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# Gelatin-based materials: fabrication, properties and applications in the food packaging system

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As a natural biodegradable polymer derived from animal proteins, gelatin exhibits significant potential in environmentally friendly food packaging. This review briefly outlines the preparation and cross-linking methods of gelatin, explores the regulatory mechanisms of different cross-linking strategies on material properties, and analyzes the multi-form application characteristics of gelatin in packaging, elucidating its structure–property relationships. Additionally, this review summarizes intelligent active packaging systems based on gelatin, which integrate bioactive substances (e.g., antimicrobial agents, pH indicators) to achieve real-time food freshness monitoring and shelf-life extension. By deeply analyzing the interplay between the composition, structure, and performance of gelatin-based materials, this review provides innovative insights for designing intelligent and sustainable gelatin-based packaging materials, while highlighting future challenges in scalability and regulatory standardization.

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## 1. Introduction

Due to the increasing concern for the environment, as an alternative to traditional plastics, people have gradually developed a large number of food packaging based on environmentally friendly substances such as proteins and polysaccharides. Found primarily in the bones hides and skin of birds, fish and mammals, gelatin is inexpensive and biodegradable, making it an ideal food packaging material. Gelatin mainly comes from mammals, primarily from the skin of pigs and cattle (accounting for 46% and 29.4% respectively) and bones (accounting for 23.1%), as well as other species (accounting for 1.5%).<sup>1</sup> However, gelatin from mammals is subject to certain religious restrictions. As an alternative to mammalian resources, researchers have been paying increasing attention to gelatin extracted from other species. For example, gelatin is extracted from *Chitala striata*,<sup>2</sup> codfish,<sup>3</sup> silver carp skin, salmon skin,<sup>4</sup> *Nemipterus japonicus*,<sup>5</sup> and fish waste.<sup>6</sup> Fish gelatin has been proven to have similar properties to porcine gelatin and holds great potential in the pharmaceutical field and food industry. Furthermore, avian gelatin is also an important research direction. Said and Mhd Sarbon demonstrated that chicken skin gelatin films exhibit enhanced color properties, light transmittance, and mechanical strength compared with mammalian gelatin films.<sup>7</sup> The major commercial gelatins are extracted by acid and alkali treatments and are generally described as type A and type B gelatins, which have isoelectric points of pH 6–9 and pH 5, respectively.<sup>8</sup>

Gelatin, due to its rich content of proline, glycine and hydroxyproline, has excellent film-forming ability, which helps to form flexible films.<sup>9</sup> In addition, gelatin also possesses functional characteristics such as gel formation, water vapor barrier, biocompatibility and biodegradability.<sup>10</sup> However, gelatin-based materials exhibit a dual characteristic in moisture regulation in food packaging: on the one hand, the dense network structure formed by intermolecular hydrogen bonds can effectively reduce the evaporation of moisture from food inside the package, playing a role in moisture retention; on the other hand, gelatin itself is rich in hydrophilic groups (amino groups, hydroxyl groups), and is sensitive to environmental humidity, with a relatively high water vapor transmission rate (WVP), and is prone to moisture absorption and swelling in high humidity environments, leading to a decline in material mechanical properties. This contradiction essentially stems from the balance between the hydrophilicity and structural compactness of gelatin. Through crosslinking modification (e.g., with genipin) or blending with functional materials/active components (such as proteins, carbohydrates, and phenols), the network structure can be optimized to enhance polymer hydrophobicity, reduce membrane water absorption, and lower water vapor permeability (WVP), while maintaining food moisture retention. This approach mitigates the sensitivity to environmental humidity and enhances mechanical strength.<sup>11</sup>

The degradability of gelatin is one of its significant advantages as a food packaging material. Hydrolysis is the main degradation mechanism for biopolymer-based films and their composites. As gelatin is derived from collagen, its molecular structure contains a large number of peptide bonds. In acidic or alkaline environments, as well as at high temperatures, water molecules can attack these peptide bonds, leading to the

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breakage of  $\alpha$ -subunits and the triple helix structure.<sup>12</sup> For instance, in gelatin/ $\kappa$ -carrageenan composite films, long-term storage or exposure to a humid environment can trigger the hydrolytic cleavage of peptide bonds, thereby reducing the molecular weight and weakening the mechanical properties of the material. Infrared spectroscopy analysis may reveal a shift in the amide band (such as the amide I band at approximately  $1650\text{ cm}^{-1}$ ), indicating the disruption of the protein backbone structure.<sup>13</sup> Additionally, the amino acid residues in gelatin (such as proline and hydroxyproline) are prone to oxidation by reactive oxygen species (such as  $\cdot\text{OH}$  and  $\text{O}_2^-$ ), generating carbonyl derivatives. This process disrupts intermolecular interactions (such as hydrogen bonds), thereby affecting the overall structure and stability of the film.<sup>14</sup> The synergistic effect of the above-mentioned hydrolysis and oxidation degradation mechanisms eventually leads to the gradual degradation of the film.

With the high pursuit of food quality and safety by consumers, research on multifunctional food packaging such as intelligent packaging and active packaging is becoming increasingly widespread. Gelatin has excellent gel-forming, film-forming and water vapor barrier properties, and also features biodegradability, non-toxicity and edibility, which are environmentally friendly and safe. It has great application potential in food packaging. Through reasonable modification methods, its inherent defects can be effectively overcome, and its application scope under different environmental conditions can be expanded. Therefore, this paper reviews the methods of gelatin performance improvement, different forms in food packaging and applications in food packaging, aiming to provide theoretical and technical references for in-depth research on the application of gelatin in the field of food (Fig. 1).

## 2. The extraction method of gelatin

Different extraction methods (acid, base, enzyme treatment) will affect the molecular weight and amino acid composition of gelatin, and thus affect the molecular structure, physical properties, chemical properties and functional properties of gelatin. At present, gelatin extraction techniques can be divided into two major categories: traditional extraction methods and emerging extraction technologies.

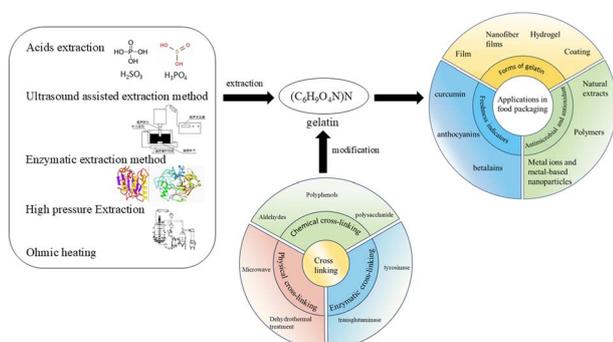


Fig. 1 Gelatin extracted according to different extraction methods and the application of gelatin in food.

### 2.1 Traditional extraction methods

The traditional extraction methods mainly include acid extraction. Acidic treatment enhances the swelling of collagen, resulting in better hydrolysis and a higher yield percentage.<sup>15</sup> The swelling power and solubility of the collagen material are highly influenced by the concentration and type of acid used, which may lead to changes in the molecular weight distribution of the resulting gelatin. The important acids used are phosphoric acid and other organic acids. However, their costs gradually increase and will have adverse effects on the odor and flavor of the generated gelatin.<sup>16</sup>

### 2.2 Emerging extraction technologies

In recent years, to overcome the shortcomings of traditional methods, emerging technologies such as ultrasonic-assisted extraction, enzymatic extraction, high-pressure extraction and ohmic heating have gradually been applied in the extraction of gelatin. These methods have shown significant advantages in improving yield, preserving functional properties and shortening processing time.

**2.2.1 Ultrasound-assisted extraction method.** Ultrasonic treatment destroys cells by causing acoustic cavitation, which increases the mass transfer of cell contents, resulting in higher gelatin extraction rates than other methods or techniques. The ultrasonic treatment of gelatin protein can expand the tertiary structure of gelatin protein and promote the hydrophobic interaction of gelatin,<sup>17</sup> This extraction method can increase the yield and recovery rate (higher than that of traditional CT (gelatin extracted by a typical process without ultrasound)).<sup>18</sup> Therefore, the gelatin film formed from the gelatin extracted by ultrasound can have higher antioxidant activity and flexibility, but lower water vapor and oxygen permeability. Li *et al.* modified gelatin emulsion by ultrasonication, and then prepared gelatin films by modified gelatin emulsion. The results showed that: under the condition of 400 W ultrasonication for 12 min, the zeta potential and viscosity of gelatin emulsion were the largest; the thickness, water vapor permeability (WVP) and water solubility (WS) of corresponding gelatin films were the lowest, and the tensile strength (TS), elongation at break (EAB), denaturation temperature ( $T_m$ ) and enthalpy ( $\Delta H$ ) were the highest.<sup>19</sup>

