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Research paper

Gelatine Extraction from Chicken Skin as Influenced by NaOH Pretreatment

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ABSTRACT

The present study was conducted to determine the effect of different NaOH concentrations for pretreatment of deserted chicken skins used thereof for gelatine extraction. The low temperature (50°C) treatment was used to extract maximum fat from minced skin and resulting skin was used to see the feasibility of gelatine extraction. The effect of NaOH pretreatment at different concentrations (0.1, 0.2 and 0.4%) on various quality attributes of chicken skin gelatine was observed. The proximate composition revealed moisture, protein, fat and ash content of chicken skin gelatine varying from 10.06 to 10.63%, 81.67 to 84.58%, 2.20 to 3.22% and 0.86 to 0.92%, respectively. The 0.4% NaOH showed highest protein, and lowest moisture, fat and ash content. The dried gelatine sheets were transparent and have mildly rancid odour; the yield varied from 4.27 to 4.81% and pH ranges from 4.89 to 4.93. A reduction in L* and a* value was observed with increasing NaOH concentration, but the percentage transmittance value was highest in 0.4% NaOH. The electrophoretic pattern showed discernible α-1 and α-2 chains with maximum distribution of proteins between 70 and 120kDa. Therefore, due to better quality attributes and maximum resemblance with control; 0.4% NaOH concentration is suggested for pretreatment of chicken skin during gelatine extraction.

Keywords: *Gelatine, NaOH pretreatment, Chicken skin, Colour, Proximate composition*

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INTRODUCTION

The demand and consumption of poultry meat and meat products has increased tremendously during last few years. The high demand for poultry meat in years to come will demand processing of more number of birds, generating higher amount of poultry slaughter waste. The by-products obtained from poultry processing has the potential to be converted into high value product (Kulkarni and Devatkal 2015). In India, most of the chicken are slaughtered in wet market and consumers prefer skinless broilers. This chicken skin is discarded as such without any treatment which causes environmental nuisance besides wasting a valuable source of animal protein and fat. The proper utilization of this skin can open new avenues for its economic disposal (Mandal et al. 2011).

The skin constitute nearly 15% of broiler carcass (Hayse and Marion 1973) and it contains approximately 20-30% of fat without adipose tissue (Sheu and Chen 2002). As per Osburn and Mandigo (1998) chicken skin contains nearly 40% fat and 9% protein, besides having about 3.5% collagen content (Cliche et al. 2003). This collagen can be converted into gelatine which can have many uses. Gelatin is a natural biopolymer produced by collagen thermo-hydrolysis (Ahmad et al., 2018). Due to its high water binding capacity, film forming, foaming and emulsifying ability, it has found varied uses in pharmaceuticals, food, cosmetic and photography industry (Vidal et al. 2020; Wang et al. 2018).

The gelatine from chicken skin has been extracted by many workers in the past (Sarbon et al. 2013; Rasli and Sarbon 2015; Sompie and Triasih 2018; Saenmuang et al. 2019; Tumerkan et al. 2019). The gelatine for poultry or fish skin is commonly carried out by two pretreatment steps. In the first step skin is exposed to alkaline pretreatment to remove noncollagenous proteins and pigments whereas in second step it is treated with the acid to cause swelling of skin and to provide necessary pH conditions for gelatine extraction (See et al. 2015). The pro[1]perties of the gelatine are affected by alkaline pretreatment, stronger alkaline conditions leads to the relatively higher viscosity but reduces yield (Schrieber and Gareis 2007). Therefore, the present study was carried out to see the effect of alkaline pretreatment on quality of gelatine extracted from low temperature rendered broiler skin.

