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A comprehensive review on gelatin: Understanding impact of the sources, extraction methods, and modifications on potential packaging applications

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ABSTRACT

Gelatin is one of the most widely used hydrocolloids; mammalian, poultry, and fish wastes are an exciting source for gelatin production. The market size of gelatin will reach 5.0 billion USD by 2025 due to the consumption perspective of gelatin in today's market. The gelatin market is predicted to reach 6.7 billion USD at the end of 2027 with a 9.29 CAGR rate, being a vital constituent of the food, pharmaceutical, cosmetic, and packaging industries owing to its foaming, emulsifying, gelling, and filmogenic properties. In the packaging sector, gelatin-based films and coatings are gaining importance owing to their eco-friendly nature. The gelatin source, amino acid composition, and extraction method play a prominent role in its packaging properties. In order to improve the packaging properties of gelatin further, physical, chemical, enzymatic, and irradiation-based modifications play an significant role. This paper reviews the impact of sources, extraction, and gelatin modifications on improvements of gelatin as packaging material, and provides detailed information on gelatin films/coatings in the shelf-life extension of food products.

1. Introduction

Gelatin is a polymeric substance and a multi-functional ingredient obtained by the limited hydrolysis/heat denaturation of skin, bones, and connective tissue collagen (Alipal et al., 2021). Gelatin contains 19 amino acids, glycine (27–35%), proline, and hydroxyproline (20–24%) being the predominant amino acids (Nurilmala et al., 2021). The gelatin composition plays an important role in film/coatings properties. The mammalian gelatin has good packaging properties, followed by poultry and marine gelatin owing to their composition (Nurul Saadah Said & Sarbon, 2022).

The gelatin sources are pig and bovine skins, demineralized hooves, and bones. Among these, one of the primary sources of gelatin is pig skin (L. Lin, Regenstein, Lv, Lu & Jiang, 2017). Porcine contributes 46%, bovine hides 29.4%, and pork and cattle skeletons 23.1% of the total gelatin production (Alipal et al., 2021). Although pig skin is the most frequently used raw material for producing gelatin on a commercial scale, the alternative raw material from fish, animals, or birds slaughtered by the halal method can be utilized, eliminating the religious apprehension about the halal method the Muslim and Jewish populace. Not only religious objections, but there are also still other concerns regarding the use of bovine, porcine skin, and bone gelatin due to BSE and mad cow disease (Kronenthal, 2015). The alternate sources of gelatin that are gaining the attention of researchers are fish and poultry by-products. Poultry wastes such as blood, viscera, feet, and bone are rich in collagenous protein and are now broadly used in food industries (Bichukale, Koli, Sonavane, Vishwasrao, Pujari & Shingare, 2018).

Gelatin is a significant additive used as a gelling, emulsifier, and thickener for various products in the food industry, such as candy preparation, bakery products, desserts, ice cream, and meat products

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(Zhang, Liang, Li & Kang, 2020). It is vital in meat products to reduce purge and fat-binding properties and prevent color discoloration, rancidity, and microbial spoilage (Umaraw & Verma, 2017). Moreover, in confectionery products, gelatin improves foam stability, texture, and chewiness and provides creaminess and mouthfeel in low-fat spreads. In dairy products, gelatin provides stabilization and texturization, and in baked goods provides emulsification, gelling, and stabilization properties (Mokrejš, Mrázek, Gál & Pavlačková, 2019). Gelatin being exploited in numerous areas of food industries also has a substantial role in developing biodegradable packaging material. The biodegradable coatings/films can protect, maintain, extend the shelf-life of food product, owing to the good filmogeniic ability of gelatin (Said & Sarbon, 2022; Amiri, Moghanjougi, Bari & Khaneghah, 2021; Mahdavi et al., 2021; Liu, Yao, Yun, Zhang, Qian & Liu, 2021). For instance, gelatin films can be used for packaging to guard plant and animal origin food and products against microbial evolution, handling abuse, and preventing lipid/fat oxidation and moisture loss (Lee, Lee, Yang, & Song, 2016; Yadav, Kumar, Upadhyay, Singh, Anurag & Pandiselvam, 2022).

The physical and mechanical properties of gelatin protein films depend on the characteristics of the raw material source and the method of extraction used (Nurul Saadah Said & Sarbon, 2022). Furthermore, the properties of gelatin are also affected by physical parameters involved in the processing of films and the inclusion of plasticizers, polymers/fillers, and cross-linkers (Nazmi, Isa, & Sarbon, 2017). Gelatin being highly hygroscopic needs the incorporation of other substances like fillers and improvers to enhance its applicability for food packaging. Adding phenolic substances, various polysaccharides, lipids, and other plant-based extracts can improve gelatin's packaging properties (Said & Sarbon, 2022). This present review paper detailed the information on the source, extraction method, type of modification on the physico-mechanical film properties of gelatin protein films and coatings, and their impact on the shelf life extension of the food products.

1.1. Chemistry and properties of gelatin

Gelatin is derived from the Latin word *gelatos*, meaning 'stiff/frozen' obtained from different animal sources by partial hydrolysis/thermal denaturation. The structure of gelatin is shown in Fig. 1. Gelatin approximately contains protein (88%), moisture (10%), and salts (1–2%), and on a dry-weight basis, the protein content is 98–99% (Valcarcel, Fraguas, Hermida-Merino, Hermida-Merino, Piñeiro & Vázquez, 2021). The protein gelatin is odorless, bland, dull, or slightly yellow in color, fragile, and translucent. It is in tasteless sheet, flake, or powdered form and is unsolvable in organic solvents but solvable in glycerol, hot water, and acetic acid. Gelatin is an amphoteric substance, depending on the nature of the solution (Nik Aisyah, Nurul, Azhar & Fazilah, 2014). Gelatin has an isoelectric point at pH 4.8–9.4; gelatins processed by acidic treatments have a higher isoelectric point than gelatin processed by alkaline treatments. It is a blend of diverse molecular weight chains such as α- chains, β- chains, and ɣ- chains having a molecular weight of (80 ~ 125 kDa), (160 ~ 250 kDa), and (240 ~375 kDa), respectively. The primary amino acids present in the gelatin are glycine (27–35%), proline, and hydroxyproline (20–24%) (H. Jafari et al., 2020). Additionally, higher contents of β-components also contributes towards better gel properties and promote strength in the gelatin films as they inspire the better ability of renaturation to the full native collagen form (Nurul Saadah Said & Sarbon, 2022).

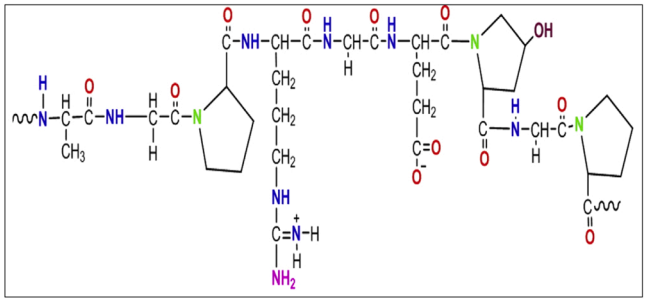

Fig. 1. Chemical structure of gelatin (Thakur, Govender, Mamo, Tamulevicius, & Thakur, 2017).

As far as its bonding is concerned, it is stabilized by diverse forms of covalent bonds, and various weak interactions govern its separation and flexibility. At low temperatures, gelatin is present in a collagen fold conformation capable of creating hydrogen bonds. Further, its double-stranded or triple helical structures are also hydrogen bond stabilized, formed by glycine residues (occurring after every third amino acid residue in the α-chain structure) inside the triple helix and form weak bonds with the carbonyl group oxygen atom (Kessler et al., 2021). Water molecules also show their involvement in the hydrogen bonding of the gelatin network (Rahman & Jamalulail, 2012). Investigations also reveal that the three-dimensional structure of gelatin gels in deuterium oxide is stabilized by hydrogen bonds of –NH group of one chain with –CO groups of other gelatin chain and hydrogen bonds made by water with chains of gelatin (Kessler et al., 2021). Similarly, hydroxyproline also forms hydrogen bonds with water by connecting hydroxyproline (–OH) groups of one chain with the (-CO) backbone of similar or other strands, thereby stabilizing the triple-helical areas or junction zones (T. Luo & Kiick, 2013). Hydrophobic interactions are known to perform an insignificant role in triple helix development of collagen molecule assembly but showed a chief effect on forming β-sheets. Experiments on skin gelatin of pigs incorporated with glycidol showed improved aggregate formation with a concentration in this phenomenon; hydrogen bonds play an essential role (Xu, Li, Tang, Qiao & Jiang, 2012). They used UV analysis to demonstrate hydrophobic interactions, which increased and competed with hydrogen bondings as a function of increasing gelatin concentrations. The protein foldings are also improved by hydrophobic interactions, which perhaps cause chain aggregations, thus affecting the physical and mechanical properties of the films. These hydrophobic interactions also cause molecular chain extensions by forming a beta-sheet structure due to increased repulsions in hydrophobic regions between charged residues (Duconseille, Astruc, Quintana, Meersman & Sante-Lhoutellier, 2015).

Salts and diverse pH also influence electrostatic interactions in the gelatin polyelectrolytic gel as it is rich in protein content bearing cationic wells and anionic groups. Studies on the swelling behavior of gelatin using diverse NaCl concentrations have shown that the swelling degree is influenced by the ionization degree of solutions attributed to the ion pairs formation between charge networks and counter ions (Vigata, Meinert, Bock, Dargaville & Hutmacher, 2021). Moreover, salt addition affects the stabilization of gel network by modifying the electrostatic interactions of gels. The studies can justify it by Haug, Draget, & Smidsrød (2004) on the mechanical properties of fish gelatin by adjusting variable pH and incorporating salts. The salts and pH variations caused stabilization of the junction zones in gelatin due to the formation of additional electrostatic interactions.