**2.2.2 Enzymatic extraction method.** In addition to physicochemical extraction conditions, partial hydrolysis of collagen can be achieved through the participation of proteolytic enzymes that disrupt the structure of natural collagen, such as pepsin, prosubtilis protease and trypsinss,<sup>20</sup> trypsin,<sup>21</sup> alcalase<sup>22</sup> and papain,<sup>23</sup> which have been used for gelatin extraction from different sources. In general, pepsin was used more frequently. Samatra *et al.* used the pepsin enzyme to assist the extraction process of gelatin. Pepsin could cut the covalent cross-linking bonds in collagen molecules and improve extraction efficiency.<sup>24</sup> Similarly, Hajlaoui *et al.* used pepsin to extract gelatin from camel skin, increasing the gelatin extraction rate. This method reduces waste and shortens processing time, but this method is more expensive than other gelatin extraction methods.<sup>25</sup>



**2.2.3 High-pressure extraction.** High pressure and acid treatment increase the extraction rate by allowing more acid to penetrate the material, thus increasing the extraction rate.<sup>26</sup> He *et al.* modified the gelatinized collagen by using high-pressure assisted extraction of the gelatinized gelatin prepared from the cowhide waste. High-pressure treatment enhanced the rearrangement of the gel structure during the gelation process and made it have better gelling properties. Compared with traditional processing, the raw material cost is reduced and the preparation efficiency of cowhide gelatin is improved.<sup>27</sup> Experiments by Zhang *et al.* have also demonstrated that the extraction time can be reduced by more than 50% using this extraction method.<sup>28</sup> However, this method is characterized by high equipment costs and complex operation, and it has strict requirements for pressure control accuracy. Moreover, when high-pressure treatment is used alone, it cannot completely degrade collagen subunits, and usually requires a combination with acid or enzyme pretreatment.<sup>29</sup>

**2.2.4 Ohmic heating.** Ohmic heating (OH) is an innovative and environmentally friendly technology. By passing a current into the extraction medium, the substance in the extraction medium is converted into resistance, thus converting electrical energy into heat energy and heating the extraction medium.<sup>30</sup> Compared with traditional methods, it can better maintain the nutritional, functional, structural and sensory properties of the product, so it is also known as a gentle processing technology. Izek *et al.* extracted gelatin from chicken skin by ohmic heating (OH). After OH treatment, the amino acid composition changed significantly, and the total amino acid content related to gel properties increased. The functional properties of chicken skin gelatin, the combination of water and oil, emulsification and foaming properties, are significantly higher than commercial gelatin, and OH treatment significantly increases these properties.<sup>31</sup> However, differences in the composition of the materials may lead to local overheating or insufficient heating, affecting the uniformity and quality stability of the gelatin extraction.<sup>32</sup>

## 3. The crosslinking of gelatin

### 3.1 Physical cross-linking

Physical cross-linking mainly refers to the physical cross-linking of molecules formed by supramolecular structures such as van der Waals forces and hydrophobic interactions. The gel formed by physical cross-linking is generally reversible.

**3.1.1 Dehydrothermal treatment.** Dehydrothermal belongs to the physical cross-linking method, which is a green and biologically safe method for cross-linking gelatin nanofiber films. Water can be extracted from gelatin by condensation reaction at high temperatures to form intermolecular cross-links. Lan *et al.* prepared electrospun nanofibrous film with gelatin/tea polyphenol/L-lysine. The water contact angle of the uncrosslinked gelatin nanofibrous films was  $16.61 \pm 7.64^\circ$ , which indicated that gelatin nanofibrous films had poor water resistance. The water contact angle of cross-linked gelatin nanofiber films increased to  $75.67 \pm 4.61^\circ$  after dehydrothermal treatment at high temperature (140 °C). The water contact angle of a series of nanofiber films made in the experiment was

increased by more than 50°. It shows that the water resistance of the nanofiber membrane is greatly enhanced after dehydrothermal treatment. However, the fiber morphology disappeared after 10 h of immersion in water. Dehydration mainly enhances surface cross-linking, delaying the initial water penetration and thus increasing the contact angle. However, the overall cross-linking density is relatively low, allowing water to gradually penetrate the bulk matrix and disrupt the loosely connected network structure, ultimately leading to dissolution. This indicates that short-term surface hydrophobicity cannot guarantee long-term bulk stability.<sup>33</sup>

**3.1.2 Microwave.** In recent years, microwave irradiation has had the advantages of green, environmental protection, energy saving and convenience, and has been widely used in the field of food. Microwave has a wavelength range of 0.1 mm to 1 m and a frequency range of 300 MHz to 3000 GHz, and is a type of electromagnetic wave.<sup>34</sup> Microwave irradiation can change the conformation of proteins due to the rapid increase in temperature and dipole rotation of polar molecules, thus affecting the physicochemical properties of proteins.<sup>35</sup> Therefore, the gel strength and texture properties of gelatin can be improved by microwave irradiation.<sup>36</sup> Microwave irradiation can affect the rotational motion of water molecules and thus change the properties of solutes. Therefore, due to the structural changes caused by microwave irradiation, the solubility and interface properties of gelatin can be improved. The outcome depends on the microwave parameters: a lower temperature may mainly expose hydrophobic groups, while a higher temperature will accelerate chain degradation and is more likely to increase solubility. Feng *et al.* treated pig skin gelatin with microwave irradiation. Due to the degradation of polymer subunits induced by microwave irradiation, the solubility of pig skin gelatin increased significantly at 25 °C. In addition, due to exposure to more hydrophobic groups, the hydrophobicity of gelatin increases with the increase of irradiation time.<sup>37</sup> Similarly, in Feng *et al.*'s experiment, an increase in microwave extraction temperature from 55 °C to 75 °C led to the degradation of  $\alpha$  subunits and the disruption of the triple-helical structure of gelatin. This structural alteration exposed more hydrophobic groups, thereby enhancing the amphiphilic properties of the gelatin, as indicated by the significant increase in the static three-phase contact angle from 58.35° to 81.65° and the elevation of surface hydrophobicity from 125.49 to 143.32.<sup>38</sup> The barrier property of gelatin is often attributed to its triple helical structure in the molecule. It is believed that the ordered helical domains can form a dense barrier, hindering the diffusion of water molecules or gases. Therefore, the exposure of hydrophobic groups when the triple helix unfolds may form a "hydrophobic layer" on the surface, counteracting the negative impact of reduced helical content and thereby reducing permeability.<sup>39</sup>

### 3.2 Chemical crosslinking

The polymer chain segments are cross-linked by covalent bonds. It is generally necessary to add crosslinking agents, and chemical reactions such as addition and condensation of compounds are used to form chemical crosslinks between each



other.<sup>40</sup> Common chemical cross-linking agents for gelatine include formaldehyde, glyoxal, glutaraldehyde, 1,6-diisocyanatohexane, butadiene dicyclo-oxide, maleic anhydride, dopamine hydrochloride and others. Natural gel-based hydrogels are used to protect food products from moisture and can be modified by cross-linking with suitable cross-linking agents to enhance the 3D structure of the gel.<sup>41</sup>

**3.2.1 Aldehydes.** Aldehydes cross-linking occurs mainly through aldimine condensation between the aldehyde group and the  $\epsilon$ -amino groups of lysine and hydroxylysine residues in the gelatin chain to form Schiff base intermediate. For instance, Liu *et al.* regulated the structure and performance of gelatin edible films through pullulan dialdehyde crosslinking. When  $\text{NaIO}_4$  was added to the pullulan solution, the peroxyanion of iodate oxidized the C-2 and C-3 of the pullulan repeat units, generating free aldehyde groups. In the presence of gelatin, the free amino groups reacted with the aldehyde groups through Schiff base reaction, leading to the formation of a three-dimensional gelatin network (Fig. 2).<sup>42</sup> In addition, glutaraldehyde is also a commonly used aldehyde crosslinking agent. However, the use of glutaraldehyde also raises concerns about the safety and cytotoxicity of the final material.<sup>43</sup> Cinnamaldehyde, an aromatic aldehyde from the cinnamon plant, can act as a cross-linking agent and has antimicrobial activity due to the presence of phenyl groups linked to unsaturated aldehydes, Mousavi *et al.* prepared active antimicrobial crosslinked composite films by doping  $\epsilon$ -poly(lysine) ( $\epsilon$ -PLL) into gelatin-chitosan mixtures. The cross-linking was performed by glutaraldehyde (G) or cinnamaldehyde (C), respectively. The cross-linking agent affected the microstructure of the composite films. The G and C cross-linked films showed smooth and rough surfaces, respectively. C induces very small pores in the cross-section of the composite films. Compared with cinnamaldehyde, the addition of glutaraldehyde reduced water solubility, moisture, and WVP, while increasing TS. The release of  $\epsilon$ -

PLL was higher in the C-crosslinked matrix, resulting in higher antimicrobial activity and better inhibition of bacterial growth. Both G and C, as cross-linking agents, improved the structural and antimicrobial properties of this composite film.<sup>44</sup>