MATERIALS AND METHODS

Skin preparation

Broiler (age 6-8 weeks) skins with feathers were collected from roadside retail shops of Bareilly city (U.P, India). The skins were washed thrice with potable water at room temperature to remove the adhering dirt or blood clots. After washing, skins were dipped in 5% salt solution for one hour to reduce the microbial load. Skins were again washed with potable water and to loosen the feathers, and then dipped in 4% acetic acid overnight at 25±1°C. The loosened feathers from skin were removed manually and the defeathered skins were washed thoroughly under running water. The skins were then frozen at -20°C till use, not later than 15 days. Then frozen skin were thawed at 4±1°C for 12 hours and then minced through a meat mincer using 8mm plate. The minced skin was then rendered in a locally designed electrically heated dry renderer at 50°C for 4 hours at atmospheric pressure with continuous agitation at the rate of 20 rpm. The rendered fat or the liquid low density phase was separated using double layered muslin cloth and pressing it 30 minutes by applying pressure @ 1 kg/cm^2. The rendered skin was then stored at -20°C till further use, not later than 15 days. The frozen rendered skin was thawed at 4±1°C for 12 hours before use.

Pretreatment

To rendered skin 0.1, 0.2 or 0.4% NaOH solution was added

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in the ratio of 1:5 and stirred slowly over magnetic stirrer for 2 hour at room temperature. This step was repeated after discarding used NaOH solution and thorough washing the skin with potable running water. After completion of NaOH treatment, the skin was again thoroughly washed. After this acidic treatment was carried out to produce sufficient swelling of skin by soaking it in 0.5 M acetic acid (1:4 w/v) for 18 hours. After which the skin was thoroughly washed with potable tap water till the running water tested neutral.

Extraction of gelatine

Thermo hydro extraction of gelatine was carried out with distilled water (1:3 w/v) at 60°C in a hot water bath for 6 hours with intermittent stirring. After extraction the liquid part was separated from residual skin by filtering through double layered nylon mesh strainer. The gelatine extract was poured into beaker and kept overnight at refrigeration temperature to see its gelling behavior. The gelatine extract was heated in water bath at 55°C to liquefy and then it was clarified by centrifuging at 10000g for 20 minutes. The drying of gelatine was carried out at °55C in hot air oven. The dried gelatine films were ground in a mixer grinder transferred to air tight polypropylene containers for further analysis. The commercial gelatine procured from market was used as control.

Physicochemical properties

Proximate analysis

The proximate analysis (moisture, protein, fat and ash) of extracted gelatine was carried out using approved methods (AOAC 1995).

Sensory evaluation

The sensory evaluation of dried gelatine was carried out for colour and odour. Gelatine sheets were ground and evaluated (Setyawaty and Triliandari 2018). The gelatine obtained after overnight refrigeration was also evaluated for quality of gel produced.

The yield of gelatine was calculated based on wet weight of minced skin using the following formula:

$$\text{Gelatine Yield (\%)} = \frac{\text{Weight of Dried Gelatine(g)}}{\text{Wet weight of Skin(g)}} \times 100$$

pH

The pH value for gelatine was determined using Hanna pH meter (Hanna 211 Intruments, Italy).

Colour and clarity

The colour measurement of dried gelatine was carried out using MiniScan EZ Hunter Lab (4500 L Spectrophotometer, Hunter Associate Laboratory, Inc., Reston) in which values L*, a*, and b* refers black-white, red-green, and yellow-blue colour, respectively. To determine the clarity, gelatin solution (1%) was heated at 60°C, and the clarity was determined by measuring transmittance using spectrophotometer (GENESYS 10 UV-Vis, Thermo scientific, U.S.A) at 620 nm (Roy et al. 2017).

Electrophoretic analysis

The molecular weight distribution was determined by SDS-PAGE analysis of dried chicken skin gelatine and control using method of Laemmli (1970). Gelatine samples (5mg) were dissolved in distilled water (1mL) at 60°C and then mixed with 2x sample buffer in 1:1 (V/V) ratio to have a final concentration of 2.5mg/mL and then boiled in water bath for 5 minutes. This chicken skin gelatine solution (10 µL) and high molecular weight protein marker (6 µL) were loaded in the gel. The SDS-PAGE was run using 5% stacking gel and 8% separating gel at 30mA. The staining was carried out using 0.15% (w/v) Coomassie brilliant blue R-250 in 45% (v/v) methanol and 10% (v/v) acetic acid and de-staining was done with 30% (v/v) methanol and 10% (v/v) acetic acid.