Despite the thermal and chemical treatments, covalent bonds remain within the gelatin molecules and impart various mechanical properties. It has been investigated that collagen covalent bonds are formed by the allysine pathway in which aldolic condensation takes place by 2 allysine residues (lysine with aldehyde group) to form a cross-link. Another probable reaction includes forming a Schiff base by allysine and lysine residues to form a lysinorleucine. Eyre, Weis, & Rai, (2019) also described hydroxylysine (found in bone tissue) and allysine pathways, which are both identified as precursors of cross-link formation. Pentosidine is a cross-link naturally found in skin proteins like collagen and gelatin due to the reaction among pentoses and arginine or lysine side chains (Vos et al., 2013). The hexoses also contribute to pentosidine development by sugar fragmentation during prolonged protein glycosylation. Pyridinoline and deoxypyridinoline are 2 pyridinium ring-like cross-links in collagen non-helical regions formed by the lysyl oxidase

pathway (Ricard-Blum, 2011). As the denaturation of collagen takes place and results in gelatin formation, other covalent bonds form due to the chemical and environmental conditions during/after the manufacturing process. The resulting cross-links are favored by higher temperatures, humidity, UV-light & chemical substances like reducing sugars & formaldehyde (Solt et al., 2019). Other types of cross-links have also been observed and characterized in gelatin. The underlying mechanism is that the free amine groups of lysine residue react with an aldehydic group, forming a hydroxymethyl amino, yielding a water molecule to generate a secondary aldimine. The resulting imine group further reacts with other lysine residues and forms dimethylene ether, which undergoes rearrangements and links two lysine residues with the methylene bond (Vistoli, De Maddis, Cipak, Zarkovic, Carini & Aldini, 2013). Therefore, the cross-links in gelatin involve multiple interactions at both intra-molecular and inter-molecular regions of the helices. However, a few more cross-links are still under observation and discussion, like disulfide linkages and pyridinoline (Duconseille et al., 2015).

2. Sources of gelatin

Mammalian sources such as pig skin and cowhides are the most available sources of gelatin, accounting for 46% of the world's gelatin source, followed by bones (23%), hooves (29%), and the remaining 1% coming from marine sources such as fish (Rakhmanova, Khan, Sharif & Lv, 2018). In Europe, 95% of gelatin is obtained from bovine hides and porcine, and 5% from their bones (Alipal et al., 2021). Mammalian gelatin has high boiling and gelling points and a thermoreversible character. The bones, cattle hides, and pork skin are traditional mammalian sources of gelatin (Alipal et al., 2021). The gelatin from cow bone is of high quality and is preferred for industrial purposes. Due to religious and aesthetic objections, pork gelatin and gelatin obtained from other animals not slaughtered following Islamic laws are not used (Rakhmanova et al., 2018). These reasons increased the halal foods and additives market and gained researchers and industrialists (Ab Talib, Sawari, Hamid & Chin, 2016). Therefore, researchers are searching for an alternative, new, halal sources of gelatin. In recent years the market potential of fish and poultry by-product gelatin gets increased. Poultry wastes are probably the chief sources of gelatin soon but presently have limited commercial production due to low yields (Abedinia et al., 2020). Much research is being done to obtain gelatin from fish skin and poultry wastes compared to gelatin obtained from mammals. From the packaging point of view, the source of gelatin plays an important role in the physical, chemical, and functional properties of the films that can be developed from a particular source of gelatin which can be discussed by Alfaro, Balbinot, Weber, Tonial, and Machado-Lunkes (2015). Table 1 summarises the physical and mechanical properties of different sources of gelatin films.

2.1. Poultry gelatin

The poultry processing industries are one of the best-rising agro-food segments in the world. The worldwide meat production from poultry sources grew from 92.68 MT IN 2008 to around 127.29 MT in 2018, as per FAOSTAT (2019). Among this production in 2008 share of chicken, Geneva fowl, duck, goose, and turkey were 80.84, 2.27, 3.83, 2.27, and 5.7 MT, respectively, while as shear of these birds in 2018 was 114.26, 2.64, 4.46 and 5.9 MT, respectively. This tremendous increase in poultry processing can generate huge quantities of byproducts and wastes utilized for gelatin and pet meal production. Poultry processing results in numerous by-products/wastes comprising liver, gizzard, feet, skin, feathers, and head, which contains 34.2% dry matter with 51% protein, 41% fat and 6.3% ash content (Abedinia et al., 2020).

Management of these valuable sources possibly will offer economy to the countries and a solution to waste utilization. Chicken feet are underutilized by-products in the planned poultry processing industry and often thrown away without treatment, and can be a reason for environmental pollution. Chicken feet contain collagenous material that can be utilized as a good source of gelatin (Chakka, Muhammed, Sakhare & Bhaskar, 2016; Chakka, Muhammed, Sakhare & Bhaskar, 2017). Protein isolates are also obtained from low-value poultry processing wastes like bones and mechanically separated meat residues (Du, Khiari, Pietrasik & Betti, 2013). Chicken wastes like a comb, bone, cartilage, and wattle contain higher gelatin content (Fig. 2). Chicken skin gelatin contains more α-helix and β-sheet structures with hydrogen bonding; hence the gelatin has higher gel strength and elastic and viscous modulus (Soo & Sarbon, 2018).

In a study by Bichukale et al. (2018), gel strength of bone poultry

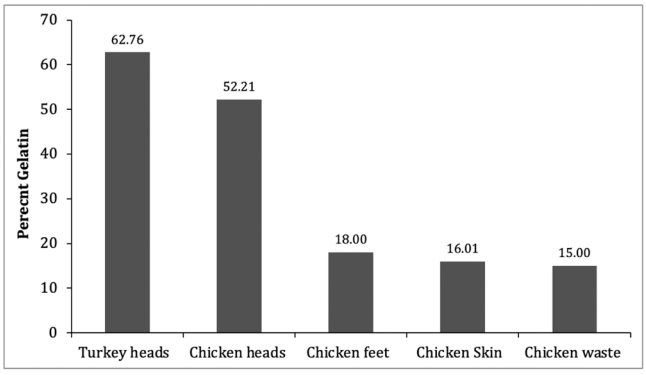

Fig. 2. Gelatin content in different parts of poultry, turkey heads, chicken heads, chicken feet, chicken skin and chicken waste (Rahman & Jamalulail, 2012; Du et al., 2013; Sarbon et al., 2013).

Table 1
Physical and mechanical properties of different sources gelatin films.

Gelatin Film	WVTR (g/ms Pa)	TS (MPa)	EAB (%)	Reference
Bovine hide/oregano essential oils	$0.81–1.21 \times 10^{-10}$	8.90–14.00	8.30–10.10	Martucci, Gende, Neira, and Ruseckaite (2015)
Bovine hide/lavender essential oils	$0.68–1.27 \times 10^{-10}$	8.80–15.40	4.30–7.60	Martucci et al. (2015)
Bovine/curcumin extract	$0.90–1.20 \times 10^{-10}$	1.90–3.40	144.30–198.60	Musso, Salgado, & Mauri, (2017)
Tilapia skin/ginger essential oils	$1.88–2.61 \times 10^{-11}$	18.58–35.73	41.70–72.03	Tongnuanchan, Benjakul, & Prodpran, (2013)
Tilapia skin/turmeric root essential oils	$1.89–2.48 \times 10^{-11}$	23.34–34.04	42.79–72.08	Tongnuanchan et al. (2013)
Fish skin/ haskap berries extract	$5.96–7.14 \times 10^{-11}$	46.70–51.50	2.87–3.69	Liu et al. (2019)
Fish skin/Origanum essential oil	$1.35–1.90 \times 10^{-11}$	3.28–6.72	87.20–151.82	Hosseini, Rezaei, Zandi, and Farahmandghavi (2016)
Feet gelatin/25% glycerol	2.04×10^{-11}	44.86	15.99	Tew et al. (2017)
Feet gelatin/35% glycerol	2.14×10^{-11}	34.20	33.30	Tew et al. (2017)
Chicken skin/rice flour	$6.83 \times 10^{-11} – 1.39 \times 10^{-9}$	2.08–2.91	58.45–79.31	Soo & Sarbon, (2018)
Chicken skin/5–20% glycerol	$4.86–6.67 \times 10^{-12}$	1.75–3.64	106.43–148.33	Nor, Nazmi, & Sarbon, (2017)
Chicken skin/CMC/Centella	$1.11–1.13 \times 10^{-4}$	5.00×10^{-2}	271.17–281.00	Nazmi & Sarbon, (2020)
Chicken skin/CMC	1.03×10^{-4}	3.00×10^{-2}	223.05	Nazmi & Sarbon, (2020)
Chicken skin	5.94×10^{-10}	1.54	48.33	Soo & Sarbon, (2018)
Chicken skin	4.17×10^{-12}	33.66	3.87	Nor, Nazmi, & Sarbon, (2017)