**3.2.2 Polyphenols.** Polyphenols are effective cross-linking agents for gelatin hydrogels and can be used in a variety of reaction pathways including hydrogen bonding, covalent bonding and hydrophobic interactions. During the formation of protein-phenolic complexes, the polypeptide chains of proteins can be rearranged by various factors. In the hydrogel experiments of anthocyanin-gelatin cross-linking to form a mesh structure performed by Chen *et al.*, both polyphenol-gelatin solutions and lyophilized samples under optimized conditions showed a more ordered microstructure than pure gelatin. The addition of polyphenols significantly changed the secondary structure of the polyphenol-gelatin complex. This hydrogel exhibited a higher encapsulation rate than pure gelatin.<sup>45</sup> Hydrogen bond networks are one of the core mechanisms for the combination of polyphenols and gelatin: the hydroxyl groups ( $-\text{OH}$ ) of polyphenols form multiple hydrogen bonds with the amino groups ( $-\text{NH}_2$ ), carboxyl groups ( $-\text{COOH}$ ), or amide groups ( $-\text{CONH}-$ ) in gelatin molecules. Hu *et al.* added betaine-rich plant amaranth extract into the quaternary ammonium chitosan (QC)/fish gelatin (FG) blend membrane. The structural characterization showed that the interaction between the Amaranth extract and film matrix was based on hydrogen bonds, which affected the density of the film. The addition of Amaranth extract significantly enhanced the thickness, ultraviolet-visible light barrier, elongation at break, free radical scavenging activity, antibacterial activity and ammonia sensitivity.<sup>46</sup> Dong *et al.* added curcumin to an edible film of soluble soy polysaccharides (SSPS) and gelatin, hydrogen bonds were formed between the SSPS/gelatin macromolecules and curcumin (Fig. 3). The addition of curcumin significantly increased the tensile strength, maximum weight loss temperature, yellow index, transparency value and water contact angle of the material, which were 2.3–5.5 MPa, 249–252 °C, 2.04–183.07, 0.51–1.63 and 70.7–98.6°, respectively.<sup>47</sup> Secondly, covalent cross-linking is also a key action pathway for some polyphenols. Tannic acid (TA) is a non-toxic and harmless plant polyphenol, which is an effective crosslinking agent for hydrophilic gelatin hydrogels through hydrogen bonding, covalent bonding and hydrophobic interaction. The catechol groups of tannic acid generate quinone substances under oxidative conditions and covalently bond with the amino groups of gelatin to form a rigid cross-linked network. Shan *et al.* made a functional gelatin hydrogel/ethyl cellulose bilayer film with adjustable humidity. Tannic acid (TA) was used as a green crosslinking agent for the preparation of GEL hydrogels and as a reductant for the synthesis of AgNPs *in situ* in the hydrogel network. TA cross-linked GEL hydrogel networks can be used to synthesize and immobilize AgNPs, thereby reducing their biological toxicity. In addition, in the study, the crosslinking density of TA has a significant impact on the mechanical properties of gelatin-related materials. When the TA content is 3 wt%, the tensile strength and elastic modulus of the AgNPs@GT-3/EC bilayer film increase by 47.4% and 6.9%

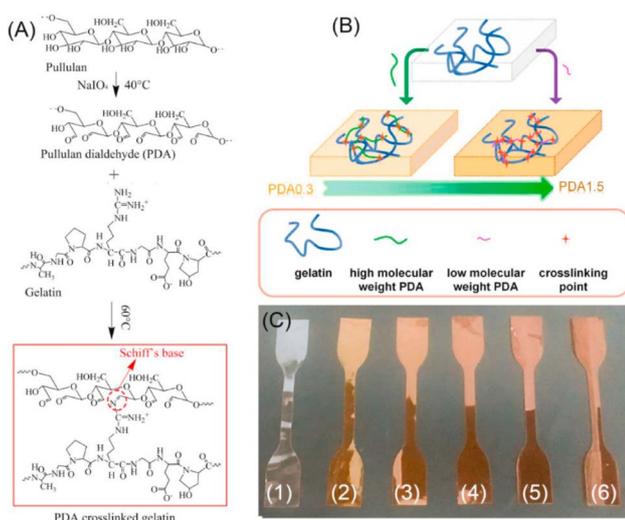


Fig. 2 (A) Periodate oxidation of pullulan and the reaction of gelatin with pullulan dialdehyde (PDA) to produce Schiff's base linkage; (B) network formation in gelatin films with application of different kinds of PDA; (C) photograph of the dumb-shaped gelatin films.<sup>42</sup>



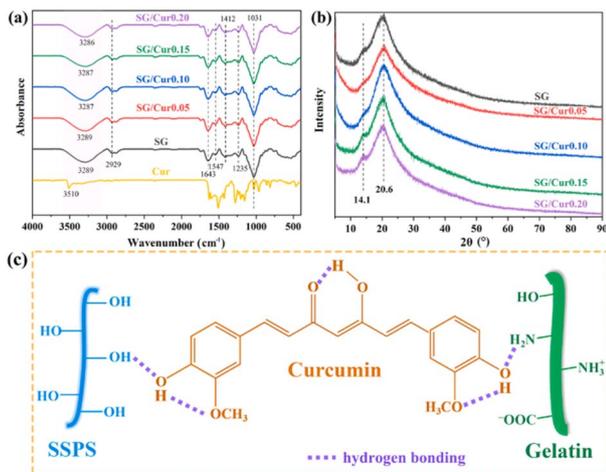


Fig. 3 (a) ATR-FTIR spectra and (b) XRD patterns of curcumin (Cur) and SG-based films; (c) illustration of the hydrogen bonding interaction between the SSPS/gelatin macromolecules and curcumin. The phenolic hydroxyl groups of curcumin and the hydroxyl groups of SSPS and the amino groups of gelatin form hydrogen bonds.<sup>47</sup>

respectively, compared with the G/EC bilayer film without TA, indicating that moderate crosslinking is beneficial to enhance the mechanical strength of the material. When the TA content is too high (exceeding 3 wt%), excessive AgNPs will be generated, interfering with the interaction between gelatin and TA, disrupting the orderliness of the crosslinking network structure, and leading to a decline in mechanical performance.<sup>48</sup> The content related to the cross-linking efficiency of more polyphenols is presented in Table 1.

**3.2.3 Polysaccharides.** The aldehyde groups of oxidized polysaccharides can form covalent amide bonds with the amino groups of gelatin, thus enhancing the mechanical, wettability, and hygroscopic properties of gelatin nanofibers. For example, Yavari Maroufi *et al.* enhanced gelatin-based nanofibers by using oxidized xanthan gum (OXG) as a cross-linker. Crosslinks were created between  $\text{NH}_2$  of gelatin and  $\text{R}-\text{CH}=\text{O}$  of oxidized polysaccharides. The addition of OXG to gelatin fibers reduced water vapor transmission rate, water solubility, and water content properties while improving thermal stability.<sup>53</sup> In another experiment, Kwak *et al.* prepared gelatin nanofibers that were environmentally stable to degradation by interacting environmentally friendly sugar cross-linkers (sucrose, glucose and fructose) with water-soluble fish gelatin. The tensile strength and modulus of gelatin nanofibers without added sugar molecules were found to be 0.65 MPa and 16.61 MPa, respectively, by tensile testing. These weak mechanical properties of gelatin nanofibers were significantly increased by sugar crosslinking. Among them, the most reactive gelatin nanofibers with 10% fructose addition (FG-F10) showed an increase in tensile strength and modulus by 383% and 331%, respectively, compared to gelatin nanofibers (FG).<sup>54</sup> The Maillard reaction (MR) is a natural cross-linking process, MR is a chemical and non-enzymatic browning reaction that occurs when proteins are mixed with sugars (reducing sugars) at high temperatures (Fig. 4). Chitosan nanocrystalline whisker-enhanced glucose-cross-linked gelatin film prepared by

Etxabide *et al.*, which promotes the chemical cross-linked Maillard reaction (MR) between glucose and gelatin after heat treatment. The films then became less soluble (from 100% to 10%), had better thermal stability, significantly improved UV-visible light absorption, significantly enhanced tensile strength (from 42 MPa to 77 MPa) and Young's modulus (from 1476 MPa to 2921 MPa), however, they also become less flexible (with elongation at break from 17% to 7%) and transparent.<sup>55</sup>

### 3.3 Enzymatic cross-linking

Biological modification is a green and clean method. Compared with physical and chemical methods, the enzymatic modification method has significant advantages such as stable product structure, high selectivity and substrate specificity, mild reaction conditions and sustainable enzyme source. Therefore, the enzymatic modification method has a broad application prospect in the field of protein modification.<sup>57</sup> Commonly used enzymes are transglutaminase (TG) and tyrosinase. Microbial transglutaminase (MTGase) widely exists in animals, plants and microorganisms in nature. It is a kind of acyltransferase, which catalyzes the acyl transfer reaction between proteins (or within them) and leads to covalent cross-linking between proteins (Fig. 5).<sup>58</sup> Buscaglia *et al.* applied chemical cross-linking and enzyme cross-linking methods to prepare three-dimensional, porous and mechanically enhanced hydrogels and sponges with different microbial transglutaminase (MTG) ratios. The results showed that the crosslinking density of gelatin was higher than 70% of that of free amine. The addition of MTG increases the mechanical properties and thermal resistance, and the resulting polymers are stable above 40 °C for at least 7 days, comparable to chemically cross-linked gelatin. On a macro level, chemically cross-linked gelatin appears orange due to the consumption of aldehydes during the cross-linking process, whereas enzymatic cross-linked hydrogels do not, which appear white.<sup>59</sup> In another experiment, XU *et al.* investigated the effects of transglutaminase cross-linking on the structural, physico-chemical, functional and emulsion stability of three types of gelatins, namely, bovine bone gelatine, pig skin gelatine and cold-water fish skin gelatine, and the results showed that TG modification increased the nanoparticle-forming behaviors of the three gelatins, and altered their physicochemical and functional properties. In addition, TG-modified aquatic gelatin showed comparable foaming properties ( $147\% \pm 6\%$  foaming capacity and  $55\% \pm 4\%$  foam stability) and higher emulsification properties ( $18 \pm 1 \text{ m}^2 \text{ g}^{-1}$  emulsion activity index and  $142 \pm 11$  min emulsion stability index) to unmodified mammalian gelatin. The TG-modified gelatin reduced the droplet size and improved the emulsion stability of the fish oil emulsions compared to the unmodified gelatin.<sup>60</sup> All these results indicate that TG modification is an effective molecular modification method for gelatin. In addition to a single enzyme crosslinking, Wang *et al.* prepared gelatine-chitosan materials modified by microbial transglutaminase and tyrosinase double enzymes. These two enzymes can trigger gel formation and covalent bonding of gelatin/chitosan blends. Compared with the unmodified material, the double-enzyme modified gelatin





Table 1 Polyphenol efficiency table

Crosslinking agent	Crosslinking principle	Crosslinking density	Swelling ratio	Mechanical strength performance
Tannic acid (TA)	Physical cross-linking can be formed with the amino, carboxyl and hydroxyl groups of gelatin through hydrogen bonding, hydrophobic interaction and $\pi$ - $\pi$ stacking; covalent cross-linking can also be formed after oxidation	FTIR: The stretching vibration peak of -OH/NH redshifts by $9\text{ cm}^{-1}$ ( $3330\text{--}3339\text{ cm}^{-1}$ ), which supports the strengthening of the hydrogen bond network	The swelling degree of gelatin/tannic acid (G-TA) in 0.1 M PBS solution at pH 7.4 is 1180%	The cross-linking of TA forms a dense but rigid structure, enhancing mechanical strength but reducing flexibility <sup>49</sup>
Catechin	Hydrogen bonding is dominant	The apparent viscosity of the solution with tannic acid (BFT-2) added is higher than that with catechin (BFC-5) added, indicating that the cross-linking degree of the polymer molecular network modified by tannic acid in the solution is higher than that of catechin	High crosslinking density reduces water molecule penetration and lowers swelling; tannic acid is more effective due to multi-point crosslinking	Tensile strength: tannic acid > catechin > blank, attributed to the enhanced crosslinking density <sup>50</sup>
Anthocyanin (AC)	Hydrogen bonds and non-enzymatic covalent cross-links	Anthocyanin (AC) has the highest cross-linking density at pH 8, which is characterized by a decrease in $\beta$ -sheet, an increase in $\beta$ -turn, a moderate particle size, and dense pores	Anthocyanin at a concentration of 0.075% has a moderate cross-linking degree, forming a low-swelling and highly stable network	Anthocyanin forms a gel skeleton with high mechanical strength at a concentration of 0.075%, and after freeze-drying, the surface is smooth and dense <sup>45</sup>
Tea polyphenols (TP)	Hydrogen bonds, hydrophobic interactions, and mild covalent cross-linking	As the TP ratio increases, the particle size of gelatine-polyphenol microgels decreases, the polydispersity index (PDI) reduces, the viscosity increases sharply, and the diffusion of water molecules is restricted, indicating a significant enhancement in crosslinking density and a denser network	The highly cross-linked network restricts the migration of free water and reduces ice crystal formation (the area of gelatin ice crystals is $783\text{--}1584\text{ }\mu\text{m}^2$ , while the area of ice crystals in the mixture with a ratio of 1:250 of tea polyphenols to gelatin is $125\text{--}214\text{ }\mu\text{m}^2$ )	The cross-linked network of TP and gelatin significantly stabilizes the structure of myofibrillar proteins <sup>51</sup>
Apple polyphenols (AP)	Hydrogen bond/covalent bond cross-linked gelatin molecular chains	FTIR: after the addition of AP, the amide A peak ( $3300\text{ cm}^{-1}$ ) shifted to a higher wavenumber, suggesting an enhancement of hydrogen bonding DSC: the melting temperature ( $T_m$ ) increased from $48.97\text{ }^\circ\text{C}$ to $55\text{ }^\circ\text{C}$ , indicating a significant increase in crosslinking density	The swelling ratio was significantly reduced (from 41.60% of gelatin to 38.72% of gelatin/apple polyphenols), demonstrating the cross-linking	The tensile strength was significantly enhanced, increasing from 11.51 MPa for gelatin to 15.16 MPa for gelatin/apple polyphenols, but the elongation at break decreased (from 42.9% for gelatin to that of gelatin/apple polyphenols) <sup>52</sup>

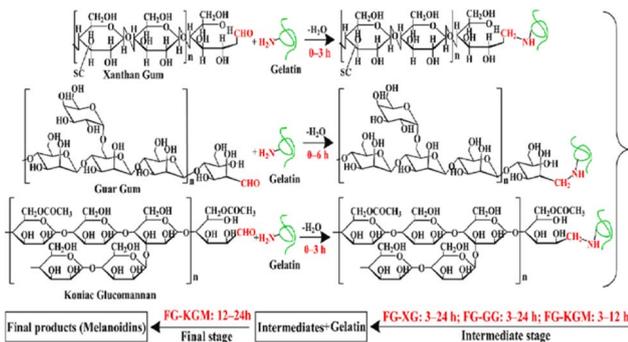


Fig. 4 Maillard reaction mechanisms of fish gelatin with polysaccharides.<sup>56</sup>

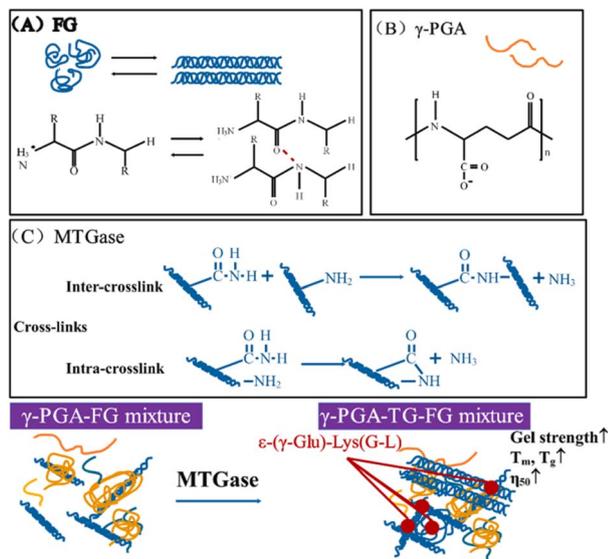


Fig. 5 Schematic diagram illustrating (A) fish gelatin (FG), (B)  $\gamma$ -polyglutamic acid ( $\gamma$ -PGA), and (C) the mechanism of MTGase catalyzed gelatin. The modification of  $\gamma$ -PGA-FG with MTGase affects the structure and rheological properties.<sup>58</sup>

chitosan showed better tensile strength, stability, and enhanced antibacterial activity against *E. coli* and *S. aureus*.<sup>61</sup>

### 3.4 Comparative analysis of cross-linking strategies

Crosslinking strategies have a significant impact on the mechanical strength, biocompatibility and functional performance of gelatin-based materials. Table 2 evaluates the three main methods of chemical crosslinking, enzymatic crosslinking and physical crosslinking from four aspects: efficiency, scalability, toxicity and potential for food packaging applications.

