



## Poultry gelatin: Characteristics, developments, challenges, and future outlooks as a sustainable alternative for mammalian gelatin

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### ABSTRACT

**Background:** Studies indicate a 30% increase in demand for all types of food and non-food grade gelatins in the world. The largest volume of gelatin production comes from mammal sources (cows and pigs). Nowadays, health, cultural, and religious concerns have arisen due to consumption of mammalian gelatin. This has prompted scientists to look for non-mammalian sources that closely resembles the desirable physicochemical, functional, and sensory characteristics of mammalian gelatins. Non-mammalian gelatin from poultry and fish by-products are also gaining importance in food industry. Over the past decade, poultry production has increased by about 37.34%. Poultry by-products have good potential for replacing mammalian sources for gelatin extraction.

**Scope and approach:** This paper reviews in detail the fundamental properties of poultry gelatins (PG), including rheological, functional and physicochemical properties. This study provides a perspective on their potential food, pharmaceutical, medical and industrial applications.

**Key findings and conclusions:** The highest quality PG was extracted through acid treatments. PG extracted in this way exhibited favorable rheological, fat replacement, film formation, foaming, emulsifying and sensory properties, and nutritional quality. PG films showed better barrier properties than mammal-origin gelatin, making them ideal for food and medical applications. The amino acids composition of PG, especially the imino acid and hydrophobic amino acids, which determine the physicochemical and functional properties of gelatin, are higher than gelatin obtained from mammals and fish that classifies them in the upper Bloom category.

### 1. Introduction

Gelatin, a partially hydrolysed of collagen, is biodegradable and exhibits good applicability in food as a texture, water-binding and creamy provider, foaming, emulsifier and fining agent, colloid stabilizer (Karim & Bhat, 2009), biodegradable packaging material, vehicle for encapsulating probiotic living cells (Soukoulis, Behboudi-Jobbehdar, Yonekura, Parmenter, & Fisk, 2014) and micro-encapsulating agents (M. C. Gómez-Guillén, Giménez, López-Caballero, & Montero, 2011). It is low in calories and is used as a supplier of protein in body-building foods and as carbohydrate reducer in diabetes patients' diets. In medicine, it is used in composites utilized to repair bone defects or for bone tissue engineering (BTE) (Ranganathan, Balagangadharan, &

Selvamurugan, 2019), nanoparticles as drug and gene delivery systems (Mahmoudi Saber, 2019), a matrix for implants, in injectable drug delivery microspheres, as alive attenuated viral vaccines stabilizer agent, in intravenous infusions, for the production of hard and soft capsules, plasma expanders, wound care and cosmetic fields and etc.

Sheela (2014) suggests an increase of about 30% in global demand for food and non-food grade gelatin, as was expected, it rose from 348.9 kilo tons in 2011 to 450.7 kilo tons in 2018, where 40% of the gelatin output in 2011 was derived from pig skin. In the past, gelatin was extracted from the skin and cartilage of pigs (46%) output, bovine hides (29.4%), bones (23.1%), and other sources (1.5%) (GME, 2020). Nowadays, owing to bovine spongiform encephalopathy (BSE) and foot-and-mouth disease (FMD) concerns, other safer sources are also

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used for gelatin extraction. Consuming the pig skin gelatin is considered unlawful in Judaism and Islam and beef gelatin is acceptable only if it has been prepared according to the religious dietary law. World's population of Muslims, Jews and Hindus is expected to increase about 53% in the next 20 years to around 3.7 billion (PRC, 2019). These concerns have even led scientists to look for analytical methods to find ways to recognize the source of the gelatin species such as chromatographic, chemisorption, mass spectrometric, spectroscopic, immunochemical, halal authenticity applying species-specific PCR (Shabani et al., 2015), molecular techniques and proteomic methods like electrospray ionization quadrupole time-of-flight mass spectrometry (nano-UPLCESI-q-TOF-MSE) (Yilmaz et al., 2013) which are expensive and time-consuming. Therefore, developing and offering gelatin alternatives to nearly more than a third of world population is highly desirable for food industries as the global market for halal foods is growing rapidly (Badii & Howell, 2006; Oladzadabbasabadi, Ebadi, Mohammadi Nafchi, Karim, & Kiahosseini, 2017).

According to Cheow, Norizah, Kyaw, and Howell (2007), the gelatin quality depends on its physical and chemical specifications, which are strongly associated with animal species and the tissue types they are extracted from. During the past decade, there has been an intensive trend in gelatin derived from non-mammalian sources, especially fish and poultry. So far, the gelatin's yield from poultry by-products (skin, bone, scale, mechanically deboned residue, and feet) has been so low that it has not reached a commercial scale (Schrieber & Gareis, 2007; Araghi et al., 2015; Hazaveh, Mohammadi Nafchi, & Abbaspour, 2015). Researchers in both industrial and academic fields have attempted to develop alternative routes to produce non-mammalian gelatin resembling unique functional properties of the mammalian ones.

Most of the studies in the past to find novel sources for gelatin production have been focused on fish. The effects of various extraction methods on properties of the gelatin obtained from different fish species, such as black and red tilapia skin (Jamilah & Harvinder, 2002), hake and cod skin (M. Gómez-Guillén et al., 2002), haddock and pollock (Zhou, Mulvaney, & Regenstein, 2006), and ribbon (Norziah, Kee, & Norita, 2014) have been thoroughly studied. Gelatin from fish has limited use because of its weaker gel strength and lower stability of its gel structure as compared with mammalian gelatin. The fishy odor is also one of the major drawbacks of the fish gelatin (Rafieian, Keramat, & Kadivar, 2013). Fish-sourced gelatin accounts for only 1% of the world's annual gelatin production (Jan Arnesen & Gildberg, 2002).

Some studies have investigated gelatin production from poultry by-products. However, there is limited information on detailed characterization of physicochemical and structural properties of these types of gelatin. Therefore, this review study aims to provide an insight into other aspects of PG as a suitable alternative to mammalian gelatin, with an emphasis on the properties of the tissues and methods used for gelatin extraction and the physico-chemical, functional and sensory properties of PG. Also, the potential difficulties and possibilities for increasing the industrial use of PG and further research pathway strategies are discussed.

## 2. Poultry gelatin

According to the Schrieber and Gareis (2007) collagen classification, about 27 types of collagen have been identified so far. Type I collagen is derived from connective tissue such as bone, skin, and tendons. Collagen of type II occurs practically and widely in cartilage tissue. Type III collagen is strongly related to age whereas very young skin contains up to 50% and sometime reduces to 5–10%. Other types of collagen are available in limited amounts, which is only and often organ-specific. Gelatin is a water soluble material obtained from the fibrous protein collagen, which is the main component of animal bone, skin and connective tissues. Therefore, the source, collagen type and age of the animal species are the factors that affect gelatin properties. Partial hydrolysis of native collagen forms gelatin, which is combined of an

important repeating strand of Gly-X-Y triplets, where X is originally proline, and Y is mainly hydroxyproline which stabilizes collagen structure or in other words are Gly-Pro-Y, Gly-X-Hyp, and Gly-Pro-Hyp, with the transition temperature of the (Gly-X-Hyp)<sub>n</sub> being higher than that of (Gly-Pro-Y)<sub>n</sub> (Schrieber & Gareis, 2007).

The quality of the amino acids in gelatin determines the unique properties and functionality of gelatin. Therefore, this parameter guarantees many physicochemical properties and causes to make differences between gelatins which are mainly owe to different amino acid composition as well as proline and hydroxyproline contents (imino acid). Hydroxyproline is a derivative of proline and both are responsible for the stability of collagen structure by forming hydrogen bonds in its structure. Imino acid contents are also responsible for the melting and gelling temperatures of gelatin. The lower the imino acid contents, the lower the melting and gelling temperatures of gelatin, so the amount of the amino acid content distinguishes and identifies different types of gelatin sources (Haug, Draget, & Smidsrød, 2004). The amount of imino acid in gelatin derived from poultry skin (Mhd Sarbon, Badii, & Howell, 2013) is higher than that of mammals (Eysturskarð, Haug, Elharfaoui, Djabourov, & Draget, 2009) and fish (Wang et al., 2015; Lin, Regenstein, Lv, Lu, & Jiang, 2017). Different types of gelatin have different physicochemical characteristics that affect the thermal and rheological properties, including melting and gelling temperatures and Bloom strength (Cheow et al., 2007).