gelatin at 40 °C, 45 °C, 50 °C, 55 °C, and 60 °C were 257.67, 274, 265.33, 263, and 260 g, while gel strength of poultry skin gelatin at similar temperatures were 258.33, 282.67, 273.67, 264.33 and 262 g respectively. The viscosity of the gelatin extracted at similar temperatures were 3.83, 5.53, 4.43, 4.07, and 4.03 cP for one gelatin, and the viscosity values of skin were 5.77, 9.10, 8.33, 7.37, and 6.53 cP respectively. This study indicates that the gel strength and viscosity were higher at 45 °C. Thus the gelatin extracted at this temperature showed improved packaging properties. Poultry wastes/byproduct gelatin exhibits good filmogenic properties owing to its higher bloom value and imino acid groups (Nurul Saadah Said & Sarbon, 2022). Several investigations have been lead on poultry wastes/byproducts based gelatin films, including the active chicken skin gelatin-Centella asiatica composites (Suderman & Sarbon, 2019) and active duck paw gelatin-cinnamon leaves essential oil biocomposite films (Yang, Lee, Beak, Kim, & Song, 2017). The single poultry-gelatin film derivative of chicken paw gelatin film showed a lower thickness (0.06 mm) almost similar to those thickness values obtained from single films of bovine gelatin (Tew, Soon, Benjakul, Prodran, Vittayanont & Tongnuanchan, 2017). On topmost of that, single poultry gelatin films were seeming as lesser yellowish compared to animal and fish gelatin films. The color values (L*, a*, and b*) of single poultry-derived gelatin (chicken feet) films showed L* value of 90.77–91.29 with a* value of − 1.40–(−1.30), and b* as 3.18–3.01 (Tew et al., 2017). This might be accredited to lower contents of amino acids cysteine (0.16%) and methionine (0.07%) in poultry based gelatin films compared to fish and animal gelatin (Nurul Saadah Said & Sarbon, 2022). Addationally, single poultry gelatin-derived films of chicken skin have been stated to have lowest UV light transmission of 0.03–4.48 compared to other sources of gelatin. The WVP of poultry gelatin films derived from chicken feet and skin were 4.17×10^{-12}–5.94×10^{-10} g/m.s. Pa compared to animal gelatin films (Nor, Nazmi, & Sarbon, 2017). The melting points (Tm) of single chicken gelatin films were reported to be in higher range (49.51–134.22 ºC) compared to single mammalian and fish gelatin films. Besides, poultry gelatin films derived from chicken feet and skin gelatin have been stated to exhibit higher tensile strength of 34.20–44.86 MPa and 0.98–33.66 MPa respectively than reported from fish gelatin films (Nurul Saadah Said & Sarbon, 2022).

2.2. Fish gelatin

The fish and its byproducts were extensively studied as a potential source of gelatin protein. The fish processing industry generates a substantial amounts of by-products and wastes while manufacturing fish fillets, as product yields are only ~30–50% (Coppola, Lauritano, Palma Esposito, Riccio, Rizzo & de Pascale, 2021). These wastes and by-products are rich in gelatin protein, but to date, fish gelatin use is limited in food industries compared to mammalian gelatin. The main reason for lower usage of fish gelatin has been reported owing to fishy off orders, and other reasons are its poorer gelling ability (Tohmadlae, Worawattanamateekul, & Hinsui, 2019).

Fish processing wastes account for 75% of the catch weight (Coppola et al., 2021). Among these, fish skin and bone represent ~30% of the weight, which contains higher collagen content that can be utilized to produce fish gelatin. The gelatin from fish processing wastes such as skin provides a substitute and serves alternative gelatin sources for markets worrying about the bovine spongiform disease. Fish gelatin has lower melting and gelling temperatures and lower gel strength than mammalian gelatin. Gelatin obtained from warm waters fishes contains normal hydroxyproline content and gel strength, but gelatin obtained from cold-water fishes has low hydroxyproline content and gel strength. Deep cold-water fish processing waste gelatin contains a lesser quantity of proline and hydroxyproline, thus forming a gel at a lower temperature of 8–10 °C (Shahiri Tabarestani, Maghsoudlou, Motamedzadegan & Sadeghi Mahoonak, 2014).

The gel strength of various fish species like *Catla catla*, *Cirrhinus mrigala*, *Labeo rohita*, *Aluterus monoceros*, *Channa striatus*, *Claris batrachus*, *Pangasius sutchi* was 367.7, 343.0, 258.0, 149.8, 311.2, 278.7, 325 and 487.6 g respectively (Nitsuwat, Zhang, Ng & Fang, 2021).

Fish gelatin exhibits good filmogenic properties, is transparent, nearly colorless, aqua-soluble, and extremely extensible (Alfaro et al., 2015). Abundant studies have been done on marine gelatin protein films, such as active fish gelatin/peppermint oil composite film and intelligent fish gelatin/haskap berry extract film (Nurul Saadah Said & Sarbon, 2022). Compared to animal gelatin films, the thickness of single fish-based gelatin films has been reported in the range of 0.05–0.12 mm (J. Liu, Yong, Liu, Qin, Kan & Liu, 2019). While, the thickness of active fish gelatin composites with incorporated natural extracts of grape seed, basil, cinnamon, and lavender oils were stated in higher range of 0.06–0.21 mm as compared to single fish gelatin films (Nurul Saadah Said & Sarbon, 2022). The color (L*, a*, and b*) values for single gelatin films derived from tilapia, unicorn leatherjacket, and catfish were reported in range of 90.32–94.25, − 2.51–(−0.80) and − 1.68–15.81, respectively (Arfat, Ahmed, Hiremath, Auras & Joseph, 2017).

Meanwhile, the UV light transmission (200–280 nm) for a single fish gelatin films derived from numerous fish species exhibited higher values of 0.01–40.73 compared to the animal gelatin film (Jridi, Abdelhedi, Salem, Kechaou, Nasri & Menchari, 2020). The single gelatin films of fish source have been described to reveal lower WVP than single animal gelatin films. The statement was also supported by the outcomes of Nurul Saadah Said & Sarbon (2022), who found that single marine gelatin films showed WVP values of 6.00×10^{-13}–2.05×10^{-10} g/m. s·Pa compared to the reported values from bovine gelatin films. The melting point of single fish gelatin film was reported to reveal a lower endothermic melting shift of 53.14–124.45 ºC compared to single animal gelatin films (Ali, Prodpran, & Benjakul, 2019). The marine-based gelatin films exhibited lower TS value (6.23–43.62 MPa) compared to marine animal gelatin films, the EAB value for a single fish gelatin films was reported in range of 2.96–76.73% (Nurul Saadah Said & Sarbon, 2022).

2.3. Mammalian gelatin

Various mammals used for gelatin production are cow, goat, buffalo, and yak. The most crucial gelatin source in the 1930 s was pig hide which accounted for primary industrial gelatin production. The mammalian collagen/gelatin sources are hides or skin, tendons, skeletons, and cartilages. The gelatin from porcine and bovine sources is generally obtained from skin or hides and, to a lesser extent, from bones, cartilages, and tendons. Buffalo processing wastes are well suited for the production of gelatin. Buffaloe's hides are 6–8 mm thick and more potent than other mammals' hides, corresponding to approximately 11.5% of total body weight, and cowhide accounts for 9.0% only (Crackers, 2011). Collagen is a significant constituent of buffalo hide, and buffalo hide has more collagen than cowhide. Due to the higher hydroxyproline content in buffalo hide collagen, collagen structure has higher complexity, heat stability, and gel strength (Mulyani, Setyabudi, Pranoto & Santoso, 2017). A study of two diverse sources of mammalian gelatins, i.e., bovine and porcine, showed that both contain diverse molecular weight components ranging between 10 and 400 kDa. The extensively used mammalian gelatin has some constraints and skepticism amongst consumers due to sociocultural and health-related anxieties (Abdalbasit Adam Marion & Fadul, 2013). For extraction of gelatin from mammalian (bovine/porcine) sources, various extraction methods are used, but the majority of bovine gelatin was extracted by acidic process and porcine gelatin by alkaline methods (Mariod, Abdelwahab, Ibrahim, Mohan, Abd Elgadir & Ain, 2011). The bovine gelatin is extra popular than other gelatin sources for film making owing to its superior gel forming capacity (gel strength and viscosity) and strong filmogenic properties. Additionally, various studies have been presented on active and intelligent animal gelatin, like active bovine-gelatin/nano chitin/corn oil composites and intelligent bovine/curcumin composite

films (Nurul Saadah Said & Sarbon, 2022).

The bloom strength of the mammalian gelatin was reported to be higher than that of the fish and animal gelatin. Chandra & Shamasundar (2015) reported the gel strength of porcine to be 466.4 g, while that of the bovine gelatin was reported to be 350 g by Atma & Taufik, (2020). The single swinish gelatin films have been reported to have higher thickness values compared to single bovine-gelatin films. This is owing to the higher protein contents in pig gelatin (91.30%) compared to bovine gelatin films (88.45–91.20%) (Aykın-Dinçer, Koç, & Erbaş, 2017). The color (L*, a* and b*) values reported for single bovine-based gelatin films were in the range of (89.07–97.30), (−1.27 to 0.07), and (2.00–5.40) respectively, whileas L* , a* and b* values of single porcine gelatin films were reported in the range of (90.00–96.97), (−0.39 to 1.11) and (2.22–3.22) respectively (Nurul Saadah Said & Sarbon, 2022). The WVP of single animal gelatin film was reported to be $8.00 \times 10 - 11$–$9.68 \times 10 - 10$ g/ms·Pa. The melting point (Tm) of single animal gelatin film derived from bovine and swinish gelatin were reported in the range of 60.42–82.20 ºC and 66.80–87.70 ºC respectively (Rawdkuen, Faseha, Benjakul & Kaewprachu, 2020). The higher Tm observed in porcine gelatin films may be owing to higher imino acids (23.70%) compared to bovine gelatin films (22.91–23.33%). It has also been reported that gelatin with higher imino acid groups require higher temperature for conversion of coil structure to helix structure, thus gelatin with higher imino acid groups is thermally more stable (Nurul Saadah Said & Sarbon, 2022). Numerous studies on TS of the animal gelatin films have been reported so for, single porcine gelatin films showed TS of 2.40–63.25 MPa. These TS values were higher as compared to TS of single bovine gelatin films with TS of 0.70–51.68 MPa (Nurul Saadah Said & Sarbon, 2022). The EAB values described from single mammalian gelatin films of bovine and porcine were reported in the range of 0.78–30.83% and 4.40–90.55% respectively (Rawdkuen et al., 2020).