## 4. Different forms of gelatin in food packaging

### 4.1 Film

Gelatin has been widely studied for its excellent film-forming properties, especially in film production, where it is one of the

commonly used base materials due to its low production cost. Gelatin-based films are produced by solution casting as well as extrusion molding methods. Casting is a convenient and environmentally friendly manufacturing process. By mixing with a plasticizer or emulsifier, the film solution is prepared to produce a composite film. The advantages of the casting method are ease of manufacture, no specialized equipment, low cost, and better particle interaction. For example, starch and gelatin blend film, starch and gelatin can dissolve at high temperatures. Starch and gelatin molecules were directly added to 70–100 °C water to dissolve, cooled and cast to prepare the mixed film.<sup>62</sup> The starch gelatin mixed film obtained by casting has the unique characteristics of high optical purity, excellent transparency and low fog degree, but this method is time-consuming and labor-consuming. Extrusion molding is a continuous manufacturing process in which materials are heated and plasticized within the extruder barrel, then mechanically advanced by the rotating screw to form products or semi-finished goods through a shaped die. Extrusion molding has high production efficiency and low energy consumption. Cheng *et al.* blow molded starch/gelatin blends at a relatively low temperature (120 °C) to produce bio-based films with strong mechanical and hydrophobic properties.<sup>63</sup> However, the extrusion molding method is not widely used. The main disadvantage of extrusion molding is that it exposes the film components to high temperatures, which can lead to thermal degradation, creating voids in the film and affecting its uniformity, strength and appearance. In addition, this machine setup requires significant initial and maintenance costs.<sup>57</sup>

### 4.2 Hydrogel

Hydrogels are hydrophilic colloidal substances composed of 3D polymer networks. The core properties of hydrogels are their ability to absorb water and the balance of swelling; the water absorption and subsequent swelling properties of hydrogels are a multi-step process. The responsive behavior of hydrogels is also one of the appropriate characteristics to consider for food packaging applications, and hydrogels also exhibit different responses to physical, chemical and biological stimuli.<sup>64</sup> Stimulus-responsive hydrogels are a good choice for delivering active compounds, especially antimicrobials or antioxidants. There are three types of hydrogels: colorimetric hydrogels, hydrogel absorbent pads and hydrogel films. Conventional gels have low mechanical strength and stiffness when subjected to external stresses, which makes them weaker materials for use in food packaging and tissue engineering applications. The mechanical properties of hydrogels can be improved by reinforcing them with electrostatically spun fibers. Liu used antimicrobial hydrogels prepared with gelatin, chitosan and 3-phenyl lactic acid. In this case, the carboxyl group in 3-phenyl lactic acid reacted with the amino group in chitosan to form an amide bond under acidic conditions. This bond enhances the water absorption and stability of the hydrogel.<sup>65</sup>

### 4.3 Nanofiber films

Bio-nanocomposite technology helps in the fabrication of high-performance materials with additional biofunctional





Table 2 Comparative analysis of different cross-linking strategies

Crosslinking type	Efficiency	Cross-linking strength	Potential for large-scale production	Toxicity	Advantages	Limitations
Physical crosslinking	It needs to be slowly formed through molecular forces	Gross-linked through hydrogen bonds or ionic interactions, but to a relatively weak extent	Higher (with simple equipment, such as microwave method which can be operated continuously)	Non-toxic (no chemical reagents required, relying solely on physical effects)	Environmentally friendly, excellent biocompatibility, suitable for direct contact with food	Low crosslinking degree, limited improvement in water resistance and mechanical properties
Chemical crosslinking	Covalent bonds can form rapidly	Strongly cross-linked through covalent bonds	Medium (the amount of crosslinking agent needs to be precisely controlled)	Some are toxic (for example, glutaraldehyde is cytotoxic; natural crosslinking agents such as cinnamaldehyde are of low toxicity)	Significantly enhance mechanical strength and water resistance	There may be residual chemical reagents, which could affect biological safety
Enzymatic cross-linking	Enzyme-catalyzed reactions are specific	By site-specific bonding. Moderate degree	Medium (enzyme cost is relatively high, and the reaction temperature and pH need to be controlled)	Non-toxic (enzymes are biological molecules and leave no residue after reaction)	The product structure is stable and it has both mechanical properties and biocompatibility	Enzymes are relatively expensive; they are sensitive to reaction conditions (such as fluctuations in temperature and pH affecting enzyme activity)

properties. The commonly used method is electrospinning, which is a very popular method of processing bio-polymers into film-forming solutions. The resulting nanofibrous films are characterized by a large specific surface area, a unique pore structure, and ease of modification. Gelatin nanofibers can be prepared by uniaxial and coaxial electrospinning methods. Tang *et al.* used uniaxial electrospinning to prepare essential oil-loaded gelatin nanofibers. The process involves preparing an electrospinning solution by mixing gelatin and essential oils, electrospinning under high voltage and collecting the nanofibers.<sup>66</sup> Using coaxial electrospinning, unique core-shell nanostructures can be created in which the shell protects the internal bioactive compounds. Gelatin-based electrospun nanofibrous films are a promising material for food packaging, but they have poor water resistance, thermal stability, mechanical strength, and limited antioxidant and antimicrobial activity.<sup>67</sup> To solve these problems, they are often combined with other biomaterials such as polysaccharides,<sup>68</sup> essential oils,<sup>69</sup> metal nanoparticles (silver, copper)<sup>70</sup> and metal oxide nanoparticles (ZnO),<sup>71</sup> *etc.* After encapsulation of bioactives, especially antimicrobial agents, the nanofibers can be used as active packaging materials for a long time.

#### 4.4 Coating

Gelatin, a substance derived from collagen, is widely used as a film-forming component of coatings for the preservation of fruits and vegetables, bread and other fresh foods. Coatings are typically made by applying gelatin in liquid form to the surface of fruits and vegetables by dipping or spraying to form a film.<sup>72,73</sup> These coatings provide protection against ultraviolet radiation, regulate moisture, and maintain an internal balance between solutes and gases involved in the ripening and breathing process of perishable commodities.<sup>74</sup> Edible coatings act as carriers for many bioactive compounds such as natural antioxidants, phenolic compounds and antimicrobial agents to enhance their effectiveness in food preservation and to limit microbial metabolism on the surface of the food product.<sup>75,76</sup> Xiong *et al.* The incorporation of *Streptomyces lactis* peptides and grapeseed extracts into a chitosan gelatin edible coating effectively delayed lipid oxidation in pork samples.<sup>77</sup>

## 5. Applications of gelatin-based smart and active packaging

Gelatin-based materials can achieve functions such as mechanical property optimization, antibacterial and antioxidant protection, and freshness monitoring by blending with polymers or adding functional components, demonstrating diversified application potential in food packaging. According to functional differences, their applications can be divided into two major categories: active packaging and intelligent packaging.

### 5.1 Active packaging

Microbial proliferation and oxidation during food storage are the main reasons for the decline in food quality and nutritional

value. Active packaging inhibits food spoilage by releasing antibacterial or antioxidant components, thereby extending the shelf life. Gelatin, due to its excellent film-forming property and compatibility, has become an ideal carrier for loading active substances.

**5.1.1 Antimicrobials.** Antibacterial gelatin-based packaging ensures food safety by inhibiting the growth of microorganisms, and its core lies in the addition of highly efficient antibacterial agents. Antimicrobial agents are substances used to prevent and control various pathogenic microorganisms, and they have the function of inhibiting or killing microorganisms. Commonly used natural antibacterial agents include chitosan,<sup>78</sup> curcumin,<sup>79</sup> plant essential oils (such as carvacrol,<sup>80</sup> cinnamon oil,<sup>81</sup> perillaldehyde,<sup>82</sup> eugenol<sup>83</sup>), and plant extracts (such as grapefruit seed extract,<sup>84</sup> pomegranate peel extract<sup>85</sup>), etc. For instance, curcumin—a polyphenolic compound with broad-spectrum antibacterial activity—exerts its antimicrobial effects by inhibiting bacterial quorum sensing, suppressing cell division, and disrupting cell wall/membrane integrity.<sup>86</sup> Research indicates that following exposure to bacterial suspensions, the bacterial log concentration on agar plates treated with the soybean polysaccharide/gelatin film containing 0.20% curcumin (SG/Cur0.20) was markedly decreased.<sup>47</sup> Similarly, the functional gelatin/curcumin composite film developed by Roy and Rhim demonstrated notable antibacterial efficacy against foodborne pathogens, including *Escherichia coli* and *Listeria monocytogenes*.<sup>87</sup> In addition, the antibacterial potential of grapefruit seed extract (GSE) has been verified. Riahi *et al.* prepared gelatin-based functional films containing GSE and TiO<sub>2</sub>, and found that the films exhibited strong antibacterial activity against both Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*Listeria monocytogenes*).<sup>84</sup> For volatile and relatively unstable plant essential oils, such as carvacrol, encapsulation within nano-carriers offers a more effective means of enhancing their functional properties. When carvacrol is encapsulated in ZIF-8 nanoparticles and incorporated into gelatin films, the dense structure of the gelatin matrix further prevents direct contact between carvacrol and the external environment, thereby reducing its oxidation and volatilization. This approach enables sustained release of the antibacterial agent, resulting in an inhibitory effect against *Escherichia coli* and *Staphylococcus aureus* that lasts for more than 72 hours, whereas free carvacrol loses its efficacy after only 24 hours. During the use of essential oils, there are differences in the antibacterial effects of different essential oils. These differences may be related to the properties and composition of the essential oils. Moreover, the ratio of essential oils to polymers in the film and the possible interactions between polymers and active compounds can also affect their diffusion in the medium.<sup>80</sup> In the study conducted by Acosta and Sandra, the inhibitory effects of three distinct essential oils—cinnamon bark, clove, and oregano—on two pathogenic fungal species, *Fusarium oxysporum* and *Colletotrichum gloeosporioides*, were evaluated. Films incorporating all three essential oils demonstrated notable inhibition of fungal growth. Overall, cinnamon essential oil exhibited the most pronounced inhibitory activity against *Fusarium oxysporum*. In contrast, both clove and

cinnamon essential oils showed stronger antifungal effects against *Colletotrichum gloeosporioides*. However, oregano essential oil consistently displayed lower antifungal efficacy compared to the other two oils.<sup>88</sup> Despite their high safety profile and compatibility with the requirements of food contact materials, natural antibacterial agents typically encounter challenges such as elevated production costs and susceptibility to degradation under the influence of temperature and light. To address these issues, it is essential to enhance their stability through advanced techniques, including microencapsulation and crosslinking modification strategies.

