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Functional Properties of Gelatin Extracted From Poultry Skin and Bone Waste

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ABSTRACT

The utilization of waste skin and bone from poultry for production of value added by-products has attracted substantial attention. Poultry skin and bone is a waste part and can be converted into value added product like gelatin. This serves as a good practice of post harvest management of waste utilisation. In order to evaluate the waste from poultry as source of gelatin, the gelatin was extracted from skin and bone. Its rheological and functional properties were examined at five different temperatures (40°C, 45°C, 50°C, 55°C and 60°C).

Proximate composition of poultry skin and bone was observed to be moisture 70.01 and 58.60%, crude protein 12.94 and 10.30%, crude fat 12.00 and 5.70%, ash 4.50 and 6.65% respectively. The proximate composition of extracted gelatin was found to be comparatively better at 45°C temperature which than what at 40°C, 50°C, 55°C and 60°C. The gelatin yield was extracted from poultry skin and bone at five different temperatures (40°C, 45°C, 50°C, 55°C and 60°C) for skin were 9.43, 10.53, 11.07, 11.80 and 12.23% for skin and 7.30, 7.49, 7.50, 7.52 and 8.03% for bone respectively bloom values of gelatin extracted poultry skin and bone at five different temperatures (40°C, 45°C, 50°C, 55°C and 60°C) of skin were found to be 257.67, 274, 265.33, 263 and 260gm and of skin were 258.33, 282.67, 273.67, 264.33 and 262 gm respectively. Similarly the viscosity, melting point, emulsifying capacity and stability of extracted gelatin at 45°C temperature from poultry skin and bone were in general greater than those of gelatin extracted at 40°C, 50°C, 55°C and 60°C. Hydroxyproline content in extracted gelatin of poultry skin and bone was found to be in the range of 5.87-9.17 and 6.17-8.23. The highest content of hydroxyproline was observed to be at 45°C temperature. It can be concluded from present study that the poultry skin and bone is a prospective source to produce gelatin of good yield and quality with desirable rheological and functional properties at 45°C temperature. These promising findings may contribute to the ongoing efforts for using poultry gelatin as an alternative source for fish and bovine gelatins.

Key words: *Poultry waste (Skin and Bone), Rheological and Functional properties*

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INTRODUCTION

Nowadays a lot of research has been conducted to make gelatine from fish, however it has been limited application due to the gel formed is less stable and had a weak rheological properties compare to gelatine extracted from land mammals[41]. However, there is too little research concerning production of gelatine from chicken skin. At present, the fish gelatine production is very low, yielding about 1% of the annual world gelatine production of 270,000 metric tonnes[20]. Nowadays, there is great request for Halal products so chicken is the best selected sources for halal product. Furthermore, the production of gelatine from chicken skin can be beneficial to the food industries since most of the chicken in Malaysia is Halal certified. Therefore, the study of gelatine from chicken, such as skin is interest as sources of collagen to extract gelatine. Instead of being waste that can cause pollution to environment, chicken skin can be used for production of gelatine. The waste not only causes pollution but also it emit defensive odour[46]. Those gelatines can be used to replace gelatines that are produced from bovine and porcine sources.

At present, India stands 5th position in the global poultry meat production. Domestic poultry meat production (broiler-carcass weight) is estimated to have increased from less than 1.0 million tons in 2000 to 3.4 million tons in 2012. As per the recent data published by the, 729.21 million numbers of poultry are present in India. The contribution of meat from poultry industry is approximately 36.68%. Slaughtering and processing of poultry birds give only one third as meat portion while the rest as poultry wastes and byproducts, which need to be effectively processed and utilized. Poultry byproducts include offal, bone, blood, viscera, head, feet and feathers, mechanically deboned poultry. In India the total availability of offal/bones, generated from large slaughter houses is estimated to be 21 lakh tones/annum[21]. Estimates of the manure excreted by 1000 birds per day (based on average daily live weights during the birds' production cycle) are approximately 120 kg for layer chickens, 80 kg for meat chickens, 200 to 350 kg for turkeys (grower females and grower heavy males, respectively), and 150 kg for ducks[51]. Export value of edible offal's from different livestock species of India was estimated to be 54751 lakh as per. Efficient utilization of byproducts has direct influence on the alleviation of environmental pollution. Therefore, various advanced technologies for large and small scale utilization of byproducts from poultry should be developed and implemented which will benefit poultry producers, poultry industries as well as the environment. The objective of this paper is to give valuable information regarding efficient utilization of poultry wastes and byproducts.

GME reports the annual world production of gelatin is nearly 326,000 tons, with source from pig skin derived gelatin is the most abundant (46%) output, followed by bovine hides (29.4%), bones (23.1%) and other sources (1.5%). Porcine skin required short time for treatment with acid prior to extraction while for bovine it is subjected to lengthy treatment with alkaline. The raw materials for this mammal are also abundant and the price is lower compared to bovine. A number of studies on developing gelatin alternatives to mammalian gelatin from fish source have been reported including fish skin[30,2]. and fish scales[55]. In South Korea, Jun *et al.*[25], investigates the feasibility of chicken feet to replace cowhides for jokpyun (traditional Korean gel type food).

