Gelatin-Based Films and Coatings for Food Packaging Applications

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Abstract: This review discusses the latest advances in the composition of gelatin-based edible films and coatings, including nanoparticle addition, and their properties are reviewed along their potential for application in the food packaging industry. Gelatin is an important biopolymer derived from collagen and is extensively used by various industries because of its technological and functional properties. Nowadays, a very wide range of components are available to be included as additives to improve its properties, as well as its applications and future potential. Antimicrobials, antioxidants and other agents are detailed due to the fact that an increasing awareness among consumers regarding healthy lifestyle has promoted research into novel techniques and additives to prolong the shelf life of food products. Thanks to its ability to improve global food quality, gelatin has been particularly considered in food preservation of meat and fish products, among others.

Keywords: edible films; food coatings; food preservation; biopolymers; antioxidant and antimicrobial agents

1. Introduction

In the last few decades, there has been a marked increase in the use of natural polymer-based film materials and coatings in packaging for food industry, which protect food from external contamination, retarding its deterioration by extending its shelf-life and maintaining its quality and safety [1]. In addition to consumer requirements and in order to substitute petroleum-based plastic packaging, a wide variety of biopolymers that come from agro-food industrial wastes and renewable low cost natural resources have emerged [2]. In this context, the formulation of films and coatings for food packaging applications must include at least one component capable of forming a cohesive three-dimensional matrix. Biopolymers directly extracted from biomass mainly used for edible films in food packaging are proteins, polysaccharides and lipids, and the physical and chemical properties of the biopolymer used determine the final properties of the developed films [3].

Proteins can be defined as natural polymers able to form amorphous three-dimensional structures stabilized mainly by non-covalent interactions. The functional properties of the final materials are highly dependent on the structural heterogeneity, thermal sensitivity, and hydrophilic behaviour of proteins. Different vegetable and animal proteins are commonly used as biodegradable polymers, such as corn zein, wheat gluten, soy protein, collagen and gelatin, casein and caseinates, and whey proteins, among others [1,4,5].

Regarding polysaccharides for material applications, the main ones used are cellulose and starch, but increasing attention is being given to more complex carbohydrate polymers produced by bacteria and fungi, especially to polysaccharides such as xanthan, curdlan, pullulan and hyaluronic acid [6]. In addition, the incorporation of lipid materials such as animal and vegetable oils and fats, waxes and
natural resins, into polysaccharide and protein matrices to form edible composite films and coatings has the potential to improve film moisture barrier [7].

The use of biopolymers, especially gelatin, in packaging of highly perishable food products such as meat and fish is based on some particular properties such as cost, availability, functional attributes, mechanical (flexibility, tension) and optical (brightness and opacity) properties, barrier effect against gas flow, structural resistance to water and microorganisms and sensory acceptability. In this article, the latest advances in gelatin-based films and coatings including composition (additives to be used in the gelatin matrix, including nanoparticles addition) and properties are reviewed, as well as new research trends for different food applications.

**Gelatin and Film-Forming Properties**

Gelatin is a natural water soluble protein characterized by the absence of an appreciable odour and the random configuration of polypeptide chains in aqueous solution. It is obtained from the partial hydrolysis of collagen; a fibrous protein mainly found in certain parts of vertebrate and invertebrate animals as bones, skins, connective tissues and tendons [8]; and its structure consists of rigid bar-like molecules that arranged in fibres inter-connected by covalent bonds.

Pig skin was used as raw material to manufacture gelatin in the 1930s and continues to be the most important material for large-scale food industrial production; whereas for more expensive uses, such as pharmaceuticals, gelatin is generally obtained from cattle bones, which is considered a more complex and costly extraction process. However, in a move to get away from porcine and bovine gelatin, the production of fish gelatin has increased in the last decade, accounting for more than 1.5% of total gelatin production [9].