Among synthetic antibacterial agents, metal ions and metal-based nanoparticles (such as silver nanoparticles (AgNPs), copper-based metal-organic frameworks (Cu-MOFs), etc.) have been the focus of research. In recent years, the significance of nanomaterials in enhancing the physical and chemical properties of packaging products has received extensive attention, driven by the need for advanced solutions in active food packaging. A variety of novel nanocomposites with multifunctional characteristics have been developed.<sup>89,90</sup> This class of materials, characterized by their precisely controllable synthesis processes and potent antibacterial activity, exhibits distinct advantages in gelatin-based packaging systems. The potent antibacterial activity of metal-based nanoparticles stems from their small size (typically 1–100 nm) and high specific surface area, which enables them to rapidly bind to bacterial membranes and kill bacteria, viruses and fungi through multiple mechanisms such as disrupting membrane integrity, inducing oxidative stress, interfering with protein function and degrading DNA.<sup>91</sup> Gelatin, as a biocompatible carrier, can solve the problem of nanoparticle agglomeration and achieve the controlled release of antibacterial components through the coordination of its active groups (amino, carboxyl, and hydroxyl groups) in the molecular structure with metal ions/nanoparticles. It is a key medium for the safe application of such synthetic antibacterial agents in food packaging. For instance, when Rangaraj *et al.* incorporated silver-doped sepiolite composites (Ag-Sep) into a gelatin matrix, the amino groups of gelatin molecules formed coordination bonds with Ag<sup>+</sup>, which not only prevented the aggregation of silver nanoparticles but also regulated the release rate of Ag<sup>+</sup> through the swelling property of the membrane. As the content of Ag-Sep increased, the diameter of the inhibition zone (IAZD) of the gelatin/DSWE blend film significantly increased, and the inhibitory effects on both Gram-negative and Gram-positive bacteria were simultaneously enhanced.<sup>92</sup> In the work of Roy *et al.*, the prepared gelatin/agar-based multi-functional films of copper-doped zinc oxide nanoparticles and clove essential oil Pickering emulsion showed strong antibacterial activity. The eradication of 100% *Listeria monocytogenes* was detected and the *E. coli* population was reduced by 50%.<sup>93</sup> Similarly, titanium (Ti) doped with CuO nanoparticles prepared by Sooch and Mann had better antibacterial activity and lower cytotoxicity on animal cells, and the shelf life of tomatoes (*Solanum lycopersicum* L.) was greatly extended (up to 18 days at 40 ± 3 °C).<sup>94</sup> Metal-organic frameworks (MOFs) are a type of crystalline hybrid porous structure, with metal ions as nodes and organic linkers as bridges.<sup>95</sup> When Han *et al.* incorporated copper-based



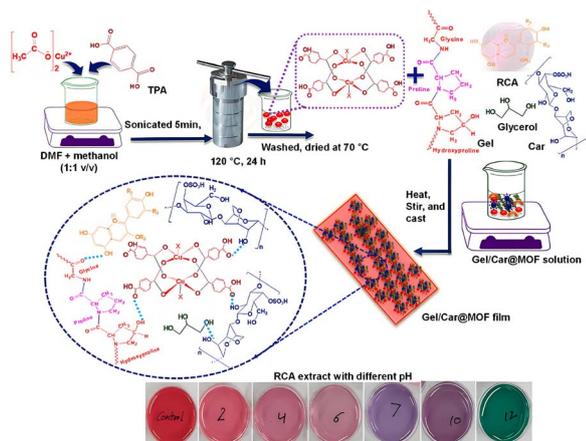


Fig. 6 The preparation process of Cu-MOF and Gel/Car@MOF-based smart packaging films. The functional groups on the surface of MOF (such as Cu–O bonds, carboxyl groups of TPA) form hydrogen bonds with the N–H of gelatin and the O–H of carrageenan.<sup>96</sup>

metal–organic frameworks (Cu-MOF) into gelatin/karaya gum-based films, functional groups on the MOF surface (such as copper–oxygen bonds and carboxyl groups of TPA) formed hydrogen bonds with the N–H of gelatin and the O–H of karaya gum (Fig. 6). Meanwhile, XPS analysis indicated that  $\text{Cu}^{2+}$  might form weak coordination with oxygen atoms in the matrix, supplemented by van der Waals forces, which enabled the MOF to be uniformly dispersed in the matrix, stabilizing the MOF structure and preventing its dissolution in water. The film containing 3 wt% Cu-MOF could maintain 98.5% UV-A and 99.9% UV-B blocking capacity, and continuously release  $\text{Cu}^{2+}$  through the porous structure of MOFs, achieving a 100% kill rate of Gram-positive and Gram-negative bacteria within 3 hours. At the same time, it could safely come into contact with food due to the compatibility of gelatin.<sup>96</sup> The commercial application of metal-based nanoparticles and MOFs faces dual challenges of production cost and regulatory compliance. From a commercial perspective, the synthesis of MOFs relies on high-purity metal ions and organic ligands, and the large-scale production process is complex (such as solvothermal method), resulting in high costs. In terms of regulations, the EU EFSA's "Nanomaterial Guidance" requires that nanoparticles (such as AgNPs, Cu-MOFs) provide particle size distribution, surface charge, migration amount ( $<1 \text{ mg kg}^{-1}$  food), and long-term toxicity data to prove that they will not accumulate through the food chain.<sup>97</sup>

**5.1.2 Antioxidants.** Antioxidant gelatin-based packaging works by delaying food oxidation and deterioration (such as fat oxidation and color deterioration), mainly relying on antioxidants. Important components in food, such as proteins, polyunsaturated fatty acids, and liposoluble substances, can undergo oxidative degradation. Antioxidants are substances that can inhibit or delay oxidation, and they can suppress the activity of free radicals in the body and prevent tissue damage. Gelatin can not only serve as a carrier for antioxidants but also

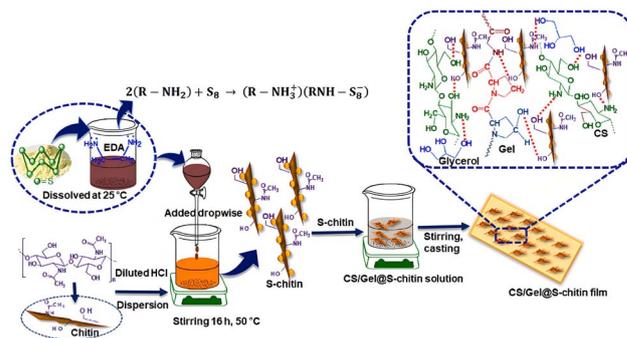


Fig. 7 Schematic illustration of S-chitin preparation and CS/Gel@S-chitin-based active packaging films.<sup>101</sup>

enhance the stability and efficiency of antioxidant components through its structural characteristics, making it a key carrier connecting antioxidants with the demand for food preservation. Natural antioxidants include anthocyanins,<sup>13</sup> procyanidins,<sup>98</sup> curcumin,<sup>99</sup> green tea carbon dots,<sup>100</sup> etc. For instance, The Ch/Gel-based multifunctional film containing sulfur-functionalized chitosan prepared by Khan *et al.* exhibited excellent antioxidant, antibacterial and UV-blocking properties (Fig. 7). The film had a strong antioxidant effect, with the scavenging rates of ABTS and DPPH radicals reaching 100% and 95.9%, respectively.<sup>101</sup> Khan *et al.* used green tea (GT) as a raw material to prepare blue light-emitting carbon dots (CDs) by the hydrothermal method and applied them to prepare chitosan/gelatin (Ch/Gel) based functional films. The antioxidant capacity of green tea carbon dots (GT-CDs) and Ch/Gel films added with GT-CDs was determined by DPPH and ABTS methods. The results showed that the Ch/Gel film added with 3 wt% GT-CDs exhibited high antioxidant activity (ABTS radical scavenging rate 99.2%, DPPH radical scavenging rate 63.87%).<sup>100</sup> Similarly, in the experiment of gelatin/watercress seed gum-based films containing chitosan nanoparticles (CSNPs) loaded with pomegranate peel extract (PPE) prepared by Soltanzadeh *et al.*, pure gelatin and gelatin/chitosan films showed no significant antioxidant activity, while the composite films containing PPE-loaded CSNPs exhibited higher antioxidant activity.<sup>85</sup> As for synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), although they have high antioxidant efficiency, there are potential toxicity controversies, which limit their application. Other antibacterial and antioxidant substances are shown in Table 3.