Global demand for gelatin industry is increasing especially in food and pharmaceutical industry. Major productions of gelatin are from mammalian (porcine and bovine) and the rest are from fish. There are halal issues and health related concerns associated with mammalian gelatin, while for fish gelatin is about allergic reactions. New potential gelatin sources such as poultry skin, feet, and bone has risen to replace mammalian resources. The objective of this review is to present the potentials of gelatin extracted from poultry as an alternative sources of gelatin. Poultry as an alternative doesn't have any

dietary concern and can be accepted if it follows the religious requirement. Limited studies on gelatin produced from chicken show that it has higher glycine, Hydroxyproline and proline content and exhibited higher thermal stability compared to mammalian and fish gelatin. Poultry gelatin from skin and feet are considered as by-products from poultry, so apart from gelatin extraction, it can minimize the product from poultry waste. The present study focuses on alternatives sources of gelatin and the challenges of poultry gelatin for future commercial use. Good waste management practice leading to additional economic benefit. Therefore, aims of this work were to determine the potential of poultry waste for the production of gelatin and evaluating functional properties. Now there is a huge increase in global demand for different poultry products in India due to its price competitive nature, nutritional value and because, it is free from social restrictions in eating point of view. Due to large number of birds are being slaughtered for the production of variety of poultry meat and meat products, huge quantities of wastes and by-products are generated. These are causing environmental pollution and health hazards, attract flies and harbours multifarious microbes of pathogenic nature. Poultry by-products can act as a source of energy, livestock feed, fertilizer, liming components for soil, collagen, gelatine and calcium. After suitable processing, poultry by-products can be converted into feather meal, hydrolized feather meal and poultry by-product meal. Under utilization of by-products not only lead to loss of potential revenues but also lead to the increasing cost of their disposal. Besides pollution and hazard aspects, poultry meat industry wastes have a potential for recycling raw materials into products of higher value.

In this regards this paper reviews the multiple uses of poultry by products for the self sustainability. Alternative sources for the production of gelatin have attracted the attention of researcher in the last decades as reviewed by Gomez-Guillen *et al.*[15]. The chicken and duck based poultry can be considered as one of the best income produced poultry since it can provide eggs and meat. Although the total production of ducks is still less than chickens, duck production increased more rapidly.

India is bestowed with vast livestock wealth and it is growing at the rate of 6% per annum. The contribution of livestock industry including poultry and fish is increasing substantially in GDP of country which accounts for more than 40% of total agricultural sector and >12% of GDP. This contribution would have been much greater had the animal by-products been also efficiently utilized. Efficient utilization of by-products has direct impact on the economy and environmental pollution of the country. Non-utilization or under utilization of by-products not only lead to loss of potential revenues but also lead to the added and increasing cost of disposal of these products. Non-utilization of animal by-products in a proper way may create major aesthetic and catastrophic health problems. Besides pollution and hazard aspects, in many cases meat, poultry and fish processing wastes have a potential for recycling raw materials or for conversion into useful products of higher value. Extraction of gelatin from chicken feet has been done by using alkaline treatment where the higher extraction percentage yield of chicken feet gelatin powder was obtained at 18% w/w[35]. While Srabon et.al[37] reported that 16% gelatin was obtained from chicken skin by using acid treatment. The acid treatment usually used for extract gelatin or collagen from young animal such as pig, fish and poultry within short period between 10 to 48 hrs. While alkaline treatment frequently used for mature animal with complex cross structure, such as bones and cartilage Cattle and buffalo which takes place long to extract the gelatin from 6-20 day[35]. According to Hao *et al.*, the quality of gelatin depends on its physical properties, which is influences by both species and tissue from which it is extracted and from the extraction method.

The waste generated in industries of meat products are frequently very large and represent a serious problem due to the high

content of organic matter, and these wastes can serve as sources of microorganisms' proliferation. Thus, according to Pelizer *et al.*[34], the environmental problems are worsened by inadequate treatment of industrial solid waste. In this way, it is necessary to minimize these wastes, even before they reach the grease and rendering plants. By keeping above mentioned reason to minimize environmental pollution through poultry waste, this waste can be utilized and extract gelatin Therefore, the objectives of the present study were to extract gelatin from poultry skin and bone from different temperature ranges i.e. 40ºC, 45ºC, 50ºC, 55ºC and 60ºC, to evaluate functional properties of the gelatin extracted from poultry skin and bone and quality evaluation.

MATERIAL AND METHODS

Raw material

Poultry skin and bone was collected from Ratnagiri poultry market. The poultry skin and bone was washed and stored at - 20^0C until further use.

Gelatin extraction from chicken skin

Gelatin was extracted from chicken skin according to the method of Badii and Howell[5], with certain modifications. For the extract gelatin, 100 gm defatted dried chicken skin was mixed with 300 ml sodium hydroxide (0.15 % w/v). The mixture was shaken well and slowly stirred at room temperature (27°C) for 40 min. This step was repeated three times. The alkaline solution was changed every 40 min to remove non-collagenous proteins and pigments. The alkaline treated pellets were rinsed with distilled water. The resulting pellets were then mixed with 300 ml sulphuric acid 0.15 % (v/v). Again, the resulting pellets were mixed with 300 ml of citric acid solution 0.7 % (w/v). The mixture was shaken well and stirred gently at room temperature for 40 min. The acid solution was changed every 40 min to denature the collagen in the chicken skin matrix. Each treatment was repeated three times and each treatment took about 2 h to complete. The pellets were then subjected to a final wash with distilled water to remove any residual salts followed by centrifuging at 3500 x g for 15 min. The final extraction was carried out in 300 ml distilled water at controlled temperature (40˚C, 45˚C, 50˚C, 55˚C, 60˚C) for overnight without stirring. The resultant mixture was filtered in a Buchner funnel with a Whatman filter paper (no.4). Resultant filtrate was dried in vacuum oven drier at 45˚C for 18 hours. The dry matter was referred to as gelatin powder.

Gelatin extraction from chicken bone

Gelatin was extracted from poultry bone according to the method Rahman, A. N. M and Jamalulail, A. K. S. A. S., with slight modification poultry bone samples was cleaned: skin, fat and cuticles were removed by soaking the samples in boiling water at 100°C for 40 minutes. The poultry bones were then dried at 50°C for 18 hours, and 1 kg dried poultry bone. After fat removal, the dried poultry bones were soaked in HCl solution at concentration of 4% with ratio 1:6 of acid per weight of sample. This process was performed at 27 °C and the solution was changed at intervals of three days for 9 to 12 days. Osein, a spongy material result from the acidic treatment (HCl solution) for the purpose of minerals and non-collagen materials removal; then immersed in 0.2 M NaOH (1:10 w/v) for 20 days and the solution was changed at intervals of 3 days. After 20 days in immersion, the samples were washed and soaked in distilled water for 24 to 48 hours and washed 7 times. Then the pH of the samples was neutralized using HCl or H_2SO_4 up to pH 5 – 7. After pre-treatment, the final extraction was carried out in 300 ml distilled water at controlled temperature (40˚C, 45˚C, 50˚C, 55˚C, 60˚C) for overnight without stirring. The resultant mixture was filtered in a Buchner funnel with a Whatman filter paper (no.4). Resultant filtrate was dried in vacuum oven drier at 45˚C for 18 hours. The dry matter was referred to as gelatin powder.

Estimation of proximate composition of raw material and extracted gelatin.

Proximate compositions were determined by method.

Determination of viscosity

Gelatin solutions at the concentration of 6.67% (w/v) were prepared by dissolving the dry powder in distilled water and heating at 60 ^{0}C for the determination of viscosity. The viscosity (cP) of 10 ml of the solution was determined using Brookfield digital viscometer (Model DV –E Brookfield Engineering, USA) equipped with a No.1 spindle at 400 C ±10 C[9].

Determination of melting point of gelatin

The melting point measurement was done by a method modified from Wainwright[47]. Gelatin solutions, 6.67% (w/v) were prepared and a 5 ml aliquot of each sample was transferred to a small glass tube (borosilicate tube, 12mm × 75mm). The samples were degassed in vacuum desiccators for 5 min. The tubes were then covered with Para film and heated in a water bath at 60 ^{0}C for 15 min. The tubes were immediately cooled in ice-chilled water and matured at 10 ^{0}C. for 18h, five drops of a mixture of 75% chloroform and 25% reddish brown dye (food colour) was placed on the surface of the gel. The gels were put in a water bath at 10 ^{0}C and the bath was heated at rate of 0.2-0.4 ^{0}C /min. The temperature of the bath was read using an electronic digital thermometer (Fisher Scientific). The temperature at which the dye drops began to move freely down the gel was taken as the melting point.

Emulsifying capacity and stability

The method of Yasumatsu *et al.*[53], was used to determine emulsifying capacity and stability. Emulsions were prepared with 1 g of each sample, 50 ml of cold distilled water (4^0 C) and 50 ml of sunflower oil. The gelatin samples were dispersed with a homogenizer/blender. Each blended samples was equally into 50 ml centrifuge tubes. One centrifuge tube was directly centrifuge at 4000 × g for 10 min while the other was centrifuged under the same conditions after heating in a water bath at 80^0 C for 30 min and cooling to room temperature (25^0 C). The height of emulsified layer, as a percentage of the total height of material in the unheated tubes, was used to calculate the emulsifying capacity and stability, using following formulae:

$$\textbf{Emulsifying Capacity} = \frac{\text{Height of emulsion layer}}{\text{Height of whole layer}} \times 100$$

$$\textbf{Emulsifying stability} = \frac{\text{Height of emulsion layer after heating}}{\text{Height of whole layer}} \times 100$$

Determination of gelatin colour and gel clarity

Colour measurement was made by using a Hunter LabScan XE colorimeter (Hunter Association Laboratory, Inc., VA, USA). The tristimulus L*a*b* measurement mode was used as it relates to the human eye response to colour. The L* variable represents lightness (L*=0 for black, L*=100 for white), the a* scale represents the red/green (+a* intensity of red and -a* intensity of green) and the b* scale represents the yellow/blue (+b* intensity of yellow and -b* intensity in blue). The samples were filled into clear Petri dish and readings were taken. Clarity was determined by measuring transmittance (%T) at 620 nm in spectrophotometer (Thermospectronic, Cambridge, U. K) through 6.67% (w/v) gelatin solution which were heated at 60^0 C for 1 h[4].

Determination of isoionic point (pI)

Isoionic point of fish gelatin was determined according to the method described by Zhang *et al.*,[55]. The pI was determined by measuring the transparency of 2 % (w/v) gelatin solution with different pH values at 660 nm spectrophotometer (Thermospectronic, Instrument). The pH value at which the solution has the lowest transparency was the pI value of the gelatin.

Hydroxyproline content

Hydroxyproline content of gelatin was determined according to the method of Bergman and Loxley[8], with a slight modification. The samples were hydrolyzed with 6 M HCL at 110^0C for 24 hrs in reflex condenser and filtrate through Whatman no.1 filter paper. The filtrate was neutralized with 1M NaOH to pH 6.0-6.5. The neutralized sample (0.1) ml was transferred into a test tube and isopropanol (0.2ml) was added and mixed well. To the mixture, 0.1 ml of an oxidant solution (a mixture of 7% (w/v) chloroamine T and acetate/citrate buffer, pH 6, at a ratio of 1:4 (v/v) was added and mixed thoroughly. Then 1.3ml of Ehrlich's reagent solution (a mixture of solution 2g of p-dimethylalamine benzaldehyde in 3ml of isopropanol) were added. The mixture was mixed and heated at 60^0 C for 25 min in water bath and then cooled for 2-3 min in running water. The solution was diluted to 5 ml with isopropanol. Absorbance was measured against water at 558nm using a spectrophotometer (Thermo spectronic, UV 10 rom 0628). Hydroxyproline standard solutions, with concentration ranging from 10 to 60 ppm, were also run simultaneously. Hydroxyproline content was calculated and expressed as mg/g sample.

Statistical Analysis

The data of percentage yield, viscosity, bloom value, water holding capacity, pH, colour, clarity, emulsifying capacity and stability of gelatin extracted from black kingfish at three different temperature (40^0 C, 45^0 C and 50^0 C) were analysed using appropriate statistical methods (Snedecor and Cochran, 1967., Zar 1999). Using ANOVA techniques significant difference between the treatments was determined. The significance of difference between means of treatments was further subjected to SNK test.

RESULTS AND DISCUSSION

Proximate composition

The composition varies considerably depending on size, weight, water, temperature and feeding habits. In present study Proximate composition of poultry skin and bone was observed to be moisture 70.01 and 58.60%, crude protein 12.94 and 10.30%, crude fat 12.00 and 5.70%, ash 4.50 and 6.65% respectively. On similar line quality of fresh skin and bone showed moisture 69.64 and 60.66%, crude protein 17.63 and 18.69%, crude fat 12.16 and 2.19%, ash 0.14% respectively. The proximate composition and yield of extracted gelatin were shown in (Table1). The gelatin extracted from poultry skin and bone at different temperatures (40°C, 45°C, 50°C, 55°C and 60°C) showed high values of proteins and low values for moisture, ash and fat content. The gelatin obtained at 45°C contained higher content of protein than other temperatures i.e. 81.73% for skin and 67.00% for bone. Jongiareonrak et al.[24], reported a protein content of 87.9% and 88.6% in gelatin extracted from the skin of big eye snapper and brown eye snapper respectively. The gelatin from skin of adult Nile pearch also obtained 88% protein when extracted at 50°C[31]. Koli et al.[28,29], reported a protein content 86.45% in gelatin extracted from the skin of Tiger toothed croaker, when extracted at 45°C. Rahman et al.35, studied protein content of 67.40% from chicken feet gelatin.

Moisture content of gelatin extracted from poultry skin and bone at five different temperatures (40°C, 45°C, 50°C, 55°C and 60°C) were 9.90, 8.07, 8.80, 8.73 and 9.20% for skin and 7.22, 6.13, 7.92, 8.17 and 8.87% for bone respectively. Moisture content of all samples was well below the limit prescribed for edible gelatin i.e. 15%. At 6-8% moisture, gelatin is very hygroscopic and it becomes difficult to determine the physiochemical attributes with the accuracy[11].

The ash content of gelatin extracted from poultry skin and bone at five different temperatures (40°C, 45°C, 50°C, 55°C and 60°C) were 2.20, 1.83, 2.10, 2.27 and 2.60% for skin and 2.13, 1.13, 1.43, 2.27 and 2.37% for bone respectively. And these values are less than the recommended maximum limit of 2.6%[23] and the limit given for edible gelatin i.e. 2%.

Gelatin yield

The gelatin yield was extracted from poultry skin and bone at five different temperatures (40°C, 45°C, 50°C, 55°C and 60°C) for skin were 9.43, 10.53, 11.07, 11.80 and 12.23% for skin and 7.30, 7.49, 7.50, 7.52 and 8.03% for bone respectively. The gelatin yield of both skin and bone was found high at 60°C. The results show that as there is rise in temperature as per treatment the yield of both skin and bone gelatin increases and the significant difference is observed. (Table1 and 2). Variations in the yield have also been reported due to differences in the diverse extraction methods followed[16,20,31]. Leaching of collagen during washing and treatments of skin could result in the lower yield of gelatin. Insufficient denaturation of soluble collagen during extraction can also result in lower yield. The maximum swelling of skins of black kingfish was observed during pre-treatment with alkali and acid correlating with better yield due to opening of cross links during swelling. A high degree of cross linking via covalent bonds can cause decrease in solubility of collagen and leading to lower content of extractable gelatin.