FAOSTAT (2019) have reported that worldwide poultry meat production grew from about 92.68 million tons in 2008 (of which the share of the chicken, duck, goose and guinea fowl, and turkey were 80.84, 3.83, 2.27 and 5.7 million tons, respectively) to around 127.29 million tons in 2018 with the share of the chicken, duck, goose and guinea fowl, and turkey values of 114.26, 4.46, 2.64 and 5.9 million tonnes, respectively (Table 1). The growth of the poultry industry has led to an increase in large quantities of poultry slaughterhouse by-products and wastes. Among poultry by-products produced in the slaughtering, processing and without commercial targets, skin, feet, bone, and blood, which are considered waste, are utilized to manufacture meals. Several by-products previously considered as waste, such as skin, feet, bones, blood, among others, now are recycled and used in pet foods (Padilha, Silva, & Sampaio, 2006). Poultry slaughter waste contains about 34.2% dry matter which contains 51.8% crude protein, 41.0% fat and 6.3% ash (Koby, Senturk, & Bayramoglu, 2006), therefore, it can possibly replace mammalian resources to produce gelatin. About 375,000–400,000 tons of gelatin is produced annually worldwide (Meky, Fujii, Ibrahim, & Tawfik, 2019), of which only 2% (about 8 tons) extracted from non-mammalian sources. It is estimated that the cut components and bones of the carcass make up about 7–8% of the live weight of the broiler (Salminen & Rintala, 2002), which is about 9.5 million tons of suitable poultry by-product as an alternative to mammals to extract gelatin. The PG has been shown to contain amino acids, secondary structure, and molecular weight similar to those of mammalian gelatin. Moreover, this novel source would further encourage efforts to exploit untapped available resources and recycle industrial waste (Mhd Sarbon et al.,

**Table 1**  
Production of poultry worldwide and its portion (FAOSTAT, 2019).

Livestock primary	Production value (million tonnes)	Period of years	Production changes (%)
Meat, chicken	80.84 to 114.26	2008 to 2018	41.34
Meat, duck	3.83 to 4.46	2008 to 2018	16.48
Meat, goose and guinea fowl	2.27 to 2.64	2008 to 2018	16.26
Meat, turkey	5.7 to 5.9	2008 to 2018	3.38
Meat, Poultry (Total)	92.68 to 127.29	2008 to 2018	37.34

2013).

Generally, gelatin can be extracted from various parts of poultry that contain significant amounts of protein, including the heads and feet with protein levels of 16% (Okanović, Ristić, Kormanjoš, Filipović, & Živković, 2009) and bones (Cheng, Liu, Wan, Lin, & Sakata, 2008; H.; Cheng, Zhu, et al., 2008) with 23–24%. D. Liu, Lin, and Chen (2001) and Huda, Seow, Normawati, and Aisyah (2013) reported that the collagen contents in the chicken and duck feet were 30.74% and 28.37% (wet basis), respectively, which are comparable to that extracted from fish waste material include skin, bone and fins with average of 50% (dry basis or 33.3% wet basis) reported by Nagai and Suzuki (2000). Jun, Lee, Lee, and Kim (2000) studied the feasibility of using chicken feet to replace cow hides, moreover, a number of studies were focused on poultry skins (Cliche, Amiot, Avezard, & Garipey, 2003; Mhd Sarbon et al., 2013; Bichukale et al., 2018; T.-K.; Kim et al., 2020), feet (Almeida, da Silva Lannes, Calarge, de Brito Farias, & Santana, 2012; Yeo, Song, Ham, He, & Kim, 2013; Widyasari & Rawdkuen, 2014; Y.-H.; Kuan, Nafchi, Huda, Ariffin, & Karim, 2016; Abedinia, Ariffin, Huda, & Nafchi, 2017), bones (Haroun, Beherei, & El-Ghaffar, 2010; Bichukale et al., 2018; Dewi; Yuliani, Awalsasi, & Jannah, 2019; D; Yuliani, Maunatin, Jannah, & Fauziyyah, 2019), mechanically deboned residue (Fonkwe & Singh, 1997; Rammaya, Ying, & Babji, 2012; Rafieian et al., 2013; Rafieian, Keramat, & Shahedi, 2015) and processing by-product (Almeida, da Silva, da Silva Lannes, de Brito Farias, & Santana, 2013; Gál et al., 2020) to develop gelatin as an alternative source for mammalian gelatin.

It is worth mentioning that in the year of 2017 world poultry meat had about 27.5 million tonnes of production more than 2009 (FAOSTAT, 2019), resulting in significant volume of organic waste generation in different production stages. Due to the high content of organic materials, these wastes can be hosted microorganisms' proliferation and environmental problems are raised by inadequate treatment of this industrial solid waste (Alireza Seidavi, 2019; Pelizer, Pontieri, & Moraes, 2007).

2.2. Materials and methods used for gelatin extraction

Depending on the pre-treatment procedure, under acid and alkaline pre-treatment conditions, two types of gelatin are obtainable that are commercially known as type-A (isoelectric point at pH ~ 8–9) and type-B (isoelectric point at pH ~ 4–5) (M. C. Gómez-Guillén et al., 2011). Raw materials derived from fish and poultry are still new to the market. Therefore, gelatin manufacturers must precisely adjust the process and extraction parameters to obtain a product with maximum desired properties (see Table 2).

Table 3 lists the various available reports on extraction and characterization of poultry and marine gelatin as alternative sources.

For PG, as the normally young poultry are slaughtered, the material can be pre-treated using the acid process. The poultry skins contain a large volume of fat and low concentration of collagen and it is preferred to use other organs such as feet. The poultry bone is not demineralized before conditioning, so during the extraction concentration of salts is high and a precipitation step after extraction is necessary. Additional steps, such as ultrafiltration and deionization, also help remove excess

Table 2 Raw material conditioning (Schrieber & Gareis, 2007).

Raw material type	Raw material conditioning	
	Acid	Alkali
Bones	*	*
Cattle hide splits	*	*
Pigskin splits	*	*
Pigskin	*	
Fish skin	*	
Poultry skin	*	
Poultry feet	*	

Table 3 Selected reports on extraction and characterization of alternative gelatin.

Sources	Reference
Poultry:	
Turkey and chicken heads	Du et al. (2013)
Chicken heads	(Gál et al., 2020), (Ee et al., 2019)
Chicken deboned residue	(Masood & Chen, 1995), (Rammaya et al., 2012), (Rafieian et al., 2013), (Rafieian et al., 2015), (Erge & Zorba, 2018)
Turkey deboned residue	Fonkwe and Singh (1997)
Chicken feet	(Jun et al., 2000), (Almeida et al., 2012), (Rahman & Jamalulail, 2012), (Almeida et al., 2013), (Almeida et al., 2013), (Choe & Kim, 2018) (Saenmuang et al., 2019), (Santana et al., 2020)
Duck feet	(Yeo et al., 2013), (Park et al., 2013), (Y. H. Kuan, Nafchi, Huda, Ariffin, & Karim, 2017), (Abedinia et al., 2017), (Nik Muhammad et al., 2018)
Chicken skin	(Mhd Sarbon et al., 2013), (Bichukale et al., 2018), (Aykin-Dinçer et al., 2017), (Saenmuang et al., 2019)
Duck skin	(S.-J. Lee, Kim, Kim, et al., 2012), (T.-K. Kim et al., 2020)
Poultry bones	(Dewi Yuliani, Awalsasi, & Jannah, 2019), (Dewi Yuliani, Awalsasi, & Jannah, 2019), (Bichukale et al., 2018), (Haroun et al., 2010)
Marine:	
Nile perch ( <i>Lates niloticus</i> ) skins and bones	Muyonga, Cole, and Duodu (2004)
Yellowfin tuna ( <i>Thunnus albacares</i> ) skins	(S. M. Cho et al., 2005)
Bigeye snapper skins	Nalinanon et al. (2008)
Lizardfish ( <i>Saurida</i> spp.) scales	Wangtueai and Noomhorm (2009)
Hoki ( <i>Macruronus novaezelandiae</i> ) skins	Mohtar, Perera, and Quek (2010)
Mackerel ( <i>Scomber scombrus</i> ) heads	Khari, Rico, Martin-Diana, and Barry-Ryan (2011)
Marine snail ( <i>Hexaplex trunculus</i> ) meat	Zarai, Balti, Mejdoub, Gargouri, and Sayari (2012)
Cobia ( <i>Rachycentron canadum</i> ) skins	Silva, Bandeira, and Pinto (2014)
Skin of octopus ( <i>Octopus vulgaris</i> )	Jridi et al. (2015)
Comparative studies:	
Tuna, frog and chicken skins	Aksun Tümerkan, Cansu, Boran, Regenstein, and Özoğul (2019)

salts (Schrieber & Gareis, 2007). The PG has been extracted using different methods, which are summarized in Table 4.