## 5.2 Smart packaging

Due to its non-toxicity, biocompatibility and biodegradability, gelatin has been used as a functional biopolymer in food packaging materials and is an ideal matrix for loading functional indicators in smart packaging. Its three-dimensional network structure not only provides a uniform microenvironment for various indicators but also optimizes the response performance of the indicators through structural regulation,





**Table 3** Antimicrobial and antioxidant applications in gelatin packaging

Films	Preparation method	Antioxidant effect	Antibacterial activity	Reference
The electrospun fish gelatin nanofiber containing fucoxanthin	Electrospinning technology	The free radical scavenging rate of nanofibers loaded with 5% and 10% fucoxanthin was close to 61%		102
Edible chitosan-gelatin coating with $\beta$ -cyclodextrin/lemon grass essential oil	Coating		The high antimicrobial activity extended the shelf life of the fruit samples by 20 d during refrigeration	103
Gelatin/agar-based multifunctional films doped with copper zinc oxide nanoparticles and clove essential oil emulsion	Solution casting method	The addition of clove essential oil increased the DPPH and ABTS radical scavenging activities to 33% and 61%, respectively	It shows strong antimicrobial activity, where 100% eradication of <i>Listeria monocytogenes</i> was detected and <i>E. coli</i> populations were reduced by 50%	93
Gelatin nanofibers loaded with eugenol	Electrospinning technology	The antioxidant activity and free radical scavenging activity of the membranes increased with the addition of eugenol ( $2-4 \text{ mg g}^{-1}$ ). Nanofibers containing $4 \text{ mg g}^{-1}$ eugenol showed the highest antioxidant activity ( $32.99 \pm 4.20 \text{ mg DPPH g}^{-1}$ dry weight) at $12.15-32.99 \text{ mg DPPH g}^{-1}$ dry weight and $17.83-43.80\%$ at $17.83 \text{ mg DPPH g}^{-1}$ dry weight, respectively. Free radical scavenging activity ( $43.80 \pm 3.67\%$ ) showed significantly higher antioxidant activity, 6% gel film showed significant DPPH free radical inhibition (69.4%)	It has antibacterial properties against <i>Escherichia coli</i> and <i>Staphylococcus aureus</i>	104
Chitosan nanoparticles coated with pomegranate peel extract enhanced active gelatin/cress seed gum base film	Solution casting method	The free radical scavenging activities of gel/AGAR/ZnO films in ABTS and DPPH methods were 22.7% and 11.4%, respectively. The addition of anthocyanins increased the antioxidant activity of the films to 99.4% and 48.6%, respectively	The membrane showed obvious antibacterial activity against <i>E. coli</i> and <i>Listeria monocytogenes</i>	85
Gelatin/AGAR base containing anthocyanins and zinc oxide nanoparticles	Solution casting method	Adding CD to the film increased the antioxidant activity from 16.66% to 94.08%		105
Gelatin film rich in green carbon quantum dots (CD)	Solution casting method	The Ch/Gel membrane supplemented with 3wt% GT-CD showed high antioxidant activity (ABTS free radical scavenging rate was 99.2%, DPPH free radical scavenging rate was 63.87%)		106
Chitosan/gelatin-based multifunctional membrane binding green tea carbon points	Solution casting method	The DPPH and ABTS scavenging activities of SSPS/gelatin films containing 0.20% curcumin were 42.6% and 99.6%, respectively, which were significantly higher than those of pure SSPS/gelatin films	Strong antibacterial action (100% kill <i>Escherichia coli</i> and <i>Listeria monocytogenes</i> within 3 h)	100
Curcumin incorporated soluble soybean polysaccharide/gelatin active food film	Solution casting method	The antioxidant activity of gelatin film was significantly increased to 31% and 57% by DPPH and ABTS methods, respectively	The composite membrane has antibacterial activity against <i>Escherichia coli</i> and <i>Staphylococcus aureus</i>	47
The gelatin-based functional film containing grapefruit seed extract and TiO <sub>2</sub>	Solution casting method		It shows strong antibacterial activity against Gram-negative bacteria ( <i>E. coli</i> ) and Gram-positive bacteria ( <i>Listeria monocytogenes</i> ). Both tested bacteria were destroyed and stopped growing three hours after exposure	84



Table 3 (Contd.)

Films	Preparation method	Antioxidant effect	Antibacterial activity	Reference
Chitosan gelatin with lactostreptin and grape seed extract added to food coating	Coating	The higher the TBARS value, the higher the lipid oxidation degree. The TBARS values of all coated pork samples grew much more slowly during storage and were well below the rancidity threshold by day 20	Compared with the uncoated samples, the coated samples effectively inhibited microbial growth from the fifth day. On days 15 and 20, the TVC of the coated samples was significantly lower than that of the chitosan-coated samples alone	77
Functional gelatin/curcumin composite membrane	Solution casting method	The DPPH and ABTS scavenging activities of pure gelatin film were 5.1% and 12.9%, respectively, but increased to 76.2% and 88.1% after mixing with 1.5 wt% curcumin	The gelatin/curcumin composite membrane showed significant antibacterial activity against foodborne pathogens, <i>Escherichia coli</i> and <i>Listeria monocytogenes</i>	87
Active/smart food packaging film containing amaranth leaf extract (ALE)	Solution casting method	After 3 days of storage in pure film, TBARS of fish and chicken increased rapidly and were 2.89 mg MDA kg <sup>-1</sup> and 1.25 mg MDA kg <sup>-1</sup> , respectively. When they were stored in an active membrane containing ALE, TBRS values for fish and chicken were only 1.3 and 1.07 mg MDA kg <sup>-1</sup> , respectively, after 12 days of storage	The addition of ALE to the PVA-gelatin membrane conferred the antibacterial properties of the membrane, and two Gram-positive bacteria, <i>Bacillus cereus</i> and <i>Staphylococcus aureus</i> , had the largest inhibitory zone	107
Blood orange peel pectin (BOPP) film and fish gelatin (F-G) composite film	Solution casting method	In F-G : BOPP (50 : 50), the reducing power, β-carotene bleaching inhibition and free radical scavenging activity reached 0.96%, 50.36% and 67.36%, respectively	The addition of BOPP increased the antibacterial activity of gelatin membrane against four kinds of Gram-positive and Gram-negative bacteria: <i>Micrococcus luteus</i> (ATCC 4698), <i>Bacillus cereus</i> (ATCC 11778), <i>Enterococcus faecalis</i> and <i>Enterobacter</i>	108
Electrospun fish gelatin film containing lauryl arginine ethyl ester (LAE)	Electrospinning technology		The addition of LAE increased the antibacterial activity of the electrospun film. The membrane has good antibacterial activity against <i>Staphylococcus aureus</i> and <i>Escherichia coli</i>	109
Gelatin/chitosan-based "sandwich" antibacterial nanofiber film loaded with perillaldehyde	Electrospinning technology		Due to the synergistic effect of chitosan and perillaldehyde, the film exhibited the most excellent antibacterial activity	82
Gelatin/κ-carrageenan active films through procyanidins crosslinking	Blending and drying	The antioxidant ability of films significantly improved ( $p < 0.05$ ) by adding procyanidins. The films also exhibited high ABTS radical scavenging activity		98
Gelatin/polyvinyl alcohol films loaded with doubly stabilized clove essential oilchitosomes	Solution casting method		Films showed optimal inhibition of both <i>Staphylococcus aureus</i> and <i>Escherichia coli</i>	110

while reducing the risk of toxic migration and achieving precise monitoring of food freshness. Based on the difference in indicator types, gelatin-based smart packaging can be divided into natural indicator systems and synthetic sensor systems. Both have their characteristics in response mechanisms, application scenarios, and performance optimization, and together they form the core technical path for visual monitoring of food quality in smart packaging.