Gudmundsson and Hafsteinsson[19] recorded the 14% yield of gelatin of cod fish. Gomez-Guillen *et al.*[16], recorded the percentage yield of sole fish, megrim, cod, squid and hake gelatin were 8.3, 7.4, 7.2, 2.6 and 6.5% respectively. Jamilah and Harvinder[20] reported that the yield of red tilapia gelatin and black tilapia gelatin were 7.81% and 5.39% respectively. Rahman et al.[35], recorded yield of chicken feet gelatine as 4.1% and dry gelatine powder as 18%.

Table 1: Proximate composition and yield of poultry skin gelatin extracted at different temperatures

Sr. No	Proximate Composition (%)	40°C	45°C	50°C	55°C	60°C
1	Moisture	9.90±0.12	8.07±0.09	8.80±0.23	8.73±0.22	9.20±0.17
2	Protein	77.30±0.31	81.73±0.46	80.00±0.55	79.23±0.48	78.23±0.15
3	Fat	1.33±0.15	0.87±0.09	1.60±0.15	1.63±0.12	1.80±0.15
4	Ash	2.20±0.12	1.83±0.09	2.10±0.21	2.27±0.12	2.60±0.15
5	Yield	9.43±0.18	10.53±0.20	11.07±0.15	11.8±0.12	12.23±0.24

Table 2: Proximate composition and yield of poultry bone gelatin extracted at different temperatures

Sr. No	Proximate Composition (%)	40°C	45°C	50°C	55°C	60°C
1	Moisture	7.22±0.22	6.13±0.09	7.92±0.10	8.17±0.18	8.87±0.23
2	Protein	63.96±0.40	67.00±0.79	64.23±0.55	62.68±0.30	61.91±0.37
3	Fat	2.03±0.19	0.90±0.06	1.60±0.15	2.13±0.19	2.17±0.15
4	Ash	2.13±0.18	1.13±0.09	1.43±0.19	2.27±0.12	2.37±0.09
5	Yield	7.30±0.18	7.49±0.20	7.50±0.15	7.52±0.12	8.03±0.24

Bloom value (Gel Strength)

Gelatin is highly capable of forming hydrogen bonds with water molecules to form a stable three-dimensional gel. The need to evaluate the characteristics of the gel has resulted in the concept of gel strength which is known as bloom value. In present study, bloom values of gelatin extracted poultry skin and bone at five different temperatures (40°C, 45°C, 50°C, 55°C and 60°C) of skin were found to be 257.67, 274, 265.33, 263 and 260gm and of skin were 258.33, 282.67, 273.67, 264.33 and 262gm respectively. The bloom value was significantly ($p<0.05$) higher at 45°C of poultry skin and bone gelatin compared to others (Table3 and 4). The bloom value obtained in this study were higher to that of tilapia (180.76 g)[20], sin croaker (124.94 g) and short fin scad (176.92 g) and lower than that of Nile pearch (229 g)[31], of yellow fin tuna (426

g)[9], tilapia (263 g)[17], and grass carp (267 g)[27]. The ability to form weak gels may find new application for fish gelatin as a non-gelling gelatins and it could possibly be used in refrigerated products and in products where low gelling temperature are required[18]. The gel strength of fish gelatin has been reported in a wide range 124-426 g, compared to 200-300 g for bovine or porcine gelatin[26]. The difference in gel strength among the various species could be explained by differences in extraction process used and the intrinsic properties of collagen which varies among fish species. Gudmundsson and Hafsteinsson[19], suggested that the gel strength may depend on isoelectric point and may be controlled, to certain extent, by adjusting the pH. Rahman *et al.*[35], reported that the gel strength of chicken feet skin was 264.33g and similarly[1], reported the gel strength from chicken by-product as 294.78g.

Viscosity

In present study, viscosity of gelatin extracted from poultry skin and bone at five different temperatures (40°C, 45°C, 50°C, 55°C and 60°C) of skin were found to be 3.83, 5.53, 4.43, 4.07 and 4.03 cP respectively and of bone were found to be 5.77, 9.10, 8.33, 7.37 and 6.53cP respectively (Table 3 and 4). Viscosity is the second most important commercial property of gelatin after gel strength[49]. The viscosity for the samples was in the range of 3.83 and 5.53cP in skin, 5.77 and 9.10cP in bone. The viscosity was significantly ($p<0.05$) higher at 45°C of Poultry skin and bone gelatin compared to other temperatures (Table 4.1.5.2b and 4.2.5.2b). Viscosity is partially controlled by molecular weight a molecular size distribution[43]. The viscosities of most of the commercial gelatins have been reported up to 13.0 cP[22]. Jamilah and Harvinder [20] reported that the viscosity of red tilapia gelatin and black tilapia gelatin were found to be 3.20 and 7.12 cP, respectively, whereas for channel cat fish it was 3.23 cP[52]. Park *et al.*[33], reported Viscosity of duck feet gelatin as 0.0074 in extraction using water bath, 0.0037 using electric cooker and 0.015 in microwave oven.