2.2.1. Acid treatment

In the acidic process, an acid solution is used to collagen hydrolysis, which is product of this process called type A gelatin. Pigskins are the most commonly used raw material for this process, which are treated over a period of 10–45 h. Acidic treatment causes collagen swelling to increase the efficiency of gelatin extraction throughout thermal hydrolysis (Damrongsakkul, Ratanathamman, Komolpis, & Tanthapanichakoon, 2008). To ensure the warm-water solubility of the collagen, hand-sized pieces of skin should be soaked in 2–4% dilute sulphuric or hydrochloric acid at room temperature for 24 h. Due to the mechanical agitation involved in this process, the fat is separated and is easily removed as it floats onto the surface (Schrieber & Gareis, 2007). Swelling attributes and further solubilisation of collagen are intensely affected by the type and concentration of acid used according to the durability of some of inter-relations between collagen chains, which causes the variance in the distribution of molecular weight in the resulting gelatin.

Phosphoric and organic acids are additionally reasonable for this preparation step, yet they are increasingly costly and adversely influence the smell and the flavour of the final product. However, Dewi Yuliani, Maunatin, Jannah, and Fauziyyah (2019) obtained gelatin with favorable physicochemical properties from broiler chicken bones using different concentrations and treatment times of 8–10% phosphoric acid.

**Table 4**  
Procedures used to extract poultry gelatin.

Poultry gelatin	Preparation and pre-treatment	Extraction procedure	Reference
Chicken feet gelatin (CFG)	Washing whit chlorine (2 ppm of active Cl <sub>2</sub> ) water	Extracting in 4% acetic acid solution at 60 °C for 4 h	(Almeida et al., 2013), (Almeida et al., 2012)
CFG	Washing, soaking in 0.1 N HCl at 18 °C for 24 h with a ratio of 1/10 (v/w) of tissue/solution	Neutralizing by washing, then samples were placed in polyethylene bags, vacuum-packaged and heating in water bath at 75 °C or 65, 75, 85, and 95 °C for 2	(H. Y. Kim, Song, et al., 2012), (Choe & Kim, 2018)
CFG	Washing, soaking in 4.0% and 0.318%–3.682% acetic acid for 16 h and 1–8.4 h	Rinsing in tap water and extracting with DW (1:2 w/v) for 6 h at 55C and 43.3 °C–76.8 °C.	(Almeida et al., 2013), (Santana et al., 2020)
CFG	Cleaning at 100 °C for 40 min to remove skin, fat and cuticles, then drying at 50 °C for 18 h	Soaking in HCl 4% with ratio 1:6 with changing every three days for 9–12 days, soaking osein in 0.2 M NaOH (1:10 w/v) for 20 days with changing every 3 days, then soaking in DW for 24–48 h until pH = 5–7 and heating at 60 °C for 5 h	(Bichukale et al., 2018), (Rahman & Jamalulail, 2012)
Duck skin gelatin (DSG)	Washing, soaking in 2% NaOH or 0.1 M acetic acid with a skin/solution of 1:7 (w/v)	Washing until the pH ~ 7 and extracting with distilled water (DW) for 3 h at 65 °C; skin/water 1:5 (w/v)	(S.-J. Lee, Kim, Kim, et al., 2012)
DSG	Washing, adjusting pH 1 to 14 with 0.1 N HCl, 0.1 N NaOH, and DW for 24 h, washing for 48 h and sample pH 1 was used for extraction because of highest rate of swelling.	Extracting using the following 4 extraction methods for 10 min: water bath (60 °C), sonication (60 °C with 40 kHz), superheated steam (steam temperature of 150 °C), and microwave (2450 MHz and 200 W power), then filtering and coagulating at 4 °C for 12 h and freeze drying	(T.-K. Kim et al., 2020)
Duck feet gelatin (DFG)	Washing and soaking in 0.1 N HCl in 5 times (v/w) for 24 h	Neutralizing by washing for 48 h (pH 5.5), then extracting by the ratio of 1:1 of (duck feet: DW) at 75 °C for 6 h	Yeo et al. (2013)
DFG	Thawing, cleaning, mincing and then washing with tap	Treating with 0.05 M acetic acid or 0.1 M	Abedinia et al. (2017)

**Table 4 (continued)**

Poultry gelatin	Preparation and pre-treatment	Extraction procedure	Reference
	water (1:6, w/v) at 30 °C for 10 min, 3 times	NaOH in ratio of 1:6, w/v for 3 h, washing until neutralize pH, extracting in DW for 12 h at 65 °C with a ratio of 1:2 (w/v). Enzymatic procedure were started with 0.2 M acetic acid containing pepsin 15 units/g in 1:10 (w/v), then stirring at 4 °C for 3 adjusting pH to 7.5, then heating for 12 h at 65 °C. All obtained gelatin were filtered and freeze-dried.	
Chicken skin gelatin (CSG), black-bone chicken feet and skin gelatin (CSG, BCFG and BCSG), broiler skin gelatin (BSG)	Defatted dried samples soaked in NaOH (0.15% w/v) for 40 min then mixture was centrifuged at room temperature.	Rinsing with DW and treating with 0.15% (v/v) H <sub>2</sub> SO <sub>4</sub> and 0.7% (w/v) citric acid solution, washing with DW and centrifuging, then extracting in DW at (45 °C) overnight	(Said & Sarbon, 2020), (N Suderman & Sarbon, 2019), (Bichukale et al., 2018), (Saenmuang et al., 2019), (Aykin-Dincer et al., 2017) (Mhd Sarbon et al., 2013)
Chicken deboned residue gelatin (CDRG)	Drying at 39 °C in oven, then defatting using hexane	Washing, soaking in 1% (w/v) NaCl, treating (HCl (1:2) (w/v) for 24 h), rinsing until pH ~ 7; then extracting at varying temperatures and times	(Rafieian et al., 2013), (Fonkwe & Singh, 1997)
Mechanically deboned chicken meat gelatin (MDCMG)	Washing, defatting, washing, demineralizing (3% HCl for 24 h), washing until the pH ~ 4, then soaking in 4% NaOH (1:5 w/v) for 72 h	Washing, extracting in DW at pH 4 under constant shaking for 120 min at 25 °C with ratio (2.5:1) solution/MDCM residue	Rammaya et al. (2012)
MDCMG	Cutting, grinding, defatting with DW at 35 °C for 1 h, washing, filtering, demineralization in 3 g/100 mL HCl solution for 24 h at 10 °C, washing 3 times and then filtering	Using RSM with these raging of parameters: NaOH (1.8–4.2%), extraction temperature (58–82 °C) and extraction time (30–250 min)	Erge and Zorba (2018)
Chicken head gelatin (CHG)	Mincing, frizzling, thawing, soaking in 0.1% NaOH by shaking for 45 min (4 times), rinsing, drying at 35 °C, defatting (petroleum ether and ethanol (1:1)), then filtering and purified collagen material left to dry	Dried matter was ground and conditioned by proteolytic enzyme: mixing with DW for 15 min, adjusting pH, adding proteolytic enzyme of 0.4% or 1.6%, shaking for 24 or 72 h, then, filtering,	Gál et al. (2020)

(continued on next page)



Table 4 (continued)

Poultry gelatin	Preparation and pre-treatment	Extraction procedure	Reference
		rinsing, mixing with DW, heating to 80 °C, inactivating enzyme, then extracting at 95 °C, then drying at 45 °C for 48 h	
Mechanically deboned turkey residue gelatin (MDTRG)	Treating with 1% NaCl at pH of 10.7 for 30 min, then pretreating by 5% H <sub>2</sub> SO <sub>4</sub> for 24 h	Washing in running tap water for 15 min, then extracting in DW with 1:3 (v/w) at 55 °C for 5, extracting the residue at 70 °C with 1:3 (v/w) DW for another 5 h, then heating the residue at 85 °C	Fonkwe and Singh (1997)
Bird bones gelatin (BBG)	Cleaning, demineralizing using 3% HCl at room temperature for 9–12 days, with the liquor changing of every 3 days	Treating with H <sub>2</sub> SO <sub>4</sub> to a pH of 2.5–3.0 for 16 h, heating, then filtering using activated carbon column, pH adjusting to 5.0 and drying extracted gelatin at 40 °C	Haroun et al. (2010)
Chicken bone gelatin (CBG)	Soaking in NaOH 5% for 2 days 1:4 (v/v). Osein was neutralized by tap water and acetic acid 5%.	The osein was extracted by water in 55, 65, and 75 °C for 4 h, then drying, gelatin purification by ammonium sulphate (40–70%) precipitation, centrifuging then dialysis.	(Dewi Yuliani, Awalsasi, & Jannah, 2019)
CBG	Washing, grounding, degreasing (water: 30 min at 70 °C), washing, drying (25 °C), soaking (phosphoric acid 8–10% (w/v) 1:4 (w/v) for 12 h and 24 h, washing and drying at 25 °C	Extraction was conducted in DW (1:4 (w/v) at 55 °C–75 °C). Treated bones were extracted at 55 °C for 4 h, then filtering. The procedure was repeated to the remained bones at 65 °C and 75 °C for 4 h. All of the filtrates were mixed, then filtering and drying.	(Dewi Yuliani, Awalsasi, & Jannah, 2019)

Bovine hide splits are ordinarily treated with acid for 48–72 h. It must be noticed that ensuing the acid conditioning, the extraction, likewise, is completed in an acid condition. After this treatment, pH is expanded to 2–4 by including alkali. As a result of the reactions, salts are formed which are then cleaned out over a time of 24 h using water. During the extraction, the firmness to viscosity proportion is balanced by the pH, extraction time, temperature just and speed. The producer must alter the ideal proportion between the ideal quick extraction and undesired chemical/thermal hydrolysis of the gelatin (Almeida et al., 2013).

According to Asghar and Henrickson (1982), when using organic

acids for treatment, electrolytes affect the biophysical properties (swelling, solubility, gelatin, viscosity and water-binding capacity) of a protein at various ionic qualities and pH amounts. Saline particles may either tie straightforwardly to the peptide spine of collagen, or influence collagen collapsing in a roundabout way by collaborating with structurally bound-water molecules. ‘Lyotropic hydration’ is described as a hydration arising from the disruption neutral salt ions with non-ionic bonds such as hydrogen bonds of collagen. Therefore, lyotropic agents can modify the structure of water about collagen molecules, interrupt internal hydrogen bonds, or interact with internal hydrophobic bonds by direct binding at similar destinations of the protein chains (Giménez, Turnay, Lizarbe, Montero, & Gómez-Guillén, 2005). For example, the use of NaCl (1.5% w/w) in fish gelatin extraction (Sow & Yang, 2015) was reduced textural properties and gel strength and adding (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and NaH<sub>2</sub>PO<sub>4</sub> to gelatin (Sarabia, Gómez-Guillén, & Montero, 2000) was improved the melting point. They was reported that the reasons for these changes are due to the effect of salts on the change, loss of molecular order or modification of gelatin structure.

T.-K. Kim et al. (2020) by examining the swelling rate of duck skin samples exposed to different pH levels, from 1 to 14, concluded that pH 1 is the most appropriate treatment for extracting DSG due to the high level of collagen type A in the duck’s skin. The remarkable result was that there was no significant difference between skin swelling between pH 1 and 11 treatments. Yeo et al. (2013) conducted a study to evaluate the effects of DFG, as a fat alternative, on the quality of low-fat sausages. Extraction was conducted by soaking in 0.1 N HCl solutions for 24 h. Extraction was conducted at 75 °C for 6 h. The addition of DFG improves cooking properties of low-fat sausages. Almeida et al. (2012) and Santana et al. (2020) characterized the CFG by FTIR spectroscopy. They demonstrated that CFG type A has very good nutritional quality compared to the commercial gelatin. H. Y. Kim, Lee, and Kim (2012) have observed the positive effect of CFG and wheat fibre content on the quality of semi-dried chicken Jerky (an old meat product which prepared with drying in the steps of 55, 60, 73 and 75 °C for 30, 150, 90 and 10 min, respectively). The CFG was obtained by 0.1 N HCl, pH = 1.31 treatment. Choe and Kim (2018) extracted CFG at four different temperatures using HCl at pH 2 and compared the physicochemical properties of their gels with a mixture of wheat fiber (WF). They suggested applying of CFG blending with WF in meat products. Almeida et al. (2013) proved that the extraction of CFG is economically feasible using 4% acetic acid at 60 °C, and it has very good nutritional and sensorial qualities. Sompie, Siswosubroto, Rembet, and Ponto (2019) studied the rheological properties and total bacteria of chicken leg skin gelatin (CLSG) extracted using HCl and acetic acid. Their findings showed that applying acids could reduce total bacteria in the final product (1.6–3.4 × 10<sup>4</sup> comparing other results 5.7 and 4.0 × 10<sup>9</sup> CFU/g).

### 2.2.2. Alkali treatment

In the alkaline media, gelatin is prepared from boned materials, treated with alkali, with or without agitation. The conditioning process takes quite long times depending on the temperatures and concentration. For example, using sodium hydroxide solution at 25 °C takes several weeks to form supersaturated milk of lime (GME, 2020). Agitation consistently accelerates the conditioning process. The most important qualitative factors of gelatin, including Bloom and viscosity, are directly influenced by the concentration, time and duration of exposure to alkaline treatment. Tougher conditions result in higher viscosity gelatin production (Mad-Ali, Benjakul, Prodpran, & Maqsood, 2016). Although the process with lime milk would seem inefficient at first glance, it shows various technological advantages. During the treatment, which may last for several months, mucopolysaccharides, sulphur-containing compounds which are non-protein materials and non-collagenous proteins, are dissolved (D.-C. Liu, 2002). For example, when using calcium hydroxide for treatment, this mild treatment also purifies the raw material while preventing loss of efficiency. If treated with too much alkali, the collagen dissolves in cold water. Thus, by

washing off the raw materials, the collagen is also washed off, reducing gelatin efficiency (Schrieber & Gareis, 2007).

Abedinia et al. (2017) investigated the effects of different pre-treatments on yield and composition of extraction, physicochemical, and rheological properties of DFG. Rheological analysis of alkaline gelatin indicated that almost the same elastic modulus and loss modulus with commercial bovine gelatin. Dewi Yuliani, Awalsasi, and Jannah (2019) extracted CBG by NaOH and characterized it. Although alkaline extraction and subsequent purification and dialysis treatment was not successful in comparison with other methods due to the amount of protein extracted, but, the accompanying purification step in this extract can eliminate large quantities of small peptide fragments and leave a large amount of  $\alpha$ - and  $\beta$ -chains in the product. The RSM study of Erge and Zorba (2018) showed that the most effective parameters on yield extraction and gel strength were extraction temperature and time. Their results indicated the optimum condition of the parameters were defined as NaOH concentration of 2.9–3.4%, extraction temperature ranging from 76 to 82 °C and 105–183 min of extraction time. Saenmuang, Phothiset, and Chumnanka (2019) characterized the BCFG and BCSG extracted using NaOH treatment and compared with commercial bovine gelatin (BG). Results indicated that obtained gelatins had two distinct  $\alpha$ -chains and high Bloom values along with lower color properties.

### 2.2.3. Enzyme aid treatment

Use of proteolytic enzymes is an alternative approach to acid method. As demonstrated by Abedinia et al. (2017) on poultry source and Abdelmalek et al. (2016) on marine source, using 15 Units pepsin/g tissue in gelatin extraction increases the yield of gelatin, but, SDS-PAGE evaluation indicated high band intensity for  $\alpha$ - and  $\beta$ -components, so that, the major components were  $\alpha_1$ - and  $\alpha_2$ -chain bands ( $\approx 100$  kDa each one). Low-molecular-weight peptides were obtained in this extraction, caused by the ample cleavage of peptides via enzyme during gelatin preparation. Thus, the size of the polypeptide chains was influenced by enzyme, which is the most important factor affecting rheological properties.

Pepsin has been mostly applied in studies to isolate collagens from many tissues (J. Cao, Duan, Liu, Shen, & Li, 2019; Hamdan & Sarbon, 2019; S. Cao, Wang, Xing, Zhang, & Zhou, 2020). In specified conditions, it can extract a fairly high amount of the triple-helical molecule intact shape of collagenous material. For example, depending on pepsin concentration and substrate ratio/enzyme, up to 80%–90% of bovine tendons collagens can be extracted (Ju, Liu, Zhang, Liu, & Yang, 2020). Ye et al. (2020) evaluated the physicochemical composition of gelatin obtained from collagen hydrolysis of chicken bones at different temperatures and times. The results of hydrolysis degree and SDS-PAGE analysis showed that the average molecular weight of collagen hydrolyzes at 50 and 70 °C were higher than hydrolyses at 90 °C, which indicates the re-formation of the three helix protein structures in their transport on chains. FTIR analysis confirmed that  $\beta$ -sheet decreased and random coil increased significantly ( $P < 0.05$ ). Gelatin hydrolysates obtained at 90 °C for 30 min showed better properties (melting point, textural and microstructural properties) compared to samples treated at 50 and 70 °C. Enzymes can be used to prepare active gelatin hydrolysates as antioxidant activity agent (Ketnawa, Martínez-Alvarez, Benjakul, & Rawdkuen, 2016). The mechanism of gelatin radical scavenging is due to the presence of residual free amino groups (NH<sub>2</sub>) in its structure that form a stable macromolecule with ambient free radicals and form ammonium groups by adsorption of hydrogen ions (N Suderman & Sarbon, 2019).

### 2.3. Chemical, physicochemical, and functional properties of poultry gelatin

In terms of gelatin application in food industry, the most important properties of gelatin are strength of gel, viscosity, setting and melting temperatures. Table 5 shows the uses of gelatin in different food

categories with their recommended level and Bloom strength. These important attributes are influenced by several parameters such as molecular weight and distribution, gelatin solution concentration, gel cure time and temperature, concentration of H<sup>+</sup> ions and salt content.

So far, several studies have been conducted to extract and evaluate the collagen and gelatin characteristics of poultry. These studies focus on certain purposes such as the feasibility of collagen and gelatin extraction from poultry and determination of physicochemical properties (Almeida et al., 2013; Huda et al., 2013; Rafeian et al., 2015; Dewi; Yuliani, Awalsasi, & Jannah, 2019; Gál et al., 2020; T.-K.; Kim et al., 2020), rheological properties of PG (Mhd Sarbon et al., 2013; Abedinia et al., 2017; Yasin, Babji, & Norrakiah, 2017; Santana et al., 2020), as well as its emulsifying and foaming properties (H. Y. Kim, Song, et al., 2012; Du, Khiari, Pietrasik, & Betti, 2013; Rasli & Sarbon, 2015; Chakka, Muhammed, Sakhare, & Bhaskar, 2017), bioactive activity (S.-J. Lee, Kim, Kim, et al., 2012; S.-J. Lee, Kim, Kim, et al., 2012; Wan Omar & Sarbon, 2016; Said & Sarbon, 2020), fat replacement ability (Yeo et al., 2013; Almeida & Lannes, 2017), sensory quality properties (H.-W. Kim et al., 2014; Nik Muhammad, Huda, Karim, & Mohammadi Nafchi, 2018) and film-forming ability (Haroun, Beherei, & El-Ghaffar, 2010; J.-H.; Lee, Lee, & Song, 2015; Nazmi, Isa, & Sarbon, 2017; Abedinia, Ariffin, Huda, & Mohammadi Nafchi, 2018).

#### 2.2.1. Chemical and structural properties

The twenty types of amino acids found in nature can be divided into three groups:

- Hydrophobic or non-polar, including glycine, alanine, valine, leucine, isoleucine, methionine, phenylalanine, tryptophan and proline.
- Hydrophilic or polar including serine, threonine, cysteine, tyrosine, asparagine and glutamine.
- Hydrophilic electrically charged:
  - o Positive-charged (histidine, arginine and lysine).
  - o Negative-charged (glutamic acid and aspartic acid).

Imino acid contains both carboxyl and imino (CNH) functional groups and proline is classified as an imino acid. (Hanani, 2016). Table 6 gives a brief of the amino acid composition of different kinds of PG. Generally, collagens in poultry sources show a wider variety in amino acid compositions than those of mammalian collagens. Their hydroxyproline and proline contents are higher than those in mammalian collagens, as well as, glycine. In general, the imino acid content of PG is comparable to that in mammal's gelatin. The raw material from which gelatin is extracted determines the amount and sequence of amino acids in the final product. Hydroxyproline amino acid content represents the percentage of gelatin extraction in the process (Nalinanon, Benjakul, Visessanguan, & Kishimura, 2008). The results showed that alcoholic polar amino acids (serine, threonine and tyrosine) play an important role in determining the gel strength. Serine amino acid content (with a

**Table 5**

The multifunctionality of gelatin in food production (Schrieber & Gareis, 2007; Ahmad et al., 2017).

Application	Concentration and function	Bloom strength (g)
Gelatin desserts and gummy bears	7–9% as gel formation	175–275
Meat products, sausages, broths and canned meats	1–5% as emulsion stabilizer and binding agent	175–275
Dairy products	0.2–1.0% as syneresis stabilizer	150–250
Frozen foods	0.1–0.5% reducing water loss agent	200–250
Beverage industry	0.002–0.015% as clarifying agent	100–200

**Table 6**  
Amino acid content of poultry gelatins compared to mammalian gelatins (%).

Amino acids	Porcine <sup>a</sup>	Duck <sup>b</sup>	Chicken <sup>c</sup>	Turkey <sup>d</sup>	Duck feet <sup>e</sup>	Chicken feet <sup>f</sup>
Ala	8.3	9.84	10.08	12.99	8.12	10.11
Arg	8.5	7.87	5.57	5.74	5.67	ND
Asp	6.0	4.81 <sup>g</sup>	2.11	5.82	2.72 <sup>i</sup>	4.24
Cys	0.2	0.02	0.16	ND	ND	ND
Glu	10.5	10.63 <sup>h</sup>	5.84	8.66	5.53 <sup>j</sup>	3.13
Gly	20.2	26.04	33.70	35.14	29.81	31.51
His	0.8	0.97	0.30	0.58	0.7	ND
Hyl	1.2	ND	ND	ND	ND	2.25
Hyp	10.8	12.78	12.13	ND <sup>k</sup>	10.7	9.24
Ile	1.3	1.10	1.15	2.35	1.14	ND
Leu	2.9	2.70	2.63	1.36	2.5	3.47
Lys	4.0	2.88	4.66	2.99	1.75	2.41
Met	1.1	1.34	0.07	0.89	1.47	1.12
Phe	2.1	2.19	1.77	1.65	1.84	3.16
Pro	13.4	8.84	13.42	13.87	10.68	17.6
Ser	3.6	2.50	2.20	2.57	3.75	1.43
Thr	1.9	2.94	1.01	2.10	2.37	ND
Trp	ND	ND	0.04	ND	ND	1.23
Tyr	0.8	0.72	1.22	0.43	0.46	0.96
Val	2.4	1.85	1.94	2.24	2	1.38
Imino acid	24.2	21.62	25.55	ND	21.39	26.5

<sup>a</sup> Porcine skin gelatin (type A commercial) (Eysturskarð et al., 2009).

<sup>b</sup> DSG extracted by 0.1 M (0.6%) acetic acid (S.-J. Lee, Kim, Kim, et al., 2012).

<sup>c</sup> CSG extracted using NaOH (0.15% w/v) then 0.15% (v/v) H<sub>2</sub>SO<sub>4</sub> (Mhd Sarbon et al., 2013).

<sup>d</sup> MDTRG extracted by 5% HCL at 70 °C (Fonkwe & Singh, 1997).

<sup>e</sup> DFG extracted using (4%) acetic acid (Y. H. Kuan et al., 2017).

<sup>f</sup> DFG extracted using 1.5% acetic acid (Chakka et al., 2017).

<sup>g</sup> Aspartic acid.

<sup>h</sup> Glutamic acid.

<sup>i</sup> Asp + Asn.

<sup>j</sup> Glu + Gln.

<sup>k</sup> Du et al. (2013) reported the amount of 11.2% for hydroxyproline content in type B gelatin extracted from turkey head.

free hydroxyl group) was higher in non-gelling gelatin and threonine and tyrosine content in gelatin with good gel forming properties were higher than non-gelling gelatin. This is due to the presence of free hydroxyl groups that can bond with water molecules and negatively affect the strength of the gel (Jan Arnesen & Gildberg, 2002).

Overall, in conducted studies, gelatins from chicken have higher concentrations of imino acids compared to mammalian gelatins and duck gelatins (such as Pekin duck), among which, hydroxyproline content of duck gelatins is higher than mammalian and chicken gelatins, but, proline content of chicken gelatins is higher than mammalian and duck gelatins. The proline and hydroxyproline contents are approximately 21–28% for CFG (Chakka et al., 2017), 22–30% for mammalian gelatins (Haug et al., 2004), 22–25% for warm-water fish gelatins (tilapia and Nile perch) (Jamilah & Harvinder, 2002; Weng, Zheng, & Su, 2014), and 17% for cold-water fish gelatin (cod) (M. Gómez-Guillén et al., 2002).