3. Methods of extraction of gelatin

Gelatin is an essential protein obtained by partial hydrolysis of collagen. The hydrolysis is done by using acids, bases, enzymes, or by their combinations. The most common extraction method of gelatin in industries is the chemical method. However, in biological processes, the enzymatic extraction method is a more promising hydrolysis process (Noor et al., 2021). The bonds of collagen polypeptide chains are broken down. This leads breakdown of the fibrous structure of collagen to produce gelatin. Therefore, the gelatin's quality and yield depend not only on the gelatin source but also on the extraction methods of gelatin and the conditions during the extraction process.

3.1. Acidic extraction method

The acid solution is used to hydrolyze collagenous material in this extraction process. The produced gelatin has been called type A gelatin. The Pig hides were commonly used materials for this extraction method, which was treated for 10–45 h with acidic solutions (Abedinia et al., 2020). Acidic treatments enhance collagen's swelling, resulting in better hydrolysis and greater yield per cent (H. Jafari et al., 2020). The Swelling power and solubilisation of collagenous materials are highly affected by concentration and acid type used, which can cause variation in molecular weight distribution of resultant gelatin. The significant acids utilized were phosphoric and other organic acids. However, they are gradually more costly and can adversely affect the smell and the flavor of gelatin produced.

The minute pieces of gelatin source are first soaked into NaOH solution with a 0.2% concentration (w/v) to remove non-collagenous material. Then the mixture is shaken and stirred continuously at 22–28 °C for 40 min. The material is then treated with an alkaline solution three times. Undesirable components get removed during this treatment, and the material turns soft and ready for gelatin extraction. The material is placed in a solution of 0.2% acetic acid (v/v) for 40 min for additional extraction; the solution is drained and washed with water till neutral pH is attained. The final gelatin extraction is achieved at 70 °C for 90 min with 1:9 (w/v) of sample and distilled water. The gelatin extract is filtered using multilayered cheese clothes and then freeze-dried. Freeze-dried material is grounded to powder (Golpira, Maftoonazad, & Ramaswamy, 2021). The type of acid and its concentration greatly affects the gelatin's gel strength and hence the gelatin's packaging properties. Sántiz-Gómez et al. (2019) reported that the bloom strength of acetic acid (0.15 M) extracted gelatin was higher than that of HCl (0.15 M) extracted gelatin. The higher the gel strength, the higher the tensile strength and better the sealing and barrier properties of developed films.

3.2. Ultrasonic assisted extraction method

This is one of the innovative and effective methods used in the food and pharmaceutical industries (Lv, Gouda, Zhu, Ye & Chen, 2021). The ultra-sonication treatment disrupts cells by causing acoustic cavitation, which increases the mass transfer of cell contents and hence results in a higher yield of gelatin extraction than other methods or techniques used. Ming, (2013) reported that ultra-sonication aids in the cleavage of collagen fibrils, facilitating acidic and enzymatic hydrolysis. This advanced method of extraction increases the yield percentage, and extracted gelatin shows improved functional properties (Noor et al., 2021).

This method of extraction, along with the use of food-grade acids can increase the yield per cent of gelatin due to a synergistic effect. The acid-treated gelatin source is extracted using a temperature of 70 °C with an ultrasonic power of 300 W for (100 min) time. The extract is then sieved using a two-layered cheesecloth and then freeze-dried. Freeze-dried material is ground to form a gelatin powder (Mad-Ali, Benjakul, Prodpran & Maqsood, 2017). The gelatin extraction procedure is based on pretreatments with a mild acid to dissociate non-covalent, inter-and intra-molecular bonds, followed by extraction at above 40 °C in distilled water to disrupt hydrogen bonds that stabilize helix to coil transformation resulting in an alteration to soluble gelatin.

The oxhide gelatin hydrolysates treated with 300-W ultra-sonication had the extreme antioxidant activities. Ultrasonication has been reported to inhibit formation of hydrogen bond, reduction in crosslinking between collagen molecules, transformation of folded structures into a helical ones, and lowering of the heat stability of collagen molecules. Thus the gelatin films developed from gelatin of ultrasonically extracted can have higher antioxidant activities and flexibility but lower water vapour and oxygen permeabilities (He et al., 2021).

3.3. Enzymatic extraction method

This method creates less wastage and diminishes processing time, but the method is extra costlier than other gelatin extraction methods. In the enzymatic method of gelatin extraction, various protein hydrolyzing enzymes convert collagen to gelatin. For gelatin extraction by this method, the optimum parameters are treating with pepsin (547 U/g) at 46.98 °C, pH 4 for 1.27 h. Tong & Ying, (2013) reported that gelatin obtained by the enzymatic method of extraction has better gel strength, although gelatin yield is a little lower than other gelatin extraction methods. This higher gel strength is responsible for the higher tensile strength, storage modulus (G′) and barrier properties of the films, as reported by Nurul Saadah Said & Sarbon (2022), which is responsible for better sealing strength of the package. In this method, the gelatin source is washed and then chopped to a uniform minute size (0.5–1.0 cm). Further, chopped material is stirred with NaCI solution (3.5%) for 24 h to exclude non-collagenous materials. After this, a solution of 0.5% Na_2CO_3 is used to remove lipid-soluble materials by stirring at 200 rpm for two days. Defatted material is then neutralized with water. Next, pepsin enzyme solution is added for the extraction of gelatin. The

gelatinous solution is centrifuged for 20 min at 500 rpm to remove insoluble materials. Gelatin precipitation is done using ammonium sulfate (2.6 M). In second centrifugation, precipitated material is collected and then freeze-dried. Feng et al. (2013) reported that enzyme solutions of concentration 2.42% with liquid to solid ratio of 11.8:1 for 6.45 h are the most yielding parameters.

3.4. High-pressure extraction

High-pressure extraction is another innovative non-thermal technique in gelatin extraction (Pinheiro, Martí-Quijal, Barba, Tappi & Rocculi, 2021). The high pressure causes the protein's denaturation and distresses non-covalent interactions, making gelatin protein extract easily (C. C. Lin, Chiou, & Sung, 2015). High pressure and acid treatment have also been reported to enhance extraction yield by causing acid to penetrate more into the pretentious material, thereby increasing the extraction percentage (H.-W. Huang, Cheng, Chen & Wang, 2019). It has also been reported by Zhang et al. (2020) that extraction time is reduced by more than 50% using this extraction method. Noor et al. (2021), extracted gelatin from fish skin using this extraction method by placing alkali/acid pretreated skins in polyethylene bags containing distilled water. The sample bags were placed in the high-pressure chamber for 10 min at 250 MPa and showed a higher yield than conventional methods, as already told by (H.-W. Huang et al., 2019). Chen, Ma, Zhou, Liu, and Zhang (2014) reported that the gel strength and viscosity get improved by the application of 300 MPa pressure. Higher the gel strength of high pressure extracted gelatin can improve the film-forming properties of gelatin, as gelatin with higher gel strength showed improved tensile strength properties. Yusof, Jaswir, Jamal, Jami, and Octavianti (2017) also reported improvements in the red tilapia gelatin, which could be responsible for better packaging properties.

4. Applications of gelatin

Gelatin is known for its multifunctional properties, including its rheological (Santana et al., 2020), emulsifying and foaming capacities (Chakka et al., 2017), bioactive properties (N S Said, Howell, & Sarbon, 2021), fat replacing properties (Almeida & Lannes, 2017), and film-forming properties (Lu et al., 2022).

Gelatin is a vulnerable material with wide applicability in numerous industrial sectors. It has numerous applications in fruit and vegetable, dairy, meat, confectionery, bakery, and packaging industries. It is used as a coating, thickening, and refining agent in the fruit and vegetable industries. In the confectionery industry, it acts as gelling, stabilizing, and whipping agent. It is also a stabilizing agent in foods like ice creams, cheese, foams, and fruit salads. It has been used in desserts and gummy bears (7–9%), meat products, sausages, broths and canned meats (1–5%), dairy products (0.2–1.0%), frozen foods (0.1–0.5%) and beverages (0.002–0.015%) (Abedinia et al., 2020).

Gelatin plays a prominent role in the medical industry as it is used in coating pastilles, tablets, and capsules and encapsulates nutritional supplements. Gelatin has been reported to have higher health-promoting properties. The gelatin possesses biodegradability, biocompatibility and lower antigenicity. Hence can be used as a potential constituent in healing wounds and regeneration of tissues (Lv et al., 2021). Fish gelatin has showed higher potential of treating diverse diseases owing to its composition. Osteoporosis is a major problem nowadays in older people due to calcium loss which results in porosity of bones by causing a lowering of bone marrow density. In another study, Noma et al. (2017) showed that gelatin is utilized to reduce bone brittleness. The gelatin can be exploited in diets of hypertensive people as an anti-hypertensive representative (C. C. Lin et al., 2015). Table 2 summarizes gelatin-based films and coatings applications on food and food products.