Melting Point

In present study, melting point of gelatin extracted from poultry skin and bone at five different temperatures (40°C, 45°C, 50°C, 55°C and 60°C) were found to be 31.97, 33.03, 32.63, 32.60 and 32.50°C for skin and 28.90, 31.70, 30.40, 29.87 and 28.97°C for bone respectively. The melting point of poultry skin and bone gelatin was significantly higher ($p<0.05$) at 45°C compared to temperatures (Table 3 and 4). It is known that fish gelatin has lower melting point than mammalian gelatin[32]. The melting point of bovine gelatin and porcine gelatin has been reported as 29.7 and 32.3°C, respectively[18]. The melting points observed in the present study are far higher than those reported for cold water fishes such as cod (13°C), hake (14°C) [16], and hoki (16.6°C)[30]. However, these melting points were lower than that of black tilapia (28.90°C)[20], which was warm water fish. Park *et al.*[33], reported melting point of duck feet gelatine was 38.69, 33.06 and 39.38 using three different treatments.

Emulsifying capacity and stability

Emulsifiers are surface active materials that absorb to interface and facilitate the production of small droplets by lowering the interfacial during homogenization[48]. The amphoteric nature with hydrophobic zones on the peptide chain make gelatin to behave as an emulsifier and it is being use in the manufacture of toffees and water-in-oil emulsion such as low fat margarine, salad dressing, and whipped cream[7]. In present study, emulsifying capacity and stability of gelatin extracted from poultry skin and bone at five different temperatures (40°C, 45°C, 50°C, 55°C and 60°C) and significant difference in (Table 3 and 4). Emulsifying capacity of gelatin extracted from poultry skin and bone at five different temperatures (40°C, 45°C, 50°C, 55°C and 60°C) in skin were found to be 43.70, 48.73, 47.77, 46.90 and 45.33 % respectively and in bone 36.53, 4**3.73,** 39.63, 38.57 and 37.07% while emulsifying stability in skin were found to be 41.27, 47.20, 45.20, 43.23 and 42.77% respectively and in bone 27.37, 31.53, 29.93, 29 and 28.30%. There is a

growing trend within food industry to replace synthetic emulsifiers with more natural sources[14]. Protein extracted from a variety of natural sources can be used as emulsifiers in food because of their ability to facilitate the formation, improve the stability, and produce desirable physio-chemical properties[12]. Emulsifying capacity and stability was more in poultry skin and bone gelatin which was extracted at 45°C temperatures. The functional properties of gelatin depend on several factors including the method of preparation and the intrinsic characteristics of collagen[5].

Table 3: Functional properties of gelatin extracted from poultry skin

Sr. No	Functional properties	Gelatin extracted by different temperature				
		40°C	45°C	50°C	55°C	60°C
1	Gel strength (gm)	258.33±4.41	282.67±2.19	273.67±2.33	264.33±1.76	262.00±2.65
2	Viscosity (cP)	5.77±0.41	9.10±0.10	8.33±0.15	7.37±0.18	6.53±0.15
3	Melting point (0C)	31.97±0.19	33.03±0.12	32.63±0.09	32.60±0.12	32.50±0.06
4	Emulsifying capacity (%)	43.70±0.36	48.73±0.32	47.77±0.0.15	46.90±0.12	45.33±0.27
5	Emulsifying stability (%)	41.27±0.49	47.20±0.21	45.20±0.38	43.23±0.24	42.77±0.46

Table 4: Functional properties of gelatin extracted from poultry Bone

Sr. No	Functional properties	Gelatin extracted by different temperature				
		40°C	45°C	50°C	55°C	60°C
1	Gel strength (gm)	257.67±1.45	274.00±2.31	265.33±3.18	263.00±2.52	260.00±3.21
2	Viscosity (cP)	3.83±0.18	5.53±0.09	4.43±0.24	4.07±0.09	4.03±0.15
3	Melting point (0C)	28.90±0.23	31.70±0.15	30.40±0.26	29.87±0.15	28.97±0.09
4	Emulsifying capacity (%)	36.53±0.30	43.73±0.09	39.63±0.33	38.57±0.75	37.07±0.28
5	Emulsifying stability (%)	27.37±0.27	31.53±0.20	29.93±0.09	29.00±0.21	28.30±0.42

Gelatin colour and gel clarity

Colour of gelatin extracted from poultry skin and bone at five different temperatures (40°C, 45°C, 50°C, 55°C and 60°C) were expressed in terms of L*, a* and b* and there were significant differences in colour characteristics among the samples studied (Table 5 and 6) respectively. 81.17 L*, 1.65 a*, 8.35 b* and 47.99 L*, 1.55 a*, 7.48 b* for 40°C 84.68 L*, 2.70 a*, 7.96 b* and 57.56 L*, 1.81 a*, 8.42 b* for 45°C 83.47 L*, 2.67 a*, 7.38 b* and 54.75 L*, 2.37 a*, 7.96 b* 50°C 83.51 L*, 2.91 a*, 6.93 b* and 52.70 L*, 2.77 a*, 7.09 b* for 55°C 82.42 L*, 2.49 a*, 7.72 b* and 50.35 L*, 2.76 a*, 7.68 b* for 45°C respectively. The skin and bone gelatin was extracted at 45°C showed the greatest lightness value (L*) as compared to 40°C, 50°C, 55°C and 60°C temperatures. Similar results were found to redness (a*) and there was no significant difference with respect to yellowness (b*). It can be concluded that factors such as raw material influence the colour characteristics of extracted gelatin. Both colour and clarity of a gelatin gel are important aesthetic properties, depending on the application for which the gelatin is intended. While the skin and bone gelatin was