In recent years, by-products obtained from the fishing industry, such as heads, skin, bones, fins, muscle pieces, scales, viscera and others, are considered potential sources of exploration, rather than disposable waste. However, one of the main drawbacks of fish gelatin is its rheological properties, being less stable than the obtained from mammalian sources [10]. Moreover, since the production of gelatin from fish and poultry is still limited, the obtained products are less competitive in price than those from mammalian gelatins.

Soluble gelatin is produced by the destabilization of the collagen triple-helix. In general, gelatin properties are influenced by two main factors: the characteristics of the initial collagen and the extraction process. In this sense, the degree of collagen conversion into gelatin is dependent on the pre-treatment with warm-water extraction, temperature, pH, and extraction time. Interstitial collagen molecules are composed of three polypeptide α-chains intertwined and stabilized by hydrogen bonding and hydrophobic interactions. The destabilization is produced by breaking hydrogen and covalent bonds as a result of the heat treatment, resulting in helix-to-coil transition and subsequent conversion into soluble gelatin. Previously, the insoluble native collagen must be pre-treated to break non-covalent bonds so as to disorganize the protein structure, thus producing adequate swelling and collagen solubilisation, suitable for extraction [9].

Gelatin is a heterogeneous polypeptide mixture of α-chains (one polymer/single chain), β-chains (two α-chains covalently crosslinked) and γ-chains (three covalently crosslinked α-chains). Figure 1 shows a typical amino acid composition of gelatin: Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-Pro--; with an elemental composition of 50.5% carbon, 25.2% oxygen, 17% nitrogen and 6.8% hydrogen [11].

Depending on the processing method, gelatin can be classified into two types: (1) type A: with an isoelectronic point at pH ~8–9, obtained from acid treated collagen; and (2) type B: with an isoelectronic point at pH ~4–5, derived from an alkali treated precursor which converts asparagine and glutamine residues into their respective acids, resulting in higher viscosity. Gelatin derived from pig skin is normally referred as type A and that derived from beef skin or pig cattle hides and bones is referred as type B [8].
Basic physico-chemical properties, such as solubility, composition parameters, colour, transparency, odourless and tasteless, are the main attributes that best define the overall commercial quality of gelatin. Also, gel strength (expressed in the normalized “bloom value”) and viscosity should be considered as the most important physical properties since they influence gelatin quality and potential applications. In addition, gelatin can be produced in powdered or granulated form.

Gelatin has also distinctive functional properties that can be divided into two groups: (i) properties associated to surface behaviour such as protective colloid function, emulsion and foam formation and stabilization, adhesion and cohesion and film-forming capacity and (ii) properties related to gelling behaviour like gel formation, thickening, texturizing and water binding capacity [12]. Therefore, a wide number of final applications and uses can be obtained, in food, packaging, pharmaceutical, cosmetic and photographic industries (Figure 2) [13]. In particular, gelatin is used to provide gelling, stabilization, texturization and emulsification for bakery, beverages, confectionary and dairy products in food industry [10]. However, the limited thermal stability and mechanical properties of gelatin especially during processing, limit its potential applications.

Film-forming properties have been extensively used to protect food during its shelf life, as an outer film, from dryness, exhibition to light and/or exposure to oxygen. Due to the highly hygroscopic nature of gelatin, it has a tendency to swell or be dissolved when putting in contact with the surface of foodstuffs with high moisture content. Several research studies have been conducted to evaluate the overall effect of the addition of different substances, such as crosslinkers, strengthening agents, plasticizers or additives with antimicrobial or antioxidant properties, in gelatin-based products to improve the functional properties of gelatin and the shelf-life of food products [14,15]. The improvement in these properties occurs when intermolecular forces of protein chains are reduced by the action of molecular structures modifying their hydrophilic character or promoting the formation of strong covalent bonds in the protein network of the film [10]. Zhao et al. demonstrated the viability of using a natural extract as a new natural crosslinker for the modification of gelatin (type B, from bovine bone) by hydrogen bonding formation between water and free hydroxyl groups of amino or polyphenol groups. The results showed that the incorporation of this extract into gelatin significantly increased gel strength compared to the untreated gelatin [16]. The combination of gelatin with other biopolymers with different characteristics, such as whey proteins [17], starch [2], chitosan [18–20] or pectin [21], could be a good strategy for the development of films with improved mechanical and water resistance properties.

![Representative gelatin structure according to its typical amino acid composition (adapted from N. Hanani et al. [11]).](image-url)
The film properties of gelatin are also determined by the gelatin used, since the molecular weight distribution and amino acid composition vary greatly between gelatins obtained from different sources. In this sense, fish gelatin extracted at high temperature exhibits lower molecular weight profiles that gelatin from extraction at lower temperatures [10]. Muyonga et al. used sodium dodecyl sulphate gel electrophoresis (SDS–PAGE) to determine the molecular weight distribution of Nile perch gelatins as function of \( \alpha \) and \( \beta \) chains, concluding that it could vary with the collagenous tissue used as raw material at high and low temperatures. Indeed, when gelatins from the same raw materials were compared, they found more peptides (molecular weight less than \( \alpha \) chain) and lower proportion of high molecular weight (greater than \( \beta \)) fractions at high temperatures compared to those found at low temperature extractions [22]. Some authors have reported that these parameters also play a key role in the mechanical and barrier properties of the resulting films [23]. A recent study compared films made from fish gelatin derived from the bones of red snapper and grouper, generated as wastes by the fish processing industries, and mammalian gelatin showing fish gelatin films 17%–21% lower tensile strength than mammalian gelatin films [24].

In recent years, the interest in agro-industrial by-products has gradually increased. The revalorization of these materials is an upward trend, being considered as a potential source of resources for exploration, rather than as disposable waste. For example, the use of residues from gelatin capsules generated by the nutraceutical field is increasing despite the treatment and disposal of this residue imply economic and environmental issues [25]. Thus, the residues coming from gelatin, mainly used as encapsulating materials to deliver bioactive food compounds with active principles, can be revalorized as a potential source for the development of biodegradable films mainly composed of gelatin, glycerin and water [26,27].

Gelatin can be considered as a competitive alternative biopolymer in the market, being its use directly correlated to novel technological developments in order to improve its functional properties.

### 2. Gelatin-Based Films and Coatings for Food Packaging

Gelatin-based edible films and coatings have already been proposed to protect, maintain or extend the shelf-life of food products. Factors that should be considered when designing this type of system include the chemical nature of food, controlled release mechanisms, food organoleptic characteristics and additive toxicity, storage and distribution, physical and mechanical properties of packaging materials and regulations to be applied in this framework [14]. Consequently, different types of additives could be added to improve or modify the final properties in order to achieve suitable gelatin-based films or coatings for food packaging.

Recent studies have focused on interesting techniques to develop active packaging films and coatings, including antimicrobial, antioxidant and other agents which can enhance the biological features of food [8,14]. These components are usually essential oils or extracts obtained from plants and spices which exhibit antimicrobial and antioxidant properties, and most of them are considered...
to be Generally Recognized as Safe (GRAS) [28]. In order to reduce the use of synthetic chemical additives in the food industry, the use of natural food additives with antimicrobial and/or antioxidant properties without negative effects on human health has increased in the last years. These natural additives are able to prevent or reduce the deterioration of food caused by oxidation or microbiological effects, thus helping to preserve and extend food shelf-life [29].

2.1. Antimicrobial Agents

The use of antimicrobial additives in gelatin-based films or coatings for food packaging applications is a promising area, with the main goal being the prolongation of food shelf-life based on retarding deterioration mechanisms inside the package by using natural additives. A wide range of agents with antimicrobial properties has been proposed, e.g., organic acids, bacteriocins, spice extracts, thiosulphates, enzymes, proteins, isothiocyanates, antibiotics, fungicides, chelating agents, parabens and metals [30]. The research in this field is focused on the search for natural compounds to be used in active packaging formulations as substitutions for synthetic additives. As a result, many studies have been performed to propose the use of compounds obtained from natural sources with antimicrobial characteristics (Table 1). These additives can be obtained from different sources, including plants, animals, bacteria, algae, fungi and by-products generated during fruit and vegetable processing.

Essential oils have been extensively used in edible films. Martucci et al. developed gelatin-based films by using lavender or oregano essential oils and a mixture of them (50:50) at concentrations ranging between 0 and 6000 ppm. *Escherichia coli* (*E. coli*) as Gram-negative and *Staphylococcus aureus* (*S. aureus*) as Gram-positive bacteria were selected to evaluate the antimicrobial activity of the new films. The results showed that both microorganisms exhibited sensitivity to all active films, showing lower values of inhibition zone for *S. aureus* compared to *E. coli*, being 10.6 ± 1.5 mm and 13.7 ± 0.5 mm, respectively, at 4000 ppm [31]. Similar results were obtained by Alparslan et al. when studying the antimicrobial activity of gelatin-based films with orange leaf essential oil against five food-borne bacteria by the agar well-diffusion method. The gelatin film including 2% essential oil showed the highest antimicrobial effect against all microorganisms with inhibition zones of 14.5 ± 0.7 mm for *S. aureus* and 19.0 ± 1.4 mm for *E. coli* [32]. Antibacterial activity of fish skin gelatin films incorporating peppermint and citronella oils at 10%, 20% and 30% were studied by Yanwong et al., obtaining a growth inhibition of *E. coli* and *S. aureus* higher than 80% at 10% loading of each oil [33]. The obtained differences between Gram-negative and Gram-positive bacteria might be due to the presence of a thin peptidoglycan layer in Gram-negative bacteria that makes them more resistant against essential oils [34].

Regarding the mechanisms of action of this type of antimicrobial agent against bacteria, they have not been clearly detailed, since each compound present in the essential oil composition exhibits a unique mechanism of action that is specific to a particular range of food and microorganisms [35]. Different mechanisms have been identified: damage to the cell wall, interaction with and disruption of the cytoplasmic membrane, damage of membrane’s proteins, leakage of cellular components, coagulation of cytoplasm and depletion of the proton motive force. All these effects produce microorganism death by the modification of the structure and composition of the bacteria cells [34].

Another important feature related to the use of this type of additive in food packaging systems is their poor stability at high temperatures and the need to control their release into the food sample over time. In fact, the release rate is a key parameter to allow for good and suitable microbial inhibition. Recent works have reported the use of alternative techniques for the incorporation of these additives into gelatin by using micro- or nano-encapsulations with the purpose of improving and controlling their release rate. Wu et al. developed fish gelatin films incorporated with cinnamon essential oil nanoliposomes. An evaluation of the antimicrobial stability of the films by using the disc diffusion method in the third and thirtieth days of storage was carried out. The results showed a higher inhibition zone for the obtained film with cinnamon essential oil nanoliposomes compared to that of gelatin with cinnamon essential oil, demonstrating an improvement in antimicrobial stability along with a decrease in release rate after storage for one month [36].
Table 1. Different compounds used as active additives in gelatin-based films and coatings.

<table>
<thead>
<tr>
<th>Gelatin</th>
<th>Active Additive</th>
<th>Application</th>
<th>Main Benefits</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish gelatin</td>
<td>Origanum vulgare L. essential oil</td>
<td>Films</td>
<td>Enhancement in WVP, solubility, barrier capability to ultraviolet light</td>
<td>[37]</td>
</tr>
<tr>
<td>Fish gelatin</td>
<td>Nanoencapsulated Origanum vulgare L. essential oil</td>
<td>Films</td>
<td>Maintenance of initial thermal stability, Less resistant and more flexible films</td>
<td>[38]</td>
</tr>
<tr>
<td>Bovine gelatin</td>
<td>Bacteriocins and flavonoid ester prunin laurate</td>
<td>Films</td>
<td>Maintenance of functional properties, Enhancement in antimicrobial properties</td>
<td>[39]</td>
</tr>
<tr>
<td>Gelatin</td>
<td>Silver nanoparticles</td>
<td>Films</td>
<td>Enhancement in hydrophobicity, water vapour and UV barrier</td>
<td>[40,41]</td>
</tr>
<tr>
<td>Gelatin</td>
<td>Zinc oxide nanoparticles</td>
<td>Films</td>
<td>Crystalline structure</td>
<td>[42]</td>
</tr>
<tr>
<td>Skate skin gelatin</td>
<td>Thyme essential oil</td>
<td>Chicken tenderloin (wrap)</td>
<td>Extend shelf-life of chicken tenderloin, Increase in elongation at break</td>
<td>[43]</td>
</tr>
<tr>
<td>Grouper bone gelatin</td>
<td>Chitosan, clove and pepper essential oils</td>
<td>Fish steaks (coating)</td>
<td>Enhancement in antimicrobial properties</td>
<td>[44]</td>
</tr>
<tr>
<td>Fish gelatin</td>
<td>Cinnamon essential oil nanoliposomes</td>
<td>Films</td>
<td>Decrease in tensile strength, water soluble, water content and WVP</td>
<td>[36]</td>
</tr>
<tr>
<td>Fish skin gelatin</td>
<td>Peppermint and citronella essential oils</td>
<td>Films</td>
<td>Enhancement in antimicrobial properties</td>
<td>[33]</td>
</tr>
<tr>
<td>Fish gelatin</td>
<td>Green tea, grape seed, ginger or gingko leaf</td>
<td>Films</td>
<td>Enhancement in antioxidant properties</td>
<td>[45]</td>
</tr>
<tr>
<td>Bovine gelatin</td>
<td>Brown seaweed Ascophyllum nodosum</td>
<td>Films</td>
<td>Increase in hydrophilicity, Enhancement in antioxidant properties</td>
<td>[46]</td>
</tr>
<tr>
<td>Residues of gelatin capsules</td>
<td>Beet root residue powder</td>
<td>Films</td>
<td>Enhancement in antioxidant properties, Maintenance of initial thermal stability</td>
<td>[47]</td>
</tr>
<tr>
<td>Bovine gelatin</td>
<td>Carrot residue fibre derived from minimally processed carrots</td>
<td>Films</td>
<td>High barrier, optical and thermal properties, Capacity for protecting sunflower oil from primary rancidity reactions</td>
<td>[27]</td>
</tr>
<tr>
<td>Pork gelatin</td>
<td>Ethanolic hop extract</td>
<td>Films</td>
<td>Enhancement in antioxidant properties</td>
<td>[48]</td>
</tr>
<tr>
<td>Gelatin</td>
<td>Free/encapsulated tea polyphenols</td>
<td>Sunflower oil (coating)</td>
<td>No significant differences in visual aspect, Enhancement in antioxidant properties, Good oxidation inhibitory effect over 6 weeks of storage</td>
<td>[49]</td>
</tr>
<tr>
<td>Gelatin</td>
<td>Tea polyphenols</td>
<td>Films</td>
<td>Enhancement in antioxidant properties</td>
<td>[50]</td>
</tr>
<tr>
<td>Pig skin gelatin</td>
<td>Hydrolysable chestnut tannin</td>
<td>Films</td>
<td>Enhancement in antimicrobial and antioxidant properties</td>
<td>[51]</td>
</tr>
<tr>
<td>Beef gelatin</td>
<td>Articoat DLP 02, Artemix Consa 152/NL, Auranta FV and sodium octanoate</td>
<td>Films</td>
<td>Enhancement in antimicrobial and antioxidant properties at different degrees</td>
<td>[52]</td>
</tr>
<tr>
<td>Food grade gelatin</td>
<td>Orange leaf essential oil</td>
<td>Shrimps (coating)</td>
<td>Shelf-life extension, Enhancement in antimicrobial and antioxidant properties</td>
<td>[32]</td>
</tr>
<tr>
<td>Bovine hide gelatin</td>
<td>Oregano and lavender essential oils</td>
<td>Films</td>
<td>Enhancement in antimicrobial and antioxidant properties</td>
<td>[31]</td>
</tr>
</tbody>
</table>

Metallic nanofