different types of soaking solutions on the fishy flavour and fishy odour of gelatine from sutchi catfish skin.

MATERIALS AND METHOD

All chemicals used were of analytical grade for analysis purposes. Sutchi catfish were obtained from a local supplier in Temerloh, Pahang, Malaysia. The fish was eviscerated, washed and filleted to obtain the skin. During the process, the skin was kept in ice water and vigorously stirred in an orbital shaker to remove fat. Finally, the skin was individually packed in polyethylene bags and stored at -20°C.

Pre-Treatment of the Skin

The fish skin was prepared according to Kittiphattanabawon *et al*., [13] with slight modification. Frozen skin was thawed for 30 minutes. at chilled temperature (4°C) then cut into pieces of 2 to 3 cm and rinsed three times under tap water. The skin from GLT portion was initially soaked in lime juice (1:2 ratio of skin to solution) for 15 minutes, rinsed in distilled water and then soaked in 50% w/v tamarind solution (1:2) for 15 minutes. For GSC, the skin was soaked in 3% salt solution for 15 minutes. (1:3) and then soaked in 50% (w/v) activated carbon solution (1:2) for 15 minutes. For GC, it was soaked in distilled water (1:2) for 15 minutes. Soaking and rinsing steps were all performed at solutions' temperature of 4°C. The gelatine was extracted with distilled water at the ratio of 1:3 w/v of skin to distilled water at 50°C for 12 hours in a shaking water bath (Water Bath Shaking-1086, Germany). This was followed by filtration through Whatman filter paper No. 42. The filtrates were freeze-dried in a freeze-drier (Alpha 1-4 Martin Christ, Germany). Finally, the gelatine was ground into powder [14].

Analysis of Gelatine

Percentage of yield

Yield of gelatin was calculated based on the method of [15].

$$Yield\ (\%) = \frac{Weight\ of\ powdered\ gelatin\ (g)}{Wet\ weight\ of\ skin\ (g)}\ x\ 100$$

Gel Strength

Gelatine solution at 6.67% (w/v) was prepared by mixing 7.5 g gelatine powder in 105 ml distilled water (65°C) for 20 minutes [7]. Upon cooling at room temperature, the gel was poured into a bloom jar (59 mm diameter; 85 mm height) and then chilled (7°C) for 16 to18 hours in a refrigerator [16]. Gel strength was measured by a TA.XT2 Texture Analyser (Stable Micro Systems, Surrey UK) with the load cell of 5 kg and 0.5 diameter bottom plunger. The maximum force (g) was recorded at a rate of 0.5 mm/s.

Triplicate sample measurements were carried out at the penetration depth of 4 mm. Samples were removed from the chiller (~7°C) only when they were ready for analysis in order to maintain the temperature at cold phase during the analysis.

Setting Point and Setting Time

The setting point and setting time were determined according to Muyonga *et al*., [7]. Gelatine powder at 10% (w/v) was dissolved in warm water bath at 40°C. 20 ml of the gelatine solution was transferred into a test tube (12 mm x 75 mm) and then placed in a beaker containing warm water (40ºC). An aluminium needle (diameter 0.1 cm and length 8.5 cm) was inserted into the gelatine solution. Temperature was reduced every 2°C interval. The setting point was determined when gelatine solution could no longer drip from the tip of the rod. The setting time was determined as described for the setting point. After dissolved, 20 ml gelatine solution was transferred into a test tube (12 mm x 75 mm) and then placed in a beaker containing cold water (4°C). Aluminium needle (diameter, 0.1 cm and length, 8.5 cm) was inserted into the gelatine solution every 15 s. Setting time was recorded when the needle could not detach from the gelatine gel.

Viscoelastic Properties

Small deformation oscillatory measurement was performed according to Chandra and Shamasundar [17]. A controlled strain oscillatory rheometer (Physica Model No. MCR 300 Messtechnik GmbH, Darmstadt, Germany) was used. Gelatine powder (6.67% w/v) was dissolved in warm distilled water (65°C) and the temperature was reduced from 40 to 5°C at oscillation frequency of 1 Hz, controlled strain 2% and scan rate of 2°C/min. G' and G" values were plotted as a function of time. The crossover between G' and G" was recorded as the viscoelastic point.

Viscosity

Viscosity was measured according to the method of Ratnasari *et al*., [18]. Gelatine solution of 6.67% (w/v) was prepared similarly as

previously mentioned in gel strength analysis. Viscosity was analysed by using a viscometer (DV-1, Brookfield Engineering Laboratories, Inc., USA) equipped with a No. 1 spindle at 60 rpm. The temperature was reduced from 60 to 15°C and the readings were recorded every 3°C interval. Viscosity was expressed in centipoise (cP).

Sensory Evaluation

Sensory evaluation was carried out based on quantitative descriptive analysis (QDA) involving ten semi-trained panellists who have several experiences in sensory analysis of food [19]. Panellists were trained for fishy flavour, fishy odour, sourness, saltiness and sweetness prior to the actual sensory session. For fishy flavour and odour, different concentrations of commercial fish sauce were used at the dilutions of 100, 80, 50, 30 and 10% (v/v) in distilled water. Orange cordial, salt and sugar were diluted similarly at the above-mentioned dilutions to evaluate for sourness, saltiness and sweetness, respectively. A 15 cm line scales anchored from 'absent' to 'strong' was defined and used for the evaluation [20]. Gelatine sample kept in an air-tight sensory cup was coded with three-digit random number and presented along with the references. Panellists evaluated the five attributes in these samples by comparing with the references.

Statistical Analysis

Analysis were conducted in triplicate and reported as means ±standard deviation. Statistics on whole data were performed with the analysis of variance (ANOVA) procedure of Statistical Analysis System [21]. Significant differences ($p<0.05$) among means were used to determine the differences between means.

RESULTS AND DISCUSSION

Yield of Gelatine

Soaking in lime juice followed by tamarind (GLT) produced significantly higher yield (p<0.05) (Table 1). It has been suggested that acid pre-treatment facilitated collagen chain fragmentation [22]. It does not only remove some acid soluble proteins, lipids and other undesired components, but also disrupt collagen cross linkages by repulsive force so that warm water used during the extraction process could penetrate into the skin matrix effectively and increased the yield [23, 24, 4]. Previous findings showed that protein recovery increased with higher acid concentration in which at 0.01 to 0.20M citric acid concentrations, 10.52 to 22.4% protein were recovered [24]. The yield of sutchi catfish gelatine was lower than previously reported yield of Pangas catfish (22%), Asian redtail catfish (21.28%), striped snakehead (20.25%) and Nile tilapia (21.93%) [18]. However, extraction from black and red tilapia skin yielded 5.39 and 7.81 %, respectively [11]. Differences in yield were probably due to different methods of extraction, pre-treatment and species [11, 25, 18].

Table 1*:* Yield (%), Gel Strength (g), Setting Point and Setting Time of Gelatine from Sutchi Catfish Skin Soaked in Distilled Water (GC), Lime Juice Followed by Tamarind (GLT) and Salts Followed by Activated Carbon (GSC)

	Gelatine		
	GC	**GLT**	**GSC**
Yield (%)	15.81 ± 0.23^{b}	19.72 ± 1.26^{a}	15.01 ± 0.11^{b}
Gel strength (g)	427.92± 1.92^{a}	282.29± 5.33^{c}	401.42± 2.82^{b}
Setting point (°C)	15.68±0.20^{a}	10.46±0.29^{c}	13.60±0.21^{b}
Setting time (min.)	3.51±0.10^{c}	5.77±0.15^{a}	3.99±0.18^{b}

Means within row followed by different superscript are significantly different at p<0.05

Gel Strength

The gel strength of sutchi catfish gelatine is in the following sequence: GC > GSC > GLT (Table 1). Both acid and alkali affects the crosslinking in collagen [26]. In a study on the effect of pH on gelatine, it was observed that gelatine gel strength decreased markedly at pH less than 4 and pH slightly above 8 with maximum gel strength around pH 8 [27]. According to Zhou & Regenstein [28], high gel strength can be obtained at neutral or weak acid conditions. At pH 6, the gels were closer to the isoelectric points [29]. When pH approaches the isoelectric point, the gelatine polymers are nearer to being neutrally charged and the polymers are closer to each other, thus being able to form more compact and stiffer gel [29]. The gel strength of several fish gelatines were 426 g (yellowfin tuna skin), 360.86 g (sutchi catfish skin), 438.34 g (pangasius catfish skin), 206 g (shark skin), 124 g (rohu skin), and 177 g (tuna skin) [30, 4, 31, 14].

Setting Point and Setting Time

The setting point of GLT and GSC were lower than GC (Table 1). The result obtained was in agreement with Sarbon *et al*., [33] who reported that the addition of CaCl2 lowered the gelling temperature of sin croaker, shortfin scads and bovine gelatine solutions. They suggested that the small ion radius (chlorides) are more readily approached and hence interact to the centre of the positively charged protein chain, therefore interrupt with the gel formation. Treating with lime and tamarind lowered the setting point probably due to the coagulation of gelatine, where the resulting ordered structure failed to be formed. Lower temperature for gelation was required probably due to the decrease in the number of chemical junctions which are responsible for the formation of the amide bonds [34]. Research showed that gelatine solution added with 5 mg of ethanolic extract from coconut husk which is rich in tannic acid reduced the gelling temperature from 15.53 to 14.36°C, respectively [34]. Tamarind is rich in tartaric acids while lime juice is rich in citric acids [35]. The pH of tamarind and activated carbon are 2.5 and 6, respectively.

GLT took the longest time to form gel compared with GSC and GC. Previous study showed that when acid was used in the pre-treatment, the

resulting gelatine comprises mostly of low molecular weight protein bands [23]. Gelatine molecules with shorter chain are unable to form the strong inter-junction zone [32]. It is assumed that acid in lime and tamarind used during the pre-treatment exerted similar effect on the gelatine structure. The setting time to form gel was longer for GSC compared to GC. NaCl breaks the hydrogen bond and interfere with hydrophobic interaction which stops the formation of a rigid gel thus the formation of a gel network of gelatine needed a longer time for alignment and connection between chains [36, 37]. The setting point and setting time for gelatine from the skin of sea bass based on different weight were 17.09°C (2 kg), 18.43°C (4 kg) and 19.01°C (6 kg) and the setting time at 4°C were 2.13, 2.80, and 3.60 min., respectively [38]. As for Nile perch gelatine extracted at 50°C, the setting point and setting time were 19.5°C and one minute, respectively [7]. The difference in gelling temperatures might be due to the different species of fish, the difference in temperature surrounding the fish and differences in culture water temperature [39].

Viscoelastic Properties and Viscosity

GC had higher value of G' and G" which indicates that it had higher viscoelastic properties than the GLT and GSC (Figure 1(a)). As the elastic modulus (G') value is higher than the loss modulus (G"), it shows a solid-like behaviour. When exceeding the viscoelastic point, it can be assumed that the gelatine became gel as the storage modulus (G') were higher than the loss modulus (G") [40].

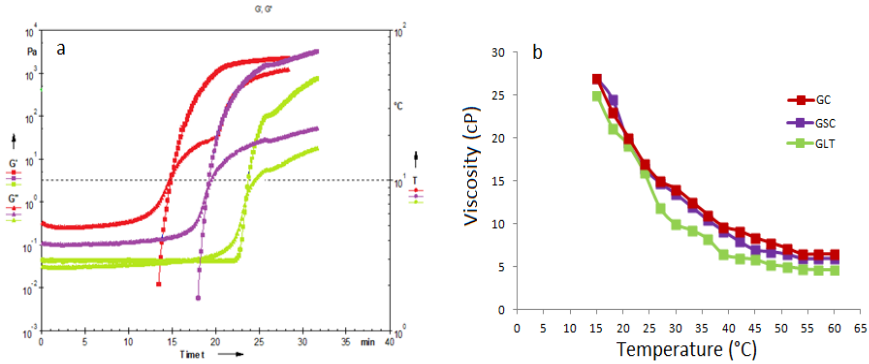

Figure 1: **Viscoelastic Properties (A) and Viscosity (Cp) (B) of Gelatine from Sutchi Catfish Skin Soaked in Distilled Water (GC) , Lime Juice Followed by Tamarind (GLT) and Salts Followed by Activated Carbon (GSC)**

During cooling, gelation occurs by entanglement of gelatine molecules as a result of renaturation of the triple-helix [41]. The time taken to reach viscoelastic point for GC was 14.5 min. (22.2ºC) while GLT and GSC were 23.6 min. (14.1ºC) and 19.2 min. (18.9ºC), respectively. This shows that GC took shorter time to solidify followed by GSC and GLT. The result obtained was in agreement with Giménez *et al*., [42]. In the study, the skins of Dover sole which were treated with 50 mM lactic acid exhibited the least gelling ability. The gelatine took longer time to reach the viscoelastic point probably due to the high fragmentation of alpha-chains which impairs the growth of the nucleation sites by further annealing of collagen chains during cooling or maturation [42]. Previous study showed the decreased of the viscoelastic properties of sin croaker, shortfin scads and bovine gelatine after the addition of $CaCl_2$ salt [33]. Salts causes the protein in the gelatine to compete with the salts for water during hydration and destabilising the structure, thus forming a weak gel [33]. Besides, it may also decrease the ability for alpha-chains to come into contact and form electrostatic bridges [43].

The viscosity of gelatine produced from the different pre-treatments are shown in Figure 1(b). As the temperature increased, the viscosity decreased and finally levelled off. At the temperature where the viscosity became almost constant the gelatine gel completely melt. The viscosity of GLT was lowest among the three pre-treatments. This is probably because of the over-hydrolysis of the collagen during the pre-treatment step, where acid breaks the peptide bonds into short-chain molecule thus resulted in the peptide chains with lower molecular weight [44]. The lower viscosity of GSC was probably due to the interruption of protein–protein interactions in the presence of salt which weakened the electrostatic interaction between the protein molecules [45, 46]. The result was in agreement with previous findings where the addition of 1% calcium acetate resulted in lower viscosity (7.3 cP) than those without calcium acetate addition (8 cP) [46].

Quantitative Descriptive Analysis

The intensity of fishy flavour and odour of GSC were lower than GC (Figure 2). As for GLT, these attributes were almost absent indicated by scores of 1.68 and 1.74, respectively where the values were below the references; 1.87 (fishy flavour) and 2.71 (fishy odour) which denotes ‘absent’

to 'weak'. This indicates that pre-treatment reduced the intensity of fishy attributes which was reflected by low score obtained during the sensory analysis. Research done to remove fishy odour of Nile perch gelatin using activated carbon showed that the odour did not differ from bovine bone or commercial fish gelatin as the fishy odour was absent while putrid odour was mild [7]. In another study, shrimp dipped and rubbed with bilimbi (*Averrhoa bilimbi L*) and tamarind (*Tamarindus indica* L) showed the reduction in off odour which was characterised by acidic lemony smell [47].

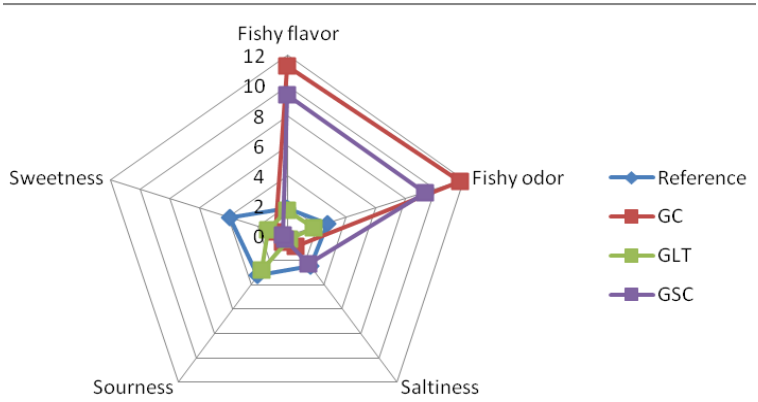

Figure 2: **Quantitative descriptive analysis (QDA) scores for gelatine gel from Sutchi catfish skin soaked in distilled water (GC) , lime juice followed by tamarind (GLT) and salts followed by activated carbon (GSC)**

Activated carbon had been shown to remove the trimethylamine (TMA) which is responsible for the fishy odour due to its high affinity towards the non-polar odorant more than the polar substances [12]. Meanwhile, soaking for 30 min. in 5% salt solution leached out some of the muddy taste [35]. Tamarind (pH 2.50) and lime juice has been used on fish in order to remove off-odour in which washing tilapia fillet with tamarind alone gave a score of 6.3 which is close to 7 (like very much) [35]. However, for fillet washed with lime juice alone and those washed with tamarind and lime juice, the score was reduced to 5.8 and 5.9, respectively.

The score for saltiness, sourness and sweetness were significantly lower (p<0.05) than the reference solution for all the gelatine ranging from 'absent' to 'weak' which were 0.23 to 2.29 for saltiness, 0.28 to 2.82 for sourness and 0.29 to 1.30 for sweetness. GLT was most sour among

the three samples as it was pre-treated with lime and tamarind while salt pre-treatment caused the intensity of saltiness for GSC to be the highest.

CONCLUSION

Gelatine from sutchi catfish skin was successfully extracted by using warm water. Pre-treatment with lime and tamarind (GLT) produced the highest yield and least fishy odour and flavour. However, the gelatine had lowest gel strength, viscoelasticity, viscosity and setting point compared GSC and GC. Pre-treatment with lime and tamarind appears to be more effective in removing fishy flavour and odour compared to salt and activated carbon since these attributes were almost absent.

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