extracted at 45°C showed the highest transmittance (T%) as compared to 40°C, 50°C, 55°C and 60°C temperatures (Table 4.1.6 and 4.2.6). The turbidity and dark colour of gelatin is commonly caused by inorganic, protein and mucosubstance contaminants, introduced or not removed during its extraction[13]. Koli *et al.*[28,29], reported that Tiger-toothed croaker skin gelatin colour i.e. 75.41 (L*), 2.79 (a*), and 19.25 (b*) for lightness, redness and yellowness respectively, while clarity in transmittance T(49.43%). While for Pink pearch skin gelatin colour 71.74 (L*), 2.74 (a*) and 22.07 (b*) for lightness, redness and yellowness respectively, while clarity in transmittance T(44.30%). Koli *et al.*[28], reported that Tiger-toothed croaker bone gelatin colour i.e. 65.44 (L*), 1.65 (a*), and 22.50 (b*) for lightness, redness and yellowness respectively, while clarity in transmittance (40.50 %T). While for Pink pearch bone gelatin colour 62.50 (L*), 1.97 (a*) and 22.60 (b*) for lightness, redness and yellowness respectively, while clarity in transmittance T(40.13%). See *et al.*[40], reported that gelatin colour of four different fish species i.e. Catfish (44.36 L*, 0.56 a* and -3.65 b*), red tilapia (40.40 L*, 0.71 a* and -2.86 b*). (Widyasari & Rawdkuen 2014) reported chicken feet gelatine colour as 61.45L*, 0.36a*, 8.49b* using acid extraction method.

Table 5: Gelatin colour and gel clarity of extracted gelatin from Poultry Skin

Gelatin colour and gel clarity	40°C	45°C	50°C	55°C	60°C
Lightness (L*)	81.17±0.44	84.68±0.42	83.47±0.15	83.51±0.23	82.42±0.15
Redness (a*)	1.65±0.21	2.70±0.15	2.67±0.18	2.91±0.14	2.49±0.20
Yellowness (b*)	8.35±0.34	7.96±0.38	7.38±0.21	6.93±0.40	7.72±0.82
Transmittance (%)	39.17±0.18	40.47±0.61	38.57±0.09	37.83±0.18	36.47±0.20

Table 6: Gelatin colour and gel clarity of extracted gelatin from Poultry bone

Gelatin colour and gel clarity	40°C	45°C	50°C	55°C	60°C
Lightness (L*)	47.99±1.23	57.56±1.01	54.75±1.85	52.70±2.49	50.35±1.59
Redness (a*)	1.55±0.16	1.81±0.35	2.37±0.17	2.77±0.17	2.76±0.08
Yellowness (b*)	7.48±0.08	8.42±0.13	7.96±0.22	7.09±0.07	7.68±0.44
Transmittance (%)	38.83±0.18	39.93±0.55	37.60±0.26	36.80±0.18	35.67±0.20

Isoionic point

In the present study, isoionic point of gelatin extracted from poultry skin and bone at five different temperatures (40°C, 45°C, 50°C, 55°C and 60°C) was found to be in the range of 4-7pI (Table 7 and 8). Generally, raw materials used to extract gelatin are pre-treated with either dilute acid or alkali solution. These two pre- treated methods produce two types of gelatines Type A and Type B. The Type A gelatin, produced by the acid pre-treatment is reported to exhibit pI range from 7 to 9; whereas Type B gelatin produced by the alkali processing, has an pI range of 4.8–5.1[11]. In another study, Stainsby[44]. has reported that isoionic point of fish gelatin depending on the method in which the collagens are pre-treated, two different types of gelatin (each with

differing characteristics) can be produced. Type -A gelatin (pI at pH 6–9) is produced from acid-treated collagen, and type B gelatin (pI at approximately pH 5) is produced from alkali-treated collagen. According to Eastoe and Leach[13], de-amidation of asparagines and glutamine occur during prolonged exposure of collagenous material to alkali, leading to decrease in pI value. In this experiment, the skins of black king fish were pre-treated with alkali solution resulting the gelatin having pI value of about 4 to 5 which is in accordance with the value reported for Type B gelatin[11], However, Gudmundsson[18]. reported isoionic point (pI) values for megrim (9.5), tilapia (9.1), and cod (8.9). These values of pI were very high as compared to black king fish gelatin.

Hydroxyproline

In present study, hydroxyproline content of poultry skin and bone gelatin extracted at different temperatures (40°C, 45°C, 50°C, 55°C and 60°C) for skin and bone were 5.87 and 6.17% for 40°C, 9.17 and 8.23% for 45°C, 8.37 and 7.50% for 50°C, 7.40 and 7.17% for 55°C, 6.57 and 6.93% for 60°C respectively. Hydroxyproline content was significantly ($p<0.05$) higher at 45°C of poultry skin and bone gelatin compared to 40°C, 50°C, 55°C and 60°C (Table 7 and 8). Gelatin with high levels of amino acids tends to high gel strength and melting point[31], as imino acids are important in the denaturation of gelatin subunits during gelling[22]. Koli *et al.*[29], reported that hydroxyproline content in Tiger-toothed croaker skin and bone gelatins were 7.77 and 7.51 mg/g. While in Pink pearch skin and bone gelatin were 7.63 and 7.41 mg/g. Strength of gelatin gel is influenced by amino acids composition and molecular weight distribution of the gelatin itself, the strength of gelatin also varies with gelatin concentration, thermal history (gel maturation temperature and time), pH and presence of any additives[10]. Cho *et al.*[9], stated that, the stability of collagen and gelatin is proportional to their total amino acid (Proline and Hydroxyproline) and glycine content. Ultimate gel strength is related to its amino acid and glycine content.

Table 7: Isoionic point and hydroxyproline of extracted gelatin from poultry skin and bone

Functional Properties	40°C	45°C	50°C	55°C	60°C
Isoionic point (pI)	5 pH	4 pH	5 pH	6 pH	7 pH
Hydroxyproline (%)	5.87±0.32	9.17±0.09	8.37±0.18	7.40±0.17	6.57±0.1

Table 8: Isoionic point and hydroxyproline of extracted gelatin from poultry bone

Functional Properties	40°C	45°C	50°C	55°C	60°C
Isoionic point (pI)	5pH	4pH	6pH	7pH	7pH
Hydroxyproline (%)	6.17±0.12	8.23±0.38	7.50±0.23	7.17±0.18	6.93±0.15

Microbiological characteristics (Total Plate Count)

In the present study, microbiological characteristics (TPC) from poultry skin and bone at five different temperatures (40°C, 45°C, 50°C, 55°C and 60°C) of skin were found to be 0.83×102, 0.76×102, 0.88×102,0.96×102, 1.1×102 (cfu/g) and of bone were 0.80×102, 0.72×102, 0.84×102, 0.93×102, 0.98×102 (cfu/g) (Table 9 and 10). There was no major difference observed due to no storage period. Gelatin is an excellent nutrient for most of bacteria; hence the extracting processes have to carefully avoid contamination. Most countries have microbiological specifications for gelatin, but generally they are not very onerous. Total plate counts of 1000 cfu/g are generally

accepted with various countries limiting the presence of Coliforms including *Escherichia coli, Salmonella*, Clostridial spores, Staphylococci and sometimes Pseudomonas[11]. In the present study, TPC of poultry skin and bone gelatin was recorded of skin were found to be 0.83×102, 0.76×102, 0.88×102,0.96×102, 1.1×102 (cfu/g) and of bone were 0.80×102, 0.72×102, 0.84×102, 0.93×102, 0.98×102 (cfu/g). The results of present study were agreed with the microbiological standard adopted for Community legislation on gelatin.

Poultry skin and bone used as raw materials for gelatin extraction are usually contaminated with microorganisms originating from chicken market. These organisms consist of potentially pathogenic and non-pathogenic vegetative cells and spores. The initial washing and cleaning of raw materials used plenty of tap water will remove a substantial number of the containing microbes. The subsequent treatment at low or high pH over substantial periods of time will effectively kill contaminating microorganisms[36]. The level of potentially surviving microorganisms will be further reduced due to the high temperature for drying. The combined effect of exposure to high or low pH in combination with heat treatment ensures that viable microorganisms would not be present in the final product[39]. The present study also reveals that except for some bacteria that might have cause non-sterile condition of processing no other pathogen.

Table 9: Total plate count of poultry skin gelatin extracted at different temperature

Microbiological status	40°C	45°C	50°C	55°C	60°C
TPC (Skin)	0.83×10^2	0.76×10^2	0.88×10^2	0.96×10^2	1.1×10^2
TPC (Bone)	0.80×10^2	0.72×10^2	0.84×10^2	0.93×10^2	0.98×10^2

CONCLUSIONS

Poultry waste is one of the major pollutants if not properly disposed. The utilization of animal by-products are often ignored, however, these items contribute a significant value to the livestock and meat industries. Non-utilization of animal by- products in a proper way may create major aesthetic and catastrophic health problems. Value addition of animal by- products has additional revenue for poultry entrepreneurs. Gelatin from the chicken skin and bone was successfully extracted by different temperature range i.e. 40, 45, 50, 55 and 60^0C. If the temperature increases yield will increase. Furthermore the functional properties of extracted gelatin will be affected by increasing temperature above 45^0C. On the basis of results of present study the recommended temperature for extraction of gelatin is 45^0C. Global demand for gelatin industry is increasing especially in food and pharmaceutical industry. Major productions of gelatin are from mammalian (porcine and bovine) and the rest are from fish. There are halal issues and health related concerns associated with mammalian gelatin, while for fish gelatin is about allergic reactions. New potential gelatin sources such as poultry skin and bone has risen to replace mammalian resources. Poultry as an alternative doesn't have any dietary concern and can be accepted if it follows the religious requirement. Poultry gelatin from skin and bone are considered as by-products from poultry, so apart from gelatin extraction, it can minimize the product from poultry waste.

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