Table 2
Applications of gelatin based films and coatings on shelf life of food and food products.

Product	Coating/film	Improved features findings	References
Fruits & vegetables	Gelatin and shellac	Ripening & softening gets delayed, diminished weight loss with extended shelf-life greater than 30 days	Soradech, Nunthanid, Limmatvapirat, and Luangtana-anan (2017)
Banana	Gelatin and shellac	Ripening & softening gets delayed, diminished weight loss with extended shelf-life greater than 30 days	Soradech *et al.* (2017)
Strawberries	Gelatin & mentha essential oil (MEO)	Depressed microflora growth, with retention of pH, firmness, weight, TSS and visual appearance greater than 13 days.	Aitboulahsen et al. (2018)
Grapes	Gelatin & iron oxide nanoparticles	Enhanced mechanical, barrier & physical properties of films, 20% of incorporated nanoparticles exhibited antibacterial activity against *E. coli* and *Staphylococcus aureus,* increased shelf life of grapes.	Mehmood, Sadiq, & Khan, (2020)
Grapes (red crimson)	Gelatin-corn starch	Increased mechanical and barrier properties, retained visual appearance and weight loss reduction during storage.	Fakhouri, Martelli, Caon, Velasco, and Mei (2015)
Grapes & cherry tomatoes	Gelatin, methylcellulose, Chitosan and tannic acid	Exhibited antibacterial activity against *E. coli & S. aureus*, reduced weight loss and browning of fruits and prolonged the shelf of fruits at least 14 days.	Kamari & Phillip, (2018)
Cherry tomatoes	Gelatin-Lotus stem starch	Reduced weight loss, retained color, TSS and pH, retained firmnesss, enhanced shelf life upto 15 days.	Rather, Makroo, Showkat, Majid, and Dar (2022)
Potatoes	Gelatin-alginate with *Pseudomonas fluorescens* bacteria	Diminished disease incidence, sheltered probiotics from damaging condition of soil and increased shelf- potato stored life.	Pour, Saberi-Riseh, Mohammadinejad, and Hosseini (2019)
Cucumber	Gelatin-clove oil with chitosan nanoparticles	Strong antibacterial properties against	Cui, Bai, Rashed, and Lin (2018)

(*continued on next page*)

Table 2 (*continued*)

Product	Coating/film	Improved features findings	References
		E. coli O157:H7 biofilms, retention of original color and flavor of cucumber during storage.	
Olive oil	Gelatin with anthocyanins	Reduced oxidation during storage and prolonged shelf-life of olive oil.	Wang et al. (2019)
Extra-virgin olive oil Fish & meat	Gelatin, Corn starch with activated guabiroba pulp	Enhanced water vapor barrier, prevented oxidation with maintained acidity index and peroxide index values of olive oil during storage.	Malherbi et al. (2019)
Fish fillets (*grass carp)*	Gelatin & curcumin/β cyclodextrin (CUR/βCD) emulsion	Prevented fish filet oxidation and proteolysis, reduced spoilage and enhanced the storage life of filets.	Sun et al. (2019)
Fish freshness evaluation	Gelatin/polyvinyl alcohol with anthocyanin	Enhanced elongation at break from 589.22% to 905.86%, anthocyanins delivered sensitive response to diverse pH & seemly in monitoring volatile compounds produced during the spoiling of fish and helped in evaluating bacteria easily & maintained the organoleptic features of shrimp.	Zeng et al. (2019)
Shrimp	Gelatin & chitosan	Suppressed growth of *P. fluorescens, Shewanella putrefaciens, Pseudomonas* spp., *L. monocytogenes* and lactic acid & flavor for minimum 10 days storage during refrigeration.	Mohebi & Shahbazi, (2017)
Beef meat	Gelatin-chitosan (monolayer and bilayer composites)	Films hindered weight loss & lipid oxidation of meat even after 6–10 days and suppressed psychrotrophic bacteria, molds and yeast growth.	Cardoso et al. (2019)
Pork meat	Gelatin nisin/ catechin with microbial transglutaminase crosslinker	Nisin & catechin prevented the growth of spoilage microorganism, diminished fat oxidation, maintained the quality of pork meat during storage.	Kaewprachu et al. (2018)
Pork		Retained normal pH, inhibited	

Table 2 (*continued*)

Product	Coating/film	Improved features findings	References
	Gelatin-chitosan with nisin and grape seed extract	oxidation of lipids and proteins and stunted microbial growth during cold storage for 20 days.	Xiong, Chen, Warner, and Fang (2020)
Pork slices	Gelatin-chitosan coating with encapsulated tarragon essential oil	The Coatings inhibited deterioration of pork slices, tarragon essential oil prevented oxidation and microbial growth. Enhanced shelf life of pork slices during 16 days of cold storage.	Zhang *et al.* (2020)
Chicken fillet	Gelatin, chitosan nanofiber & Zinc oxide nanoparticles	Showed activity against *S. aureus, E. coli*, and *P. aeruginosa*, abridged weight loss, reserved product quality and improved shelf-life significantly.	Amjadi, Emaminia, Nazari, Davudian, Roufegarinejad, and Hamishehkar (2019)
Active food packaging (Industrial scale)	Gelatin, sodium dodecyl sulfate	Inhibited lipid oxidation of products and exhibited strong activity against *E. coli* and *L. monocytogenes*.	Roy & Rhim, (2020)
Hygienic product packaging	Gelatin/sericin/ clay, glutaraldehyde & glycerol	Improved tensile strength at 8.1 MPa, presented activity against *Staphylococcus aureus, Escherichia coli* etc.	Purwar, Verma, & Batra, (2019)
Cheese	Gelatin, moringa oil with chitosan nanoparticles	Presented antimicrobial activity against *L. monocytogenes* and *S. aureus* and reserved the sensory quality of cheese for 10 days.	Lin *et al.* (2017)

4.1. Gelatin as packaging material

Packaging is an essential unit operation in the food chain, and its importance is in every type of food product. Edible packaging material such as coatings and films has great packaging demand because of their biodegradable nature, maintaining quality, and improving food and food products (Soo & Sarbon, 2018). The coatings and films act as alternatives in packaging, having excellent barrier properties and thus enhancing of shelf life of foods. These protein-based films are gaining importance nowadays over traditional petroleum-based packaging materials. Among the protein-based packaging materials, gelatin films and coatings have better properties due to their uniqueness. Gelatin coatings and films retain the quality of foods throughout storage, acting as barriers to oxygen gas, light, and moisture, preventing deterioration, retards oxidation, and protecting food from other quality losses.

Made barrier properties of gelatin coatings and film can be improved by combining gelatin with various polysaccharide substances like xanthan, chitosan, and rice flour (Ahmad, Hani, Nirmal, Fazial, Mohtar & Romli, 2015). Including antioxidant and antimicrobial substances in gelatin films and coatings improves barrier properties and preservation

of food products (Martins, Cerqueira, & Vicente, 2012). Numerous efforts have been made for natural antioxidants to prevent oxidative deterioration of food products. Plants such as phenols, tannins, flavonoids, and plant extracts have been included in gelatin films with antimicrobial and antioxidant properties, thus increasing shelf life and improving food quality. The added antimicrobials of essential oils remain in films, prevents microbial spoilage for a longer duration, and extends shelf life (Khorshidian, Yousefi, Khanniri & Mortazavian, 2018). Tea's polyphenols can be incorporated in free and encapsulated form into gelatin films to improve packaging properties. Polyphenols in the free form are distributed homogeneously in gelatin films and released fast, while encapsulated ones would be released slowly and uniformly from nanoparticles to the gelatin film matrix. Initial oxidation of food products is prevented by the free tea polyphenols packaged in these gelatin films, whereas encapsulated ones are released slowly from the nanoparticles and extend overall shelf life. Encapsulated and free antioxidants guarantee the long-term storage life of foods (F. Liu et al., 2015). Adding nisin antimicrobial in gelatin films in free and encapsulated forms shows promising results in preserving and retaining the quality of foods (Imran et al., 2012). These edible coatings and films with incorporated essential oils help in the preservation of fish and meat (Sánchez-González, Vargas, González-Martínez, Chiralt & Chafer, 2011). Alparslan, Baygar, Baygar, Hasanhocaoglu & Metin, 2014, concluded that laurel essential oil as an agent of antimicrobial and antioxidant extends the storage period of refrigerated rainbow trout fresh fillets. Applying modified gelatin films to different food products protects food from various deteriorations. The application of gelatin coating and films as packaging in different foods is as follows:

4.2. Meat and meat products coating

Various preservative methods protect meat and meat products, such as smoking, refrigeration, and freezing. Besides, the coating of meat products is also a technique of preservation. Collagen and gelatin have been extensively used as a surface coating on these products to reserve color, reduce aroma deterioration, improve the sensory properties, and slow microbial and chemical spoilage. The beef steaks coated with gelatin and stored for weak showed lower purge than the uncoated beef steaks (Gedarawatte et al., 2021). Beef cubes packaged in collagen films for 20 weeks showed a slight variance in lipid oxidation to control ones because the collagen wrapping showed less oxygen permeability than plastic wrapping (Sánchez-Ortega, García-Almendárez, Santos-López, Amaro-Reyes, Barboza-Corona & Regalado, 2014). In another study, the sausages were free of mold growth when dipped in gelatin solution (23%), sodium hexametaphosphate (6%), hydrochloric acid (2%), and water (69%) at the end of three-week storage. In another study, Jridi, Mora, Souissi, Aristoy, Nasri, and Toldrá (2018) coated the beef meat with gelatin-henna extract and reported a decreased level of protein and lipid oxidation, and lower weight loss in the coated samples. Additionally, Rasul, Asdagh, Pirsa, Ghazanfarirad, and Sani (2022) reported enhanced storage life of minced meat coated in gelatin-chickpea protein coating with incorporated nanoparticles. Gallego, Arnal, Talens, Toldrá, and Mora (2020) reported enhanced storage life of pork meat coated with gelatin-tomato byproduct coating. Wulandari, Erwanto, Pranoto, Rusman, and Sugiyanto (2020), reported lower weight loss, retention of pH, color, and enhanced shelf life of chicken sausages packaged in gelatin-soy protein film with transglutaminase as crosslinker.

4.3. Fruits coating/packaging

Fruits and vegetables are highly perishable commodities, as 80–90% of water needs proper attention during storage. Most quality and quantity losses of fresh vegetables and fruits occur between the harvesting and consumption. To prevent quality and quantity changes in fruits and vegetables, two main techniques, modified atmosphere storage and controlled atmosphere storage, have been used (Rajapaksha, Gunathilake, Pathirana & Fernando, 2021). Nowadays, coating by various edible substances such as lipids, proteins, polysaccharides, or their combinations is applied to these horticultural products. The application of coatings on horticultural commodities provides a substitute for modified atmosphere storage for quality retention through alteration and regulation of the internal atmosphere of the different fruit and vegetable. Biodegradable coatings reduce moisture loss, oxidative reactions, solute migration, and gaseous exchange, and decrease or reduce physiological disorder problems (Dhall, 2013). Gelatin-based coating solutions prevent weight loss, prevent degradation of Vit C, and darken the color of fruits. These gelatin coatings may be introduced with other natural substances such as plant extracts containing antioxidant and antimicrobial components to enhance the shelf-life of these horticultural commodities. The gelatin coatings incorporated with extracts of tea and Aloe vera maintain and prolong the fresh-cut orange quality. Coating apple slices with gelatin containing plant extracts of tea and Aloe vera increases their storage life (Radi, Firouzi, Akhavan & Amiri, 2017). Yousef, El-Moniem, & Mahmoud (2020) reported enhanced storage life of date fruits coated with soy protein-gelatin coating and showed lower weight loss, retained pH and firmness than control ones. Additionally, Pellá et al. (2020), reported extended storage life of guava fruit packaged in gelatin-casein-statch films, and showed lower senescence and overall quality of the guava fruit packaged in these films. R. Jafari, Zandi, & Ganjloo, (2022), reported reduced weight loss, firmness, volume changes and lower increment of TSS and pH of zucchini fruit coated with gelatin-alginate with incorporated anise oil. Instead, Makroo, Showkat, Majid, & Dar (2022), also reported increased shelf life up to 15 days of cherry tomatoes coated with gelatin-lotus stem starch coating.

4.4. Fish packaging

The fish industry is also vital for enhancing the economy of several coastal regions and countries. Due to its biological and chemical composition, fresh fish is a highly perishable product (Kazemi and Rezaei, 2015). In order to inhibit pathogenic spoilage, various strategies have been developed, including incorporating natural antimicrobial substances in edible coatings and films to prevent spoilage and contamination of fish and fish products (Gyawali & Ibrahim, 2014; Kazemi & Rezaei, 2015). The edible films for shelf-life extension of fish are prepared from lipids, proteins, and polysaccharides (Kocira, Kozłowicz, Panasiewicz, Staniak, Szpunar-Krok & Hortyńska, 2021). Gelatin is a coating material for fish owing to its high film-forming ability, abundance, and low manufacturing cost of gelatin films (Peña, Mondragon, Algar, Mondragon, Martucci & Ruseckaite, 2013). Gelatin with other edible ingredients enhances the properties of packaging material for fish and fish products. The coatings of 8% gelatin/chitosan in a ratio of 3:1 incorporated with clove oil (7.5%) (Socaciu, Semeniuc, & Vodnar, 2018) and coatings of 1%, 1.5%, and 2% chitosan increases the shelf life of fish fillets by 6 days. Song, Lee, Al Mijan, and Song (2014) reported a reduction in TABRS (28%), peroxide value (36%) and reduction in *E.coli* and *Salmonella* count of smoked salmon packaged in chicken gelatin film with incorporated clove oil. Nessianpour, Khodanazary, & Hosseini (2019) also reported that gelatin coatings with propolis extract extended the shelf life of *Saurida tumbil* fillet by approximately 4 days by reduction of TABRS and enhancing sensory quality.

4.5. Probiotic encapsulation

In food industries, microencapsulation has numerous applications such as core material stabilization, oxidative reaction control, the temporal and time-controlled release of substances, flavor, color or odor masking, shelf-life extension, and protection of the components against nutrient loss. Various food-grade polymers are used in microbial encapsulation, such as gelatin, pectin, carrageenan, chitosan, alginate, and CMC (carboxymethyl cellulose) (Riaz and Masud, 2013). Probiotic

bacterial cells are most commonly encapsulated by spray drying, emulsion, and extrusion methods. These bacteria get entrapped in a gel matrix (Riaz & Masud, 2013; Solanki et al., 2013). Due to its thermoreversible and amphoteric nature, gelatin is an excellent encapsulating agent mixed with polysaccharides such as gellan gum. These polysaccharides have a net negative charge, repel each other, and are miscible at pH above 6, while gelatin has a positive at pH below the isoelectric point, and hence it has a strong attraction with negatively charged hydrocolloids. Gelatin at a higher concentration (24% w/v) encapsulates lactic acid bacteria when cross-linking with toluene-2, 4-diisocyanate is used to produce biomass (Huq, Khan, Khan, Riedl & Lacroix, 2013). de Almeida Paula, Martins, de Almeida Costa, de Oliveira, de Oliveira, and Ramos (2019) encapsulated *Lactobacillus Plantarum* with gelatin-alginate coating solution and showed that 1% gelatin, and 0.1% alginate showed a higher amount of viable cells (4.2×10^9 CFU/g). Albadran, Monteagudo-Mera, Khutoryanskiy, and Charalampopoulos (2020), also reported encapsulation of *Lactobacillus Plantarum* in chitosan-gelatin and gel protected the *Lactobacillus Plantarum* for 2 hrs in simulated gastric fluid (pH 2). Sengsaengthong & Oonsivilai, (2019), reported microencapsulation of *Lactobacillus sp. 21C2–10* using gelatin-maltodextrin as wall material and incorporated in ice cream showed no significant effect on ice cream sensorial properties. The exposure of encapsulated probiotics in ice cream was simulated to gastro-intestinal juices (pH 2) for 5 h. The ice cream with encapsulated cells showed higher probiotic survival than ice cream with free probiotic cells.

4.6. Other applications

Gelatin has wide applications in various types of food products. Gelatin gels melt at a lower temperature than body temperature, making gelatin a more favorable food component than other gelling agents (Abedinia et al., 2020). In a few parts of the world, water dessert gels are made of carrageenan gum and do not liquefy in the mouth, so they are to be chewed. Although both gelatin and carrageenan-made gels are called water dessert gels, they vary in sensory attributes. In the dairy industry, gelatin acts as a stabilizer and modifies the texture of dairy products. Gelatin has applications in ice cream, yogurt, and other dairy products. In yogurt, gelatin is used to diminish syneresis and raise firmness. The gelatin is companionable with milk proteins, not masking the product's flavor compared to other gums, improving the sensory quality of foods. Food processors obtain broad ranges of textures in foods by using diverse concentrations of gelatin. In confectionery products like marshmallows and soft gummy-type candies, gelatin is an essential ingredient. The gelatin is the main ingredient in these confectionery products and is used in 3% concentration and acts as a stabilizer and whipping agent (Boran & Regenstein, 2010). A significant proportion of gelatin in the medical industry is used in pastilles, tablets, and capsules (Chakka et al., 2017).

The main use of gelatin in the beverage industry is for clarification and sedimentation. Gelatin induces clearness of suspended beverage particles and stabilizes this clearness by partial or complete flocculation and sedimentation of particles in suspension. The gelatin for this purpose is used in beverages containing tannin substances, as gelatin reacts with tannins and forms complexes. In bakery industries, gelatin acts as a setting and stabilizing agent or foam-producing material in bread and cakes, and in icings, gelatin is used as a stabilizer (Widyasari & Rawdkuen, 2014).

5. Modification of gelatin for enhancing packaging properties

The main technological properties of gelatin are viscosity, bloom strength, and melting temperature. These properties depend on gelatin composition, molecular weight, the ratio of α- and β-chains of gelatin, bloom or gel strength, and viscosity, affecting the fibrogenic properties of films (da Trindade Alfaro, Balbinot, Weber, Tonial & Machado-Lunkes, 2015). The melting temperature, bloom strength, and gelling properties of mammalian gelatin are 28–31 °C, 100–300 bloom, and 20–25 °C, respectively, while that of fish gelatin is 11–28 °C, 70–270 bloom, and 8–25 °C, respectively. The gelatin in food products is usually chosen for gelling, rheological, chemical, surface-active properties, and packaging properties, so modification of gelatin is of greater importance to improving these properties. Table 3 summarizes some methods of gelatin modification for improving the properties of films/coatings. Various methods of gelatin modification are as under:

5.1. Enzymatic modification

Enzymes such as transglutaminase, tyrosinases, and laccase improve the techno-functional properties. Compared to tyrosinases and laccase, transglutaminase is used to improve gelling and rheological properties of the gelatin (Bode, Da Silva, Drake, Ross-Murphy & Dreiss, 2011; T. Huang et al., 2017). Transglutaminase modification of gelatin results in the development of ε-(γ-glutamyl)-lysine (G-L) cross-links by three steps, namely acyl transfer, cross-linking and deamidation reactions (Savoca, Tonoli, Atobatele & Verderio, 2018). Transglutaminase from microbes results in acyl transmission between ϒ-carboxamide group of the peptide with glutamine residue and various other primary amines. Covalent bond formation occurs between a ε-amino group of lysine (acyl acceptor) and gelatin (G-L bond). This covalent bond G-L is formed between intra and intermolecular cross-links. By forming these isopeptide bonds, protein structure changes and results in stable and robust network formation, improving viscosity, solubility, gelation, and emulsification properties of gelatin. Water acts as an acyl receptor in the deamination process, and deamination of glutamine results in charge changing and solubility of gelatin (Gaspar & de Góes-Favoni, 2015). The appropriate enzyme concentration is used to modify gelatin, as increased concentrations result in hardening of gel and lower strength due to inhibition of uniform network. Non-thermal reversibility also results from using higher concentrations of transglutaminase in gelatin modification (Wu, Liao, Zhang & Chen, 2019). Microbial transglutaminase modification of gelatin improves textural properties like elasticity, cohesiveness, and adhesiveness. This dense and ordered structure also depends on gelatin's incubation time and temperature during the modification process. The effect of transglutaminase (TG) and pectin on the microstructure of fish gelatin gels are shown in Fig. 3. (Huang et al., 2017) concluded that fish gelatin films exhibit a loose network in microstructures, with modification, show enhanced network structures due to covalent bond formation and substantial aggregations. Increased TG enhances these networks and hence results in good barrier properties of the films. Compared to control gelatin, incorporating fish gelatin with pectin enhances networks and fish gelatin properties. TG increases linkage, improves mechanical strength, reduces moisture and UV light sensitivity, and reduces film solubility (Q. Luo et al., 2022).

5.2. Chemical modification

The functional properties of protein and polysaccharides-based films can be enhanced by applying various treatments such as chemical cross-linking, phosphorylation, and various natural phenolic substances. For enhancement in the technological properties of proteins, phosphorylation has played an important role (Z. Xiong, Zhang, & Ma, 2016). Polysaccharides and hydrocolloids are also modified to enhance functional roles (Shukri & Shi, 2017). Various chemical agents are used to phosphorylating proteins to enhance functional properties (Lili, Huan, Guangyue, Xu, Dan & Guangjun, 2015). Through the phosphorylation reaction, hydroxyl groups of proteins get attached to the phosphate group, resulting in improved functional properties of films. The addition of phosphates to the gelatin improves the emulsion stability by promoting hydrophobic interaction at the interface and surface of oil droplets (Z. Xiong et al., 2016). Hence the introduction of phosphate groups to gelatin improves its emulsification properties.

At this stage of development, the information regarding the impact of

Table 3
Methods of gelatin modification and improvement in properties of films/coatings.

Modification	Functional ingredient	Applications	References
Chemical/physical	Chitosan and ZnO	Antimicrobial	(Z.Liu, Lv, Li & Zeng, 2016)
	Aloe vera extract	Antimicrobial	(Radi *et al.* 2017)
	Black and green tea extract	Antioxidant	(Radi et al., 2017)
	Beeswax and Aloe vera	Enhance barrier properties/antioxidant	(Mudannayaka, Rajapaksha, & Kodithuwakku, 2016)
	Borage extract	Enhance barrier properties/antioxidant	(Gómez-Estaca, López de Lacey, Gómez-Guillén, López-Caballero & Montero, 2009)
	Sweet basil and lemongrass extract	Thermal strength/higher gel strength	(Yasin, Babji, & Norrakiah, 2017)
	Aloe vera and green tea extract	Antioxidant	(Amiri, Akhavan, Radi & Branch, 2017)
	Seaweed extract	Improves mechanical properties	(Rattaya, Benjakul, & Prodpran, 2009)
	Glutaraldehyde	Improves mechanical/thermal properties	(Bigi, Cojazzi, Panzavolta, Rubini & Roveri, 2001)
	Tea polyphenols	Antioxidant	(Liu et al., 2015)
	Epigallocatchin gallate	Antioxidant/enhance mechanical and barrier properties	(Nilsuwan, Benjakul, Prodpran & de la Caba, 2019)
	Laurel oil	Antimicrobial	(Alparslan *et al.* 2014)
	Oregano oil	Antimicrobial	(Kazemi & Rezaei, 2015)
Physical	Rice flour	Enhance barrier properties	(Soo & Sarbon, 2018)
	Ammonium sulphate	Increase/decrease strength(concentration Dependent)	(Sha et al., 2014)
Enzymatic modification	Transglutaminase	Improve barrier/mechanical properties	(Lim, Mine, & Tung, 1999)
	Transglutaminase	Improve barrier/mechanical properties	
Enzymatic/ chemical	Transglutaminase/gly oxal/ formaldehyde	Improve barrier and mechanical properties	(De Carvalho & Grosso, 2004)

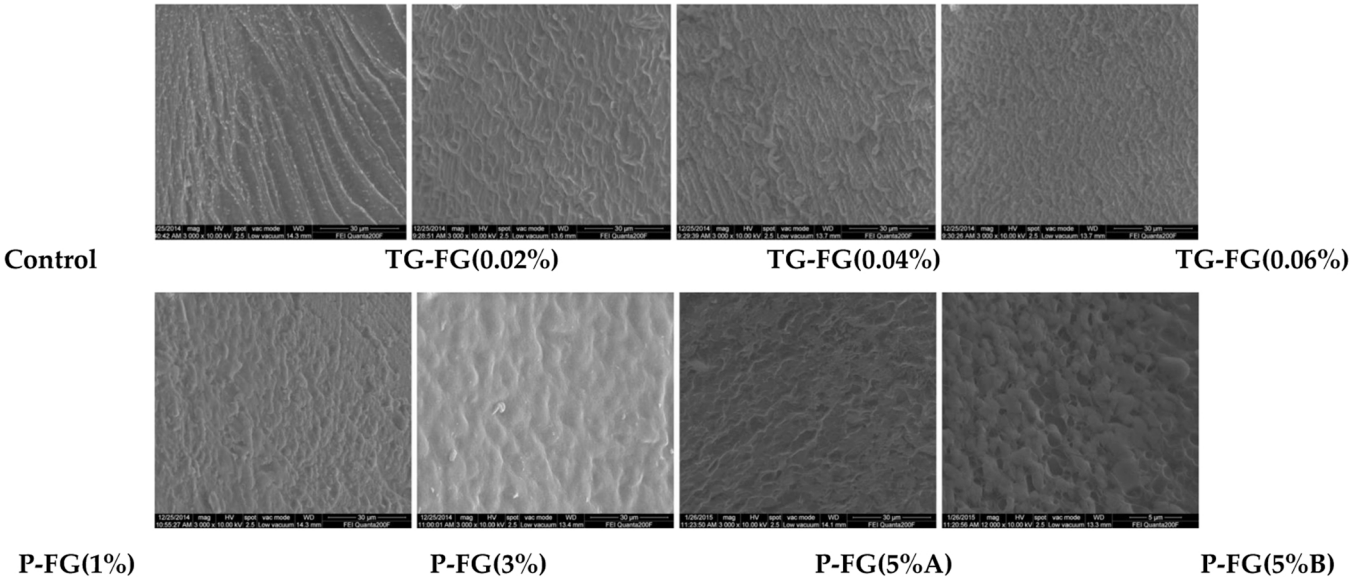

Fig. 3. Microstructures of unmodified/modified gelatin gels with different concentrations of trans gultaminase and pectin (Huang et al., 2017).

different phosphorylation methods on the gel properties of gelatin is scarce. So further research is needed to know the actual size and extent of phosphorylation at every site on gelatin. Various factors govern the phosphate linkage nature, such as the concentration of phosphates, pH, and reaction time. The introduction of excess phosphates increases repulsive forces with protein molecules and hence lowers gel strength, hence poor packaging properties. Gelatin chains aggregate to form large bundles that disturb the configuration of uniform fine gel networks due to long-time phosphorylation. There is acceleration in accessible OH and NH_2 groups on gelatin chains during alkaline conditions to react with phosphate groups. Various chemical cross-linking agents are utilized to modify gelatin and other proteins, but aldehydic agents show greater efficiency by improving various properties such as mechanical, thermal, and moisture resistance by introducing covalent bonds among gelatin chains (Skopinska-Wisniewska, Tuszynska, & Olewnik-Kruszkowska, 2021). Formaldehyde easily migrates between gelatin chains and forms new covalent bonds with lysine, cysteine, and histidine groups on gelatin chains, enhancing its film-forming properties (Benbettaïeb, Gay, Karbowiak, & Debeaufort, 2016; Azeredo and Waldron, 2016). Adjacent residues of amino acids such as ε-NH_2 of hydroxylysine and lysine groups react with glutaraldehyde, forming similar bonds like Schiff base, hence increasing water resistance and strength of the structure formed (Farris, Song, & Huang, 2010).

Nowadays, phenolic substances like ferulic acid, caffeic acid, tannic acid, and rutin have been exploited to alter the properties of gelatin and increase antibacterial properties (Bouarab Chibane, Degraeve, Ferhout, Bouajila & Oulahal, 2019). The OH group of phenolics interacts with COO groups of gelatin molecules by hydrogen bonding. Hydrophobic interactions result in hydrophobic gelatin side chains and phenolic aromatic rings, resulting in reduced barrier properties of films (Kaewdang & Benjakul, 2015). Rutin and gallic acid enhance thermal stability and gel strength but diminish swelling properties (Yan, Li, Zhao & Yi, 2011). Gelatin modified with rutin shows thermal stability and viscoelastic modulus but lower swelling with higher cross-link networks. This is

because of higher binding sites in xerogels. However, increased phenolic concentration decreases gelation property because of more likely interaction with gelatin as aggregates leading to disordered structure (Kaewdang & Benjakul, 2015). One more important property of phenolics is that they increase gelatin protein's antioxidant and emulsifying capacity (Haddar, Sellimi, Ghannouchi, Alvarez, Nasri & Bougatef, 2012). Thus phenolic substances incorporated in gelatin films can enhance the antioxidant properties of the developed films.

5.3. Physical modifications

The simplest and most commonly used method of gelatin modification is the physical method in which electrolytic and non-electrolytic solutes are used. Salts are common electrolytic solutes that have been

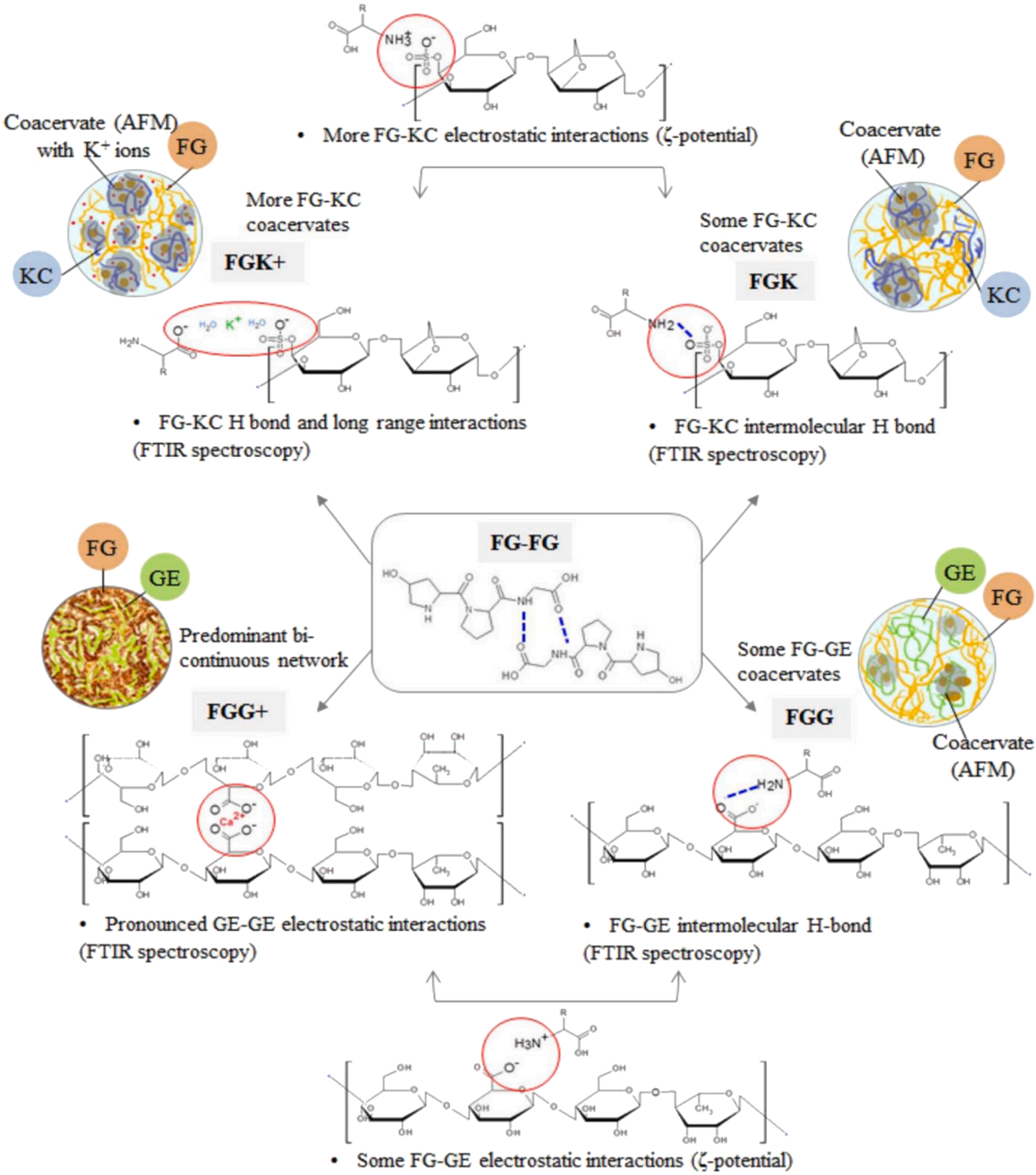

Fig. 4. Schematic diagram depicting the modification of fish gelatin (FG). FGG (FG + 0.040% GE), FGG+ (FG + 0.025% GE + 3 mM CaCl2), FGK (FG + 0.20%KC) and FGK+ (FG + 0.18% KC + 5 mM KCl). PG: Pork gelatin; FG: Fish gelatin; GE: Low acyl-gellan; KC: κ-carrageenan (Sow et al., 2018).

used for modification for a long time. According to Sow & Yang (2015), salts affect gelatin protein by modifying electrostatic forces and salt bridge formation. Karayannakidis and Zotos, (2015) observed that salts like calcium chloride, magnesium chloride, and NaH_2PO_4 improve the melting point and strength of gel fish gelatin, and a higher concentration (0.5 mol/L) of NaH_2PO_4 shows higher efficiency. The positive and negative ion salts affect gel properties depending on the composition of gelatin, salt type, and conditions of experimentation (Masuelli & Sansone, 2012). Amino acid hydroxyl groups form coordinate links with Mg^{2+}, so they promotes ordered triple helical structure and hence efficient mechanical and physical properties of films. Due to higher hydroxyproline content in tilapia skin gelatin than megrim skin gelatin, tilapia fish gelatin with $MgSO_4$ has higher temperatures of melting and gelation. Sow and Yang (2015) reported that larger monovalent aniocs such as chloride anions hinder hydrogen bond formation and interfere with hydrophobic interactions. There is a melting and gelation temperature decrease when gelatin is treated with $(NH_4)_2SO_4$ at higher pH (10).

Sugars, polysaccharides, and glycerol as non-electrolytes also improve the functional characteristics of gelatin (Sow, Kong, & Yang, 2018) by enhancing cross-linking, as shown in Fig. 4. There are hydrogen and electrostatic interactions between protein-polysaccharide, which contribute to improvement in gelation and rheology properties. Higher the crosslinking, efficiency will be the barrier and mechanical properties of the films. Other factors affecting gelatin-polysaccharide systems' properties are molecular characteristics and mixing conditions of complex systems. Sow, Peh, Pekerti, Fu, Bansal, and Yang (2017) observed that calcium chloride and gellan gum break the balance among attraction and repulsion forces in the gellan-gelatin system, and its nanostructure changes, resulting in the development of hydrogen bonds among –NH gelatin groups and –COO and –OH groups of gellan gum. The introduction of gelatin with pectin and xylitol increases gelation properties by forming hydrogen bonds (T. Huang et al., 2017). Thus, incorporating other crosslinking agents like polysaccharides, salts, and sugars improves gelatin packaging properties.

6. Conclusions

At this stage of development, several sources of gelatin are available such as mammals, poultry, fish, and insects. The mammalian gelatin showed higher packaging applicability, followed by poultry and marine sources. However, due to religious concerns, mammalian gelatin is a primary concern. Thus poultry gelatin and fish gelatin are gaining importance in developing edible coatings and films. Additionally poultry gelatin shows higher packaging applicability than fish gelatin, due to its higher gel strength which is correlated to its better mechanical properties. The ultrasonication, higher pressure processing and enzymatic pretreatments during extraction enhanced the packaging properties of gelatin. The tensile strength of the mammalian gelatin has been reported to be higher, followed by poultry and fish gelatin films. However, poultry gelatin showed higher elongation at break. Comparing religious concerns and mechanical and barrier properties, poultry gelatin has been reported to show higher packaging applicability. Additionally, physical, chemical, enzymatic, and irradiation treatments have been reported to enhance the packaging applicability of gelatin. Gelatin films and coatings have the property to extend the shelf life and prevent the deterioration of numerous food products such as fish, meat, and fruits and have a vital role in encapsulating probiotics.

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Jahangir A. Rather: Conceptualization, Methodology, Writing – original draft. **Najmeenah Akhter:** Writing – original draft. **Qazi Showkat Ashraf:** Methodology. **Shabir A. Mir:** Methodology, Writing – original draft, Writing – review & editing. **Hilal A. Makroo:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Darakshan Majid:** Conceptualization, Supervision, Writing – review & editing. **Francisco J. Barba:** Supervision, Writing – review & editing. **Amin Mousavi Khaneghah:** Supervision, Writing – review & editing. **B. N. Dar:** Conceptualization, Methodology, Writing – original draft, Supervision, Writing – review & editing

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There is no conflict among authors or any other agency and authors agreed to publish in Food Packaging and Shelf life.

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