Statistical analysis

The experiments were repeated thrice and every time reading were taken in duplicate (n=6). The data generated was analyzed for Mean±SE and one way analysis of variance with SPSS (Version 20.0 for Windows; SPSS, Chicago, U.S.A.) according to the procedure of Snedecor and Cochran (1995).

RESULTS AND DISCUSSION

Proximate analysis

The proximate analysis of chicken skin gelatin showed the moisture level of 10.06±0.14 to 10.63±0.27 for treatments whereas for control it 12.15±0.11, indicating that the gelatine was dried enough to be stable at room temperature (Table 1). Moreover, the moisture level in all three treatments was lower than control sample. The water content in gelatin is influenced by humidity, drying time, storage conditions and type of packaging used (Ockerman and Hansen 2000). The presence of fat, ash and other extraneous impurities influence the quality of gelatine (Jellouli et al. 2011) and proximate analysis plays an important role to ensure that removal of impurities and hydrolysis was carried out efficiently (Muyonga et al. 2004).

Table 1: Proximate analysis of chicken skin gelatine

Treatment	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Control	12.15±0.11^{c}	86.43±0.31^{b}	0.54±0.08^{a}	0.89±0.05
0.1% NaOH	10.63±0.27^{b}	81.67±0.92^{a}	3.22±0.07^{d}	0.92±0.02
0.2% NaOH	10.34±0.17^{ab}	83.13±1.85^{ab}	2.77±0.08^{c}	0.95±0.06
0.4% NaOH	10.06±0.14^{a}	84.58±1.23^{ab}	2.20±0.09^{b}	0.86±0.05

The protein content of chicken skin gelatine in 0.1% NaOH was significantly lower than other treatments and control. The protein content in 0.4% NaOH (84.58±1.23) was slightly lower than control (86.43±0.31), but among treatments an increase in protein content was observed with increasing NaOH concentration. The high protein content indicates the purity of gelatine; the protein content of 84.23% and moisture content 6.20% was reported in native chicken leg skin extracted at 60°C by Sompie and Triasih (2018). The gelatine powder extracted from chicken feet exhibited 6.43% humidity, 1.54% ash, 67.40% protein and 0.42% fat (Rahman and Jamalulail 2012). In another study, gelatine extracted from chicken legs skin using different acetic acid concentrations showed protein level ranging from 88.10 to 89.92% and moisture content of 7.12 to 7.44 % (Sompie et al. 2019). Similarly, protein, moisture, ash, and fat contents of 84.29, 12.57, 2.13, and 0.45%, respectively were reported in gelatine extracted from broiler skin (Aykın-Dincer et al. 2017). Although, the fat content of the chicken skin gelatine was higher than control, but there was a significant reduction in fat content from 0.1 to 0.4% NaOH treatment. NaOH aids in saponification of fats which can be further removed by washing, higher level of saponification achieved at higher NaOH concentration helps better removal of fat as well as other impurities. The further reduction in fat content can be achieved by degreasing chicken skin before extraction (Boran et al. 2010). The ash content was comparable to control, indicating efficient removal of foreign materials.

Sensory evaluation, yield and pH

The assessment of dried chicken skin gelatine for its colour and odour (Table 2) revealed that after drying pale yellow flaky powder was obtained. The colour of control gelatine was light yellow and it was granular in texture. The difference in colour and texture could be due to different processing methods adopted, commercial gelatine sheets are usually thicker giving granular texture. The quality requirements for gelatine powder are colourless to pale yellowish without any offensive odour (Setyawaty and Triliandari 2018). The mildly rancid odour of chicken skin gelatine is due to high fat content as no chemical degreasing was done. The quality of gel formed is one of very important parameter to determine gel quality.