### 2.2.2. Rheological properties

Gelatin's rheological and mechanical attributes play a significant role in the development of the product and product specifications in the food, medicinal and biomedical industries. Gelatin is categorized as a physical gel, i.e., the interactions or bonds between the chains that make up the material are physical in nature (van der Waal's interactions and hydrogen bonds, with an  $E \approx 2$  kcal/mol). If we cool a homogeneous gelatin solution (gelatin in the range of one to fifty percent constitute homogeneous gel) to below the sol-gel transition point, the collagen-like helices are re-formed and the three-chain helix forms (Djabourov, Lechaire, & Gaill, 1993). As a result, the solution undergoes a three-dimensional structure that guarantees the strength and elasticity

of the gelatin. Over time, this network becomes an infinite network (Bohidar & Jena, 1993). Since the bonds involved in the gelation process are physical (hydrogen bonds and van der Waals) they give this process the nature of thermal reversibility. The source, breed and age of animals; size, number, molecular weight and breakdown position of peptide chains; the number, type and concentration of amino acid residues present in gelatin are important factors in the rheological properties of gelatin. Gel strength and melting point are a direct function of the molecular weight as well as the complex interactions that are determined by the amino acid composition and the ratio of  $\alpha/\beta$  chains present in gelatin (S. Cho et al., 2004). According to Schrieber and Gareis (2007), strength of gel is largely dependent on the fraction with a molecular weight of approximately 100,000 g mol<sup>-1</sup>. With the same reasoning Aykın-Dinçer, Koç, and Erbaş (2017) proved that the broiler skin gelatin (BSG) had low viscosity (1.35 cP) as well as low gel strength (166.6 g) values. H. Liu, Li, and Guo (2008) demonstrated that the gelatin gel strength characteristic was strongly dependent on the amount of  $\alpha$ -chain in gelatin. Given that a high proportion of peptides with molecular weights higher or lower than  $\alpha$ -chains can decrease gel strength, it can be concluded that the higher the  $\alpha$ -chains in the gelatin protein pattern, the higher the gel strength. The amount of Bloom used to express the gel strength of commercial gelatin which is the amount of weight (in grams) required to compress the surface of a thermo stated standard under standard conditions by a specified plunger to a specified depth (Schrieber & Gareis, 2007). The Bloom for commercial gelatin ranges from 100 to 300, whereas the range between 200 and 250 is most favorable.

Table 7 shows rheological properties (Bloom number, melting, gelling point and viscosity) of various PG as reported in the literature. PG typically has a Bloom value ranging from 120 to 831 (tested under the conditions of the standard Bloom test) which is categorized in term of high (>200 g) Bloom, while a ranging from as low as zero to 270 has been reported for fish gelatins and typical Bloom values ranging from 70 to 110 for cold-water gelatins (Karim & Bhat, 2009) of course, with an exception of Bloom value as high as 426 has been reported for a warm-water yellowfin tuna skin (S. M. Cho, Gu, & Kim, 2005), compared to the Bloom values for bovine or porcine gelatin, which have Bloom values of 200–240. Gelatins extracted from mechanically deboned chicken or turkey residue have been reported to exhibit highest Bloom value in PG (Fonkwe & Singh, 1997; Rafeian et al., 2013; Rafeian et al., 2015). Such high gel strength characterizes only those gelatins extracted from mechanically deboned poultry residues. For example, Bloom values 260, 294, 338, 355, 439, 520 and 831 have been reported for duck skin, chicken by-products gelatin, mechanically separated turkey meat gelatin, chicken head gelatin, chicken bone gelatin, chicken deboned residue gelatin, and mechanically deboned turkey residue gelatin respectively (Almeida et al., 2013; Du, Keplová, Khiari, & Betti, 2014; Rafeian et al., 2015; Ee et al., 2019; Dewi; Yuliani, Awalsasi, & Jannah, 2019; T.-K.; Kim et al., 2020).

Studies have shown that the most important difference in the gel strength of different gelatins is their diversity in the amount of imino acids, which are influenced by their amount in collagen present in different species as well as the extraction conditions. Studies by Badii and Howell (2006) and Montero and Gómez-Guillén (2000) conclude that another factor that even plays an important role in the development of physical properties, especially gel strength, is the amount of hydrophobic amino acids (Ala, Val, Leu, Ile, Pro, Phe, and Met). The results showed that the amount of these amino acids in the non-gelling gelatin (cod gelatin) was low. Thus, the hypothesis of a direct relationship between the amount of hydrophobic amino acids and the gel strength is confirmed. The extraction conditions may influence the distribution and composition of hydrophobic amino acids which markedly affect the temperature of gelling and Bloom. For example, applying higher extraction time (12.05 h) has been stated to end in the lower Bloom values for mechanically deboned chicken residue (Rafeian et al., 2013), demonstrating that as the extraction time for hydrolysis affects the

**Table 7**  
Gel strength, melting and gelling points and viscosity of various poultry gelatins.

Gelatin type	Concentration (%)	Gel strength (g) <sup>a</sup>	Gelling point (°C)	Melting point (°C)	Viscosity	Reference
					Method and viscosity value (in original unit)	
CFG <sup>a</sup>	10	264.3	NR <sup>a</sup>	26.7	Brookfield at 40 °C, 4.96 cP	Rahman and Jamalulail (2012)
CFG	4	~240 to 487	NR	36.38 to 38.5	HAKKE viscotester at 35 °C, 5.12–7.61 Pa s	Choe and Kim (2018)
CSG <sup>b</sup>	6.67	270.5	NR	39.83	NR	(H.-W. Kim, Song, et al., 2012)
MDCRG <sup>c</sup>	6.67	526	NR	NR	Oswald at 60 °C, 5.85 Pa s	Rafieian et al. (2013)
MDCMG <sup>d</sup>	6.67	281 to 818	25	33.7	NR	Erge and Zorba (2018)
MDTRG <sup>e</sup>	6.67	831	NR	NR	1%, Oswald, 0.720 dl/g	Fonkwe and Singh (1997)
CSG	6.67	355	24.88	33.57	Rheometer at 40 °C, 150 ml/g	Mhd Sarbon et al. (2013)
CFG	6.67	294.7	NR	NR	NR	Almeida et al. (2013)
THG <sup>f</sup>	6.67	368.4	NR	NR	NR	Du et al. (2013)
CHG <sup>g</sup>	6.67	247.9	NR	NR	NR	Du et al. (2013)
CHG	6.67	113 to 355	NR	34.5 to 42.2	Oswald at 60 °C, 1.41 to 9.45 mP	Gál et al. (2020)
MSTM <sup>h</sup>	6.67	338.4	27.0 to 27.9	31.4 to 34.5	NR	Du et al. (2014)
CDRG <sup>i</sup>	6.67	520	NR	NR	Oswald at 60 °C, 55.5 mP	Rafieian et al. (2015)
CSG	6.67	NR	23.68	32.64	NR	Rasli and Sarbon (2015)
CFG	6.67	119.2 to 204.3	NR	NR	NR	Chakka et al. (2017)
CFG	6.67	79.23 and 185	NR	NR	NR	Widyasari and Rawdkuen (2014)
CFG	6.67	294.8	NR	NR	NR	Santana et al. (2020)
DFG <sup>j</sup>	6.67 and 10	209 to 334.3	20.5 to 26.65	27.8 to 36.4	10%, Brookfield at 40 °C, 0.64 P	(Abedinia et al., 2017), (Y. H. Kuan et al., 2017)
CHG	6.67	38.6 to 355.7	25.8 to 26.0	30.8 to 32.3	NR	Ee et al. (2019)
DSG <sup>k</sup>	6.67	210 to 260	NR	31.2 to 33.8	Brookfield at 35 °C, 56.9–77.8 mPa s	(T.-K. Kim et al., 2020)
BCSG and BCFG <sup>l</sup>	6.67	239 to 263.5	NR	NR	NR	Saenmuang et al. (2019)
CLSG <sup>m</sup>	6.67	75 to 85	NR	NR	Brookfield at 40 °C, 7 to 8.9 cP	Sompie et al. (2019)
Bovine gelatin	6.67	218 to 240	24.5	28 to 29	Brookfield at 60 °C, 9.8 cP	(Cheow et al., 2007), (Mohtar et al., 2010)
Porcine gelatin	6.67	216	NR	29.1	Brookfield at 60 °C, 5 cP	(Jan Arne Arnesen & Gildberg, 2007), (Mohtar et al., 2010)

a Chicken feet gelatin

b Chicken skin gelatin

c Mechanically deboned chicken residue gelatin

d Mechanically deboned chicken residue gelatin

e Mechanically deboned turkey residue gelatin, solution of 7.14% was used for Bloom test

f Turkey head gelatin

g Chicken head gelatin

h Mechanically separated turkey meat

i Chicken deboned residue

j Duck feet gelatin

k Duck skin gelatin

l Black-bone chicken feet and skin gelatins

m Chicken leg skin gelatin <sup>a</sup>Not reported

<sup>a</sup> The difference in the values obtained from the results of Bloom strength depends on the temperature and time of extraction, the strength of the chemical treatment used in the extraction, the texture from which the gelatin is extracted which can affect the amount of product imino acids (the most important factor influencing the amount of Bloom), method applied for Bloom determining and gelatin concentration in test.

degree of cross-linking of collagen, it subsequently has a noticeable effect on the gel formation capacity. One of the most prominent features of gelatin that makes it a desirable material for food and pharmaceutical use is the melting properties of gelatin in the mouth or melt-in-the-mouth property (usually lower than human body temperature) which is due to the prominent properties of gelatin dissolvability in water and capacity to make thermally reversible gels. The rheological attributes of the thermoreversible gels are essentially a function of the temperature below the melting point and the gelatin concentration (Zhou et al., 2006). Collagen transformation to gelatin is deciphered as the crumbling of the helical structures into irregular coils. Upon cooling, simultaneously while they endeavor to change the initial structure (Mackie, Gunning, Ridout, & Morris, 1998), the irregular coils experience a coil to helix move (Kuijpers et al., 1999). The strength and integrity of the gel is the result of the three-dimensional structure obtained from this process.

Although a wide range of melting and gelling points has been reported for the PG, but the gelling and melting points of PG are relatively

similar to those in mammalian and its viscosities are relatively higher (Mhd Sarbon et al., 2013; Rafieian et al., 2015). The representative values of the gelation and melting points of the poultry by-products are in the range of 20–28 °C and 26.7 to 34.5 °C, respectively, whereas those corresponding temperatures are in the range of 20–25 °C and 28 to 31 °C, respectively, for bovine and porcine gelatins, which demonstrates the effect of gelatin origin (Table 7). Du et al. (2013) compared the rheological properties of turkey and chicken gelatin to mammalian gelatins. The results showed that the elastic modulus  $G'$  (which mainly describes the viscoelastic properties) in turkey gelatin was higher than the critical concentration of CHG but comparable to mammalian samples. The rheological, physicochemical and structural properties of the gelatin extracted from chicken skin showed a significant difference ( $p < 0.05$ ) in the melting temperature of the chicken gelatin (33.57 °C) with that of the gelatin extracted from the bovine skin (31.55 °C), but the gelation temperature of both gelatin was about 24 °C (Mhd Sarbon et al., 2013). Avoiding widespread degradation of the peptide structure at all stages of the extraction process should be considered as an important



principle in this process as extensive changes in this structure lead to a significant reduction in the resulting gel strength. M. Gómez-Guillén et al. (2002) commented that the factors affecting gelatin gel properties that are more important than amino acid composition for determining these properties are the average molecular weight and, in particular, the distribution of  $\alpha$ -,  $\beta$ -, or  $\gamma$ -chains that affect the physical properties of the resulting gelatin and, among them, any gelatin containing higher amounts of  $\alpha$ -chains exhibits greater gel resistance (H. Liu et al., 2008). T.-K. Kim et al. (2020) proved that the heating method at duck skin gelatin (DSG) extraction stage can make a difference of about two degrees at the melting point (31.25–33.88).

Rahman and Jamalulail (2012) explained the viscosity influenced by some factors during production. The CFG gelatin was significantly less viscous than the CBG, which was related to the molecular weight, molecular size distribution, and gelatin pH so that the lowest viscosity was observed at pH 6 to 8 and if during production the pH was out of this range, the viscosity increased. Greater amounts of crosslinking compounds such as  $-\beta$  and  $-\gamma$  in gelatin make the viscosity and Bloom higher (Ogawa et al., 2004). In other words, higher viscosity gelatin has a longer chain content which is resist the flow. Finally, extraction time has a negative effect on viscosity. In general, the PG viscosity is higher than mammals because their bloom content is also higher. For example, Rafieian et al. (2015) and Mhd Sarbon et al. (2013) compared the viscosity of PG and BG and the results showed the CDR gelatin of 55.5 vs. 29 mP and the CS gelatin of 150 vs. 127 ml/g, respectively.

### 2.2.3. Emulsifying and foaming properties

The remarkable active-surface properties of gelatin make it a suitable candidate for foaming, emulsifying (emulsifier in oil-in-water emulsions) and moisturizing agent in food, pharmaceutical and medical and technical applications. (Lobo, 2002; M. C.; Gómez-Guillén et al., 2011). The emulsifying and foaming properties of gelatin are the result of the existence of hydrophobic segments on its peptide chains (Huang et al., 2017). Since the gelatin emulsifier properties are lower than conventional surface-active agents such as globular proteins and gum arabic, they form large droplets if used alone in the homogenization process (Huang et al., 2020). Thus, two ways to modify the gelatin emulsifier property are to either be modified by bonding non-polar sidebands hydrophobically, or used in conjunction with anionic surfactants (Surh, Gu, Decker, & McClements, 2005).

The multifunctional properties of gelatin (emulsifier and foaming) are considered especially in cases such as emulsified powders (Chakka et al., 2017). In such powders, during the emulsification process, both the surface-active properties and gelatin film formation are successfully exploited for the purpose. Desirable properties of gelatin including gelation and stabilization are used in subsequent encapsulation processes. During marshmallows cooling, the gel-forming properties of gelatins are applied to stabilize the foam. In a gelatin-foamed food such as ice cream, the unique behavior of the gel melts at a temperature range of 10–30 °C causing the gelatin gels to melt in the mouth. The feature that makes gelatin a unique ingredient for most applications is not only its active surface properties but also a combination of surface, rheological and chemical properties (de Wolf, 2003).

The number of studies on the gelation, emulsion and foaming properties of PG is limited. Dewi Yuliani, Awalsasi, and Jannah (2019) studied the emulsion stability (ES) of CBG which were in range from 9.82 to 61.19% and concluded that higher concentration and extraction times led to higher ES. Rafieian et al. (2015) investigated foam capacity (FC) and foam stability (FS) of CDRG. The CDRG showed the same FS as analytical, food grade porcine and Shark cartilage gelatins at 290, 280, and 260 mL/100 mL, respectively (S. Cho et al., 2004), but was much higher than ( $P < 0.05$ ) that obtained for BG at 93 mL/100 mL (Hafidz, Yaakob, Amin, & Noorfaizan, 2011). The accumulation of proteins that interfere with the interaction between water and foam proteins is known to decrease the foaming properties (S. Cho et al., 2004).

Rasli and Sarbon (2015) stated that the reason for the significantly higher FS of gelatin from chicken skin (176%) than bovine gelatin (61.17%) is the higher hydrophobic amino acids such as proline, phenylalanine, leucine and isoleucine.

### 2.2.4. Film-forming and composites properties

Research on the fabrication of films from PG and their specifications, though limited, has shown that PG represents excellent film-forming properties (J.-H. Lee et al., 2015; Nazmi et al., 2017; Abedinia et al., 2018; Said & Sarbon, 2020; Norafidah Suderman & Sarbon, 2020).

Overall, mechanical properties tensile strength (TS), elongation at break (EAB), Young's modulus (YM) and heat seal strength (HS) of PG films have been reported as similar to that of bovine bone gelatin (Abedinia et al., 2018). In food packaging, one of the primary functions is to barricade or reduce moisture transition between product and the surrounding atmosphere. Low water vapor permeability (WVP) widens the utilization of the composite packaging film, especially in an environment with high moisture levels (Qi et al., 2015). In addition to the amount and ration of imino acids, the major factors that influence the structural and physical properties of the resulting gelatin include molecular weight distribution and amino acid composition, which play a key role in determining the barrier (WVP, oxygen permeability (OP), ultraviolet (UV) and transparency) and mechanical properties (M. C. Gómez-Guillén et al., 2011; Abedinia et al., 2018).

The collagen and gelatin coating or film shows desirable WVP and OP properties, making it perfect for use in food preservation, among other applications. For example, it has been used to coat a variety of meat products to reduce purge, preserve color, improve sensory properties, slow degradation and prevent spoilage as well as act as an antioxidant (Antoniewski & Barringer, 2010). The chicken skin gelatin (CSG) film shows a low WVP value ( $1.36 \times 10^{-4} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$ ) (Nazmi et al., 2017). Edible DFG-based, chicken feet protein (CP) and CFG films plasticized by glycerol show even a lower WVP than CSG and bovine films with  $5 \times 10^{-11}$ ,  $3.44 \times 10^{-9}$  and  $1.27 \times 10^{-9} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$ , respectively (J.-H. Lee et al., 2015; Abedinia et al., 2018; Norafidah Suderman & Sarbon, 2020). The lower WVP in poultry feet gelatin-based films compared to those from poultry skin or mammalian is due to the levels of proline and hydroxyproline in this type of gelatin which are explained in terms of the amino acid composition (Abedinia et al., 2018). Poultry feet gelatins have higher hydrophobicity than mammalian gelatins due to high proline and hydroxyproline contents. The results show that the properties of poultry feet gelatin films are suitable for use as an alternative material to bovine gelatin film.

Fabricating biodegradable edible films by blending polymers together, has been conducted by some researchers to investigate the properties of PG -based films. Soo and Sarbon (2018) showed that the addition of rice flour to CSG increased the WVP, transparency, thermal properties (TM) and decreased the solubility, UV-visible light transmission of the films. The crystalline nature of the films also improved. Mechanically, the films showed higher TS and EAB. Nazmi et al. (2017) showed that composite films of CSG/CMC as compared to bovine gelatin films have similar TS and WVP properties. This study demonstrates potential of films for use in packaging materials or coatings in food products. Loo and Sarbon (2020) prepared CSG/tapioca starch with different concentrations of starch (0–25%) and observed that the addition of tapioca starch increased the thickness, thermal stability and improved the water resistance of the films. X-ray diffraction analysis showed an increase in film crystallinity as a function of tapioca starch content. The resulting films (especially the best formula of CSG/10% tapioca starch) showed higher tensile strength and elongation at break values. Using RSM, Said and Sarbon (2020) obtained optimum amounts of rice starch and curcumin to make CSG-based composite films that exhibited good antioxidant (DPPH (2,2-diphenyl-1-picrylhydrazyl) activity = 85.6%), WVP ( $1.48 \times 10^{-10} \text{ g/m.s.Pa}$ ) and mechanical properties (TS = 8.47 MPa and EAB = 416.43%) for food packaging applications. Films obtained from CSG incorporated with urban extract

also showed high antioxidant properties, including total phenolic content of 10.79 mg/mg gallic acid, 68.6% of DPPH, and reducing power 14.4%. The addition of herbal extracts reduced TS and WVP and increased the EAB (N Suderman & Sarbon, 2019). Alias and Sarbon (2019) found that adding potato starch to CSG improves the viscose behavior of film forming solutions and has a positive effect on mechanical, WVP and UV inhibition properties.

### 2.2.5. Sensory properties

H. Y. Kim, Lee, and Kim (2012) evaluated the effect of CFG and wheat fiber levels on the sensory properties of semi-dried chicken jerky. They found that the tenderness slightly increased with increasing CFG. Almeida et al. (2013) studied the acceptability of samples of gelatins using sensory affective tests such as flavour, color and aroma, comparing with the sensorial qualities of a commercial gelatin. They served gelatin samples containing different levels of gelatin and sugar to panelists directly to study the sensory properties. The results indicate that if the amount of fatty acids and effective compounds in the odor is not eliminated in gelatin extraction, the resulting gel in comparison with commercial gelatin is generally less acceptable. H.-W. Kim et al. (2014) added different concentrations of DFG to duck meat jelly to study its physicochemical, textural and sensory properties. Obtained data showed that melting property in the mouth is one of the reasons for differences between gelatins. The concentration of DFG had effect on these results as with increasing of DFG concentration the color score improved, but it was cause to decreasing the hardness and dispersibility satisfaction scores. The results indicated that although overall acceptance was observed in 3% of DFG, but the increase of DFG resulted in a high satisfaction score of appearance; the addition of DFG higher than 5% resulted in the highest appearance score ( $p < 0.05$ ). On the other hand, Nik Muhammad et al. (2018) used quantitative descriptive analysis (QDA) to measure the effect of the type of acid used for extraction of DFG on its characterization and sensory property. The results of comparing the gelatin obtained from duck feet with commercial bovine gelatin showed that gelatin obtained from duck feet was brighter in appearance than bovine gelatin, as a result of different extraction methods. The strong odor of DFG gelatin was also due to the high level of fat in gelatin, so it is recommended to use methods that reduce fat by about 0.1%. In general, they concluded that the type of acid used in gelatin extraction could have a large effect on the final product. Finally, the use of optimum value of fat removers (like some alcohols, e.g. butanol 10%) before acidic extraction of PG is proposed to improve the PG sensory properties.

### 3. Application of poultry gelatin

The obtained gelatin from the poultry by-products such as skins, mechanically deboned residue and feet showed high gel strength and relatively equal gelling and melting points to mammalian gelatins, which nominates it as a replacement for mammalian consumption in the industry. As stated in Table 6, gelatin extracted from duck feet showed low gelling temperature, a property that offers novel possible applications for PG in micro-encapsulation of bioactive components.

In the past, bioactive peptides with multiple properties have been attempted to be extracted from various protein sources, with a focus on the production of these active compounds from collagen and gelatin hydrolysates, resulting in significant advances in modern analysis that have led to deeper research on novel sources of gelatin and collagen extraction and production. J.-H. Lee et al. (2015) produced a bioactive film by blending CFG and the marjoram, coriander, and clove bud oil and measured its antimicrobial and antioxidant effects in the packaging of cheddar cheese slices during storage. They observed that this package could delay oxidation and microbial growth.

Chakka et al. (2017) suggested that the CFG has potential for application in dessert/jelly-based products and due to the high value of foam formation of the CFG, it can also be used in the baking industry,

especially as a stabilizer in the production of foam in cakes, pies and breads.

### 4. Challenges associated with poultry gelatin

PG market share is still relatively small compared to cow, pig, and fish gelatin. Factors limiting the PG industry include:

- Availability of raw materials: According to reports released by FAOSTAT (2019), the amount of raw material available for gelatin preparation from poultry by-products is much lower than the raw material from cattle and pigs. According to statistics released by the FAO in 2013, the world production of poultry was 65 billion head, of which about 15% were used for gelatin production, while the figure for only hide cattle fresh was 314 million heads. Hence, poultry production is still considered exceptionally low compared to mammals, hence the restricted production of PG. Other than that, the production of reliable poultry species in satisfactory amounts is troublesome for gelatin producers. Another problem is the need to get certification on poultry raw material. Certification is for the traceability, which is the fundamental necessity for nourishment added substances particularly from animal source (Karim & Bhat, 2009).
- Price: Although the price of gelatin produced from poultry, for example from chicken feet, is cheaper than mammalian extracted gelatin in the market (Almeida et al., 2013), prices for some poultry are particularly expensive, especially for ducks, and people are less likely to buy them (Mead, 2004). Therefore, gelatin production from these poultry species is more costly due to their limited availability.
- Variety of gelatin quality: The gelatin quality varies based not only on the methods, extraction conditions, and species used, but also on the composition of the raw materials used in production, namely skin, mechanically deboned residue, feet and bone.
- Health risks: First health concerns due to H5N1 associated with diseases called highly pathogenic avian influenza virus (HPAIV) infection has been reported in Hong Kong in year of 1997 (Suarez, 2008). Human affirmed cases have moreover been detailed by Joannis, Lombin, De Benedictis, Cattoli, and Capua (2006). The HPAIV outbreak is recognized to have negative background on the deals of poultry and financial matters of production (Fasanmi, Odetokun, Balogun, & Fasina, 2017). So there are still public concerns about the transmission of the disease from poultry products to human food chain.

### 5. Prospects, conclusions and future outlook of poultry gelatin as an alternative to mammalian gelatin

Two important justifying reasons for conducting industrial studies on gelatin production from poultry sources are increasing global demand for gelatin consumption and the need to find healthy alternatives to mammalian gelatin to avoid the strict food consumption limits set by religious laws. To date, studies for commercial production of PG have been limited to initial evaluation of the essential properties of gelatin derived from skin, feet, mechanically deboned residue, neck, and head, and research is lacking on finding optimal extraction conditions, including time, temperature and pretreatment, for a mixture of poultry by-products. Since the poultry's gelatin is classified by high Bloom content (>200 g), it seems that it can be used in combination with fish gelatin to modify the physical and chemical properties of this kind of alternative gelatin.

On the other hand, gelatin extraction with active peptides with antioxidant and antimicrobial properties has been the subject of considerable research over the past decade. The findings have shown that combining the gelatin with essential oils in fruit and plants would produce films with desirable properties of DPPH, radical scavenging, ferric reducing antioxidant power (FRAP) and hydrophobic and WVP

activities. Hence the gelatin matrix would be suitable for release of bioactive compound (Tongnuanchan, Benjakul, & Prodpran, 2012; J.-H.; Lee et al., 2015).

S.-J. Lee, Kim, Hwang, et al. (2012) extracted an antioxidant peptide from poultry by-product (duck skin) with molecular weight of 941.43 Da, the sequence of His-Thr-Val-Gln-Cys-Met-Phe-Gln, IC<sub>50</sub> = 32.6 µg/ml and DPPH of 22.7%. Then, they investigated its protective effect on normal liver cells damaged by alcohol 3.5%. The results showed that this poultry-derived peptide could prevent the production of reactive oxygen species (ROS) and the cell death of these alcohol-damaged liver cells. Wan Omar and Sarbon (2016) extracted chicken skin gelatin hydrolysate (CSGH) and investigated its antioxidant and functional properties with a DPPH radical scavenging activity of 47.33%. NM and WAN (2019) also produced CSGH enzymatically using alcalase, pronase E, and collagenase with angiotensin converting enzyme inhibitory (ACEI) activity of 69.64–87.69%. S.-J. Lee, Kim, Kim, et al. (2012) also investigated biological activity of gelatin hydrolysates from duck skin by-products. Results indicated that the pepsin hydrolysate exhibited the highest free radical scavenging activity. According to the reports on the antioxidant properties of peptide products extracted from poultry skin, it can be concluded that this type of gelatin has desirable properties for application in the bioactive compounds delivery.

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