**5.2.1 Natural indicators.** Natural indicators are mainly substances extracted from plants, animals or microorganisms in nature, which can respond to environmental parameters (such as pH, temperature, gas, *etc.*) through color changes. The semi-transparency of gelatin can better display the color-changing effect. Common natural indicators include anthocyanin,<sup>111</sup> curcumin,<sup>99</sup> betaine,<sup>46</sup> *etc.* There are abundant sources of natural pigments, like anthocyanins, which can be extracted from different plants such as red kale, sweet potato, rose hips, butterfly peas, green peels, mangosteen, and dragon fruit. Anthocyanins from different extraction sources have different pH sensitivities. In a previous report, anthocyanins extracted from red kale showed a similar wide range of pH-dependent color changes, but were colorless in the neutral region and indistinguishable from the original color under acidic conditions.<sup>112</sup> In contrast, anthocyanins derived from butterfly pea flowers showed distinct color changes under acidic and alkaline conditions, facilitating the application of pH-sensitive color indicators. Kim *et al.* extracted anthocyanins from butterfly pea flowers and synthesized ZnO using a simple hydrothermal method. Butterfly pea anthocyanins (BA) and ZnO were homogeneously dispersed in a polymer matrix and formed into composite membranes with gelatin/agar polymers, which were used to monitor shrimp freshness, and as a result, BA and BA-added films exhibited excellent pH-responsive color change properties, as well as good color stability and reversibility. The unmodified color-indicating film gelatin/agar/ZnO/anthocyanin (Gel/Agar/ZnO/BA) showed a color change from pink at pH 2 to purple at pH 4–5, to cyan at neutral, and to green at higher pH. The color change of the film according to pH change is consistent with the color change of BA anthocyanins. However, in the actual state, the color change from blue to green was rapid, limiting the detection of food spoilage. By acidifying the gelatin matrix (A-Gel/Agar/ZnO/BA film), the swelling property of the film was regulated, and the rate of color change under alkaline conditions (only partial color change at pH 9) was slowed down. This makes it more suitable for the development of color indicators. Moreover, anthocyanins, as natural substances, comply with the “Generally Recognized as Safe” (GRAS) standards of the European Food Safety Authority (EFSA) and the US Food and Drug Administration (FDA). The good biocompatibility of gelatin can also ensure the safety of the pigment in contact with food. In conclusion, freshness indicators based on natural colorants can be effectively applied to intelligent food packaging systems. The encapsulation effect of gelatin not only reduces the toxicity risk of pigments but also controls the release rate of pigments by adjusting the swelling property of the membrane, making the pH response window

more compatible with the food spoilage cycle. This enables consumers to directly assess the freshness of food with the naked eye. Other gelatin-based laminated films using colorants are shown in Table 4.

**5.2.2 Synthetic sensors.** In the field of smart packaging, synthetic sensors have become core functional components for monitoring food freshness and environmental parameters due to their high response sensitivity and stable signal output. Based on differences in their mechanism of action and detection targets, synthetic sensors can be classified into pH-responsive synthetic dye sensors and nanoscale metal-organic framework (MOFs) sensors.

Alizarin, methyl red,<sup>46</sup> bromocresol green,<sup>118</sup> bromothymol blue<sup>119</sup> and other synthetic dyes have been used to prepare pH-responsive synthetic dye sensors. This type of sensor achieves visual monitoring of pH fluctuations during food spoilage by generating color changes through its structural variations in response to pH. Wang *et al.* prepared gelatin films containing lavender essential oil Pickering emulsion (LEOP) and alizarin (ALI) for monitoring shrimp freshness, and the films showed a sensitive, visual, and high-contrast response to pH. Due to the addition of alizarin, the color change of the film shows a wider color change to the naked eye. As the pH value increases from 2 to 11, the yellow color of the film gradually changes to red and eventually to purplish red<sup>63</sup>. This type of sensor can sensitively reflect changes in food freshness. However, synthetic dyes are usually restricted to non-direct contact with food due to their potential toxicity.

Metal-organic framework (MOFs) sensors, with their porous structures, exhibit high adsorption and responsiveness to volatile gases (such as H<sub>2</sub>S, CO<sub>2</sub>) or metal ions produced by food spoilage. They can convey food quality information through structural changes or optical signals (such as fluorescence, color).<sup>120</sup> Xu *et al.* prepared cellulose acetate (CA) composite membranes loaded with Co-BIT (a kind of MOFs microcrystals) for real-time monitoring of shrimp freshness. When shrimp deteriorate, volatile ammonia is released. After the Co-BIT microcrystals interact with ammonia, the color changes from blue to brown, and the characteristic peaks of the infrared spectra shift significantly. This is because hydrogen bonds are formed between MOFs and amines, altering the coordination environment of Co elements and ultimately causing the color change. Fresh shrimp (low TVB-N) correspond to blue membranes, while spoiled shrimp (high TVB-N) correspond to brown membranes, achieving visual monitoring of shrimp freshness.<sup>121</sup> The inherent color properties of MOFs make up for the defect that natural pigments (such as anthocyanins) are prone to decomposition under heat and light conditions, improving the stability and sensitivity of the indicator film; however, the color change of MOFs-based smart films currently requires the assistance of a spectrometer for quantitative analysis, and the contrast for direct identification by the naked eye still needs to be enhanced. Its application needs to meet strict regulatory requirements (such as the EU EFSA's restrictions on the migration amount of nanoparticles), and the cost is relatively high.



Table 4 Applications of gelatin in smart packaging

Colorants	Samples	Color change	Reference
Anthocyanin	Enoki mushroom	As the pH increased from 3 to 10, the color of the SPS buffer changed from red to blue and eventually to yellow	113
Nano cerium oxide	Stern	As the concentration of hypoxanthine (HX) increases, the color of the film gradually deepens from milky white to orange	114
Amaranth leaf extract	Chicken/fish	The pink color of Amaranthus leaf extract ALE did not change significantly when pH changed from 1 to 6. When pH exceeded 6, the color of ALE changed from very light pink (pH 7–8) to yellow (pH 9)	107
Carrot anthocyanin		The colors corresponding to different pH values are: pH 2 is orange, pH 3–4 is pink, pH 5–7 is pinkish purple, pH 8–9 is purple, pH 10 is blue and pH 11–12 is yellow	111
Curcumin	Fish	At pH 7–9, the color of the hydrophilic film solution changed from yellow to reddish brown with increasing alkalinity	115
Natural pigments (saffron or red barberry anthocyanins) a	Fish	The color of the film containing the anthocyanin solution changes from red (acid pHs) to blue/purple/gray (neutral pHs) to green/yellow (alkaline pHs). The color of the film containing the anthocyanin solution changes from red (acidic pH) to light yellow (neutral pH) to yellow (basic pH)	12
Blueberry anthocyanins, Fe <sup>2+</sup>	Milk	From pH 6–7 purple-black (fresh milk) to pH 5 blue-purple (spoiled milk) and then to pH 4 purple-red (spoiled milk)	14
Mangostin	Milk	Form pH 6.6 purple to pH 5.5 purple to pH 4.5 pink	116
Black rice bran anthocyanins	Shrimp, scallop	At pH 2–12, the film colors are rose carmine, pink, purple, gray-blue and yellow-green	13
Butterfly pea flower anthocyanins	Pacific white shrimp ( <i>Litopenaeus vannamei</i> )	The transition from the initial color of the BA-containing films to red when the films were exposed to acidic pH (2–6). In contrast, the solution changed color from purple/blue at neutral pH to green at alkaline pH	117

## 6. Conclusions and prospects

To replace the traditional non-renewable petroleum-based polymers and plastic packaging, the research of biodegradable and safe food packaging is particularly important, focusing on the use of natural polymers as the substrate for food packaging utilizing films or coatings, gelatine, as a by-product of bioprocessing, is rich in sources, environmentally friendly, biodegradable, non-toxic and non-hazardous, and can be processed into various forms such as films, nano-films, hydrogels and coatings. Despite the shortcomings of simple gelatin films, they can be modified by cross-linking to make them functional. Cross-linking modification is an important means to improve the performance of gelatin, which can be improved through physical cross-linking, chemical cross-linking or enzymatic cross-linking by introducing a new 3D network structure to improve gelatin's stability, barrier properties, water resistance and so on. Future work must standardize toxicity protocols for aldehyde crosslinkers (such as clearly defining migration limits

and safety thresholds), and optimize the reactor design for enzymatic amplification reactions. In addition, gelatin film is a good substrate for the packaging of food products, the addition of active compounds (natural plant extracts, essential oils, metal ions, chitosan) can give it specific antioxidant and antimicrobial properties for the design of active biodegradable packaging materials.

The yield and quality of gelatin products obtained from different sources and different extraction methods are not the same, to expand the application of gelatin in the food industry, it is necessary to constantly explore the gelatin obtained from different sources and different preparation processes. In the synthesis of gelatin-based films, the solution casting method is still widely used, but this method is time-consuming and prone to introducing impurities. Therefore, scalable technologies (such as electrospinning, 3D printing, and spraying) need to be optimized to reduce processing time and minimize the risk of impurities. In addition, when modifying films, multiple methods are often used in combination, but this may harm the



film, so innovations need to be found and new techniques need to be explored to achieve lightweight modification of films.

## Author contributions

Conceptualization, Y. X.; writing – original draft preparation, X. X.; data collection, X. X. and Y. X.; writing – review and editing, Y. X. and Y. W.; project administration and funding acquisition, Y. W. All authors have read and agreed to the published version of the manuscript.

## Conflicts of interest

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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