Table 2: Sensory quality, yield and pH of chicken skin gelatine

Treatment	Colour	Odour	Gelation	Yield (%)	pH
Control	Light Yellow	No odour	Very Firm	-	5.74±0.05^{b}
0.1% NaOH	Transparent pale yellow	Mildly Rancid	Slightly weak	4.81±0.05^{c}	4.91±0.04^{a}
0.2% NaOH	Transparent	Mildly Rancid	Firm	4.54±0.03^{b}	4.89±0.03^{a}
0.4% NaOH	Transparent	Mildly Rancid	Very Firm	4.27±0.05^{a}	4.93±0.03^{a}

The control and chicken skin gelatine extracted using 0.4% NaOH were very firm, whereas gel from 0.2% NaOH was intermediate and 0.1% was slightly weak. Overall the quality of gel formed improved with increasing NaOH concentration. The higher protein content and purity of gelatine affects its gelation behavior. Moreover, the NaOH solution changed from transparent to cloudy during treatment indicating noncollagenous deproteinization process. The NaOH treatment help in breakdown of collagen telopeptides causing swelling of chicken skin (Jaswir et al. 2011). The yield of gelatine is an important parameter that determine the commercial value of processing conditions used. It reduced significantly with increasing NaOH concentration, which might be due to higher deproteinization and better removal of impurities in 0.4% NaOH. The high protein content and better gelling behavior indicate that although at high concentration the yield was significantly lower the quality of gelatine was better. The gelatine yield of 7.83% was reported in chicken feet skin and tendons as raw material (Almeida and Lannes 2013). In another study the on the wet weight basis gelatine yield of only 2.16% was achieved using alkaline pretreatment and consequent acid extraction (Sarbon et al. 2013). The decreased yield could be due to collagen loss during repeated washing or additionally due to incomplete hydrolysis (Jamilah and Harvinder 2002). The pH of chicken skin gelatine (4.89-4.93) was lower than control (5.74). This is due to the acidic treatment given to skin prior to hydrolysis and incomplete removal of the acidic components during washing. The pH value of gelatine is affected by the chemical process used for gelatine extraction and it affects texture profile and high bloom strength is observed when it reaches isoelectric (pH 5.0 for gelatin B) point (Gudmundsson and Hafsteinsson 1997). The pH value of 5.82 in broiler skin extracted gelatine (Aykın-Dincer et al. 2017) and 4.83 in gelatine extracted from chicken deboner residue was reported (Rafieian et al. 2015).

Colour and clarity

A significant difference was observed in L*, a* and b* value of dried chicken skin gelatine among different treatments (Table 3). The value of L* for 0.4% NaOH (25.54±0.47) was significantly lower than other treatments as well as control. Although, a* value was lowest for 0.1% NaOH and highest for control, b* value was lowest for 0.2% NaOH (13.04±0.19) and highest for control (23.60±0.06). The colour difference in gelatine depends on various processing conditions and chemicals used in extraction, the residual fat in gelatine as well as raw material used (Ockerman and Hansen 2000). However, the composition and functional properties are not much affected by the colour of the gelatine (Cheow et al. 2007). Although, the lightness value was lower it was comparable with commercial control. Rahman and Jamalulail (2012) reported L*, a* and b* values of 42.94±0.69, 2.82±0.23 and 11.42±0.20 in chicken feet gelatine, the values are comparable to those for chicken skin gelatine.

Table 3: Instrumental colour and clarity analysis of chicken skin gelatine

Treatment	*L**	*a**	*b**	Transmittance (%)
Control	28.94±0.24^{b}	3.68±0.02^{d}	23.60±0.06^{c}	79.08±0.29^{d}
0.1% NaOH	37.41±0.66^{c}	1.26±0.04^{a}	13.45±0.12^{b}	30.85±0.19^{a}
0.2% NaOH	28.32±0.53^{b}	1.41±0.03^{b}	13.04±0.19^{a}	38.15±0.51^{b}
0.4% NaOH	25.54±0.47^{a}	1.87±0.03^{c}	13.73±0.07^{b}	49.47±0.56^{c}

The turbidity or clarity of the gelatine solution is an important parameter that affects its acceptability. The clarity measurement in terms of percentage transmittance showed a significantly higher turbidity in treatments (30.85±0.19 to 49.47±0.56) than control (79.08±0.29). Among treatments there was an increasing trend in transmittance percentage from 0.1 to 0.4% NaOH. The inefficient noncollagenous deproteinization at lower concentration may be the reason for lower clarity. The gelatine extracted from Half-smooth tongue sole fish skin showed a transmittance (%T) of 60.64 ± 0.12% as compared to commercial gelatine having transmittance value of 85.49 ± 0.20% (Li et al. 2019). In commercial gelatine manufacturing the impurities are removed by chemical processes, filtration or clarification and lower transmittance of treatments observed may be due to apparent residual suspensions such as inorganic contaminants, mucous substances or protein fractions not removed during extraction process (Zarai et al. 2012).

Electrophoretic analysis

The molecular weight distribution pattern of the chicken skin gelatine is given in Fig. 1. The electrophoretic pattern of extracted chicken skin gelatine showed two clear bands at around 120 kDa, indicating the presence of α1 and α2 chains produced during collagen hydrolysis. A series of bands between 70 and 120 kDa is also observed indicating the presence of smaller peptides in this region. In case of control very faint bands were visible at around 130 kDa and in most of other areas a smearing pattern was observed. Due to breakage of inter-chain crosslinks and further splitting of peptide chains of collagen during hydrolysis, gelatine with varying molecular mass is produced (Sarbon et al. 2013).

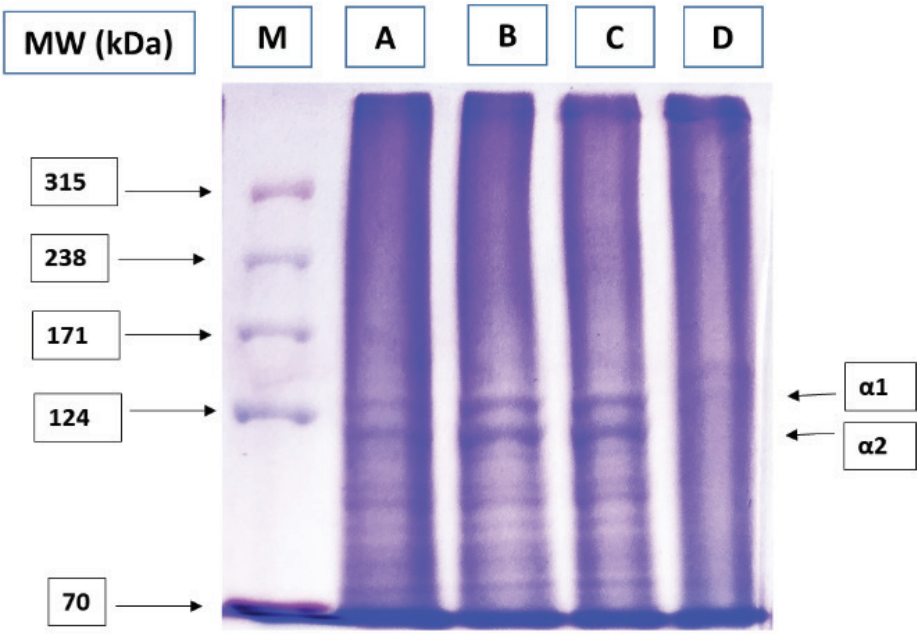

Fig. 1: Electrophoretic analysis of extracted chicken skin gelatin, A=0.1% NaOH treatment, B=0.2% NaOH treatment, C=0.4% NaOH treatment, D=control

SDS-PAGE analysis of gelatine extracted from Chinese salamander skin showed similar band pattern with two stripes at around 116 kDa (Jin et al. 2019). The concentration and type of chemicals used in extraction of gelatine causes partial loss of α- or β-chains (Niu et al. 2013), which may be the reason behind different electrophoretic pattern of control and treatments. The electrophoretic analysis of chicken feet gelatine showed major protein bands at 198 kDa and 130 kDa, whereas commercial bovine gelatin did not exhibited any identifiable protein band (Widyasari and Rawdkuen 2014). In another study, the gelatine extracted from chicken bone showed a broad range of molecular weight ranging from 15 to 300 kDa. The small peptide with less than 100kDa weight were also found and highest intensity of small fragments was at around 48 kDa (Yuliani et al. 2019).

CONCLUSION

The physical, chemical and molecular analysis of gelatine obtained from low temperature rendered chicken skin using different concentrations of NaOH for its pretreatment revealed that 0.4% NaOH is optimum for this purpose. The gelatine extracted using this treatment showed high protein percentage, low fat content, very good gelation behavior, and better clarity. Moreover, among all treatments its attributes were most closely comparable with that of control.

REFERENCES

Almeida PF, Lannes SCS (2013) Extraction and physicochemical characterization of gelatin from chicken By-Product. J Food Process Eng 36(6) : 824-833

Ahmad T, Ismail A, Ahmad SA, Khalil KA, Awad EA, Leo TK, Imlan JC, Sazili AQ (2018) Characterization of gelatin from bovine skin extracted using ultrasound subsequent to bromelain pretreatment. Food Hydrocoll 80 : 264-273

AOAC (1995) Official Methods of Analysis, 16th edn. Association of Official Analytical Chemists, Washington DC

Aykın-Dincer E, Koc A, Erbas M (2017) Extraction and physicochemical characterization of broiler (Gallus gallus domesticus) skin gelatin compared to commercial bovine gelatin. Poult Sci 96(11) : 4124-4131

Boran G, Mulvaney SJ, Regenstein JM (2010) Rheological properties of gelatin from silver carp skin compared to commercially available gelatins from different sources. J Food Sci 75(8) : E565-E571

Cheow CS, Norizah MS, Kyaw ZY, Howell NK (2007) Preparation and characterization of gelatins from the skins of sin croaker (Johnius dussumieri) and shortfin scad (Decapterus macrosoma). Food Chem 101(1) : 386-391

Cliche S, Amiot J, Avezard C, Garepy C (2003) Extraction and characterization of collagen with or without telopeptides from chicken skin. Poult Sci 82 : 503-509

Gudmundsson M, Hafsteinsson H (1997) Gelatin from cod skins as affected by chemical treatments. J Food Sci 62(1) : 37-39

Hayse PL, Marion WW (1973) Eviscerated yield, component parts, and meat, skin and bone ratios in the chicken broiler. Poult Sci 52 : 718-722

Jamilah B, Harvinder KG (2002) Properties of gelatins from skins of fish—Black tilapia (Oreochromis mossambicus) and red tilapia (Oreochromis nilotica). Food Chem 77(1) : 81-84

Jaswir I, Monsur HA, Salleh HM (2011) Nano-structural analysis of fish collagen extracts for new process development. Afr J Biotechnol 10(81) : 18847-18854

Jellouli K, Balti R, Bougatef A, Hmidet N, Barkia A, Nasri M (2011) Chemical composition and characteristics of skin gelatin from grey triggerfish (Balistes capriscus). LWT - Food Sci Technol 44(9) : 1965-1970

Jin WG, Pei J, Du YN, Pan J, Gao R, Chen DJ, Wu HT, Zhu BW (2019) Characterization and Functional Properties of Gelatin Extracted from Chinese Giant Salamander (Andrias Davidianus) Skin. J Aquat Food Prod Technol 28(8) : 861-876

Kulkarni VV, Devatkal SK (2015) Utilization of byproducts and waste materials from meat and poultry processing industry: A Review. J Meat Sci 11(1) : 1-10

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227 : 680-685

Li Y, Tang C, He Q, Li X, Zhang A (2019) Extraction optimization and characterization of gelatin from half-smooth tongue sole (Cynoglossus semilaevis Gunther) skin. J Aquat Food Prod Technol 28(6) : 637-648

Mandal PK, Cytyarasan S, Pal UK, Rao VK, Das CD (2011) Development of snacks (Murukku) by incorporation of broiler skin. J Meat Sci 7(2) : 54-57

Muyonga JH, Cole CGB, Duodu KG (2004) Extraction and physico-chemical characterisation of Nile perch (Lates niloticus) skin and bone gelatin. Food Hydrocoll 18(4) : 581-592

Niu L, Zhou X, Yuan C, Bai Y, Lai K, Yang F, Huang Y (2013) Characterization of tilapia (Oreochromis niloticus) skin gelatin extracted with alkaline and different acid pretreatments. Food Hydrocoll 33(2) : 336-341

Ockerman HW, Hansen CL (2000) Glue and gelatin, In Animal By-Product Processing and utilization. CRC Press, Pennsylvania, U.S.A pp 183-216

Osburn WN, Mandigo RW (1998) Reduced-fat bologna manufactured with poultry skin connective tissue gel. Poult Sci 77 : 1574-1584

Rafieian F, Keramat J, Shahedi M (2015) Physicochemical properties of gelatin extracted from chicken deboner residue. LWT-Food Sci Technol 64(2) : 1370-1375

Rahman MNA, Jamalulail SASKA (2012) Extractions, physicochemical characterizations and sensory quality of chicken feet gelatin. Borneo Sci 30 : 1-13

Rasli HI, Sarbon NM (2015) Effects of different drying methods on the rheological functional and structural properties of chicken skin gelatin compared to bovine gelatin. Int Food Res J 22(2): 584-592

Roy BC, Omana DA, Betti M, Bruce HL (2017) Extraction and characterization of gelatin from bovine lung. Food Sci Technol Res 23(2) : 255-266

Saenmuang S, Phothiset S, Chumnanka C (2019) Extraction and characterization of gelatin from black-bone chicken by-products. Food Sci Biotechnol 1-10

Sarbon NM, Badii F, Howell NK (2013) Preparation and characterization of chicken skin gelatin as an alternative to mammalian gelatin. Food Hydrocoll 30(1) : 143-151

Schrieber R, Gareis H (2007) Gelatine handbook, theory and industrial practice. Wiley-VCH GmbH & Co., Weinheim, Germany pp 63-71.

See SF, Ghassem M, Mamot S, Babji AS (2015) Effect of different pretreatments on functional properties of African catfish (Clarias gariepinus) skin gelatin. J Food Sci Technol 52(2) : 753-762.

Setyawaty R, Triliandari M (2018) Gelatin Production from Skin of Chicken Leg using A Variety of Naoh Concentration. Jurnal Ilmu Dan Teknologi Hasil Ternak 13(2) : 126-132

Sheu KS, Chen TC (2002) Yield and quality characteristics of edible broiler skin fat as obtained from five rendering methods. J Food Eng 55(3) : 263-269

Sompie M, Triasih A (2018) Effect of extraction temperature on characteristics of chicken leg skin gelatin. IOP Conf. Ser. Earth Environ Sci 102 : 1-4

Sompie M, Surtijono S, Tinangon M R (2019) Effect of temperature and extraction time on the characteristics of pigskin gelatin. Sci Papers Ser D Ani Sci 62(1) : 424-428

Snedecor GW, Cochran WG (1995) Statistical methods, 8th ed. Oxford and IBH publishing Co. New Delhi, India.

Tumerkan ETA, Cansu U, Boran G, Mac Regenstein J, Ozogul F (2019) Physiochemical and functional properties of gelatin obtained from tuna, frog and chicken skins. Food Chem 287 : 273-279

Vidal AR, Duarte LP, Schmidt MM, Cansian RL, Fernandes IA, de Oliveira Mello R, Demiate IM, Dornelles RCP (2020) Extraction and characterization of collagen from sheep slaughter by-products. Waste Manag 102 : 838-846

Wang L, Song X, Cui H, Man S, Li W, Muluye RA, Bian Y, Chu X, Yan D, Cai Y (2018) Anti-fatigue effects of peptide isolated from sheep placenta. Chin Herb Med 10(3) : 279-284

Widyasari R, Rawdkuen S (2014) Extraction and characterization of gelatin from chicken feet by acid and ultrasound assisted extraction. Food Appl Biosci J 2(1) : 85-97

Yuliani D, Awalsasi DR, Jannah A (2019) Characterization of gelatin profile of chicken broiler (Gallus domestica) bone Using SDS-PAGE electrophoresis. Alchemy-J Chem 7(1) : 7-12

Zarai Z, Balti R, Mejdoub H, Gargouri Y, Sayari A (2012) Process for extracting gelatin from marine snail (Hexaplex trunculus): Chemical composition and functional properties. Process Biochem 47(12) : 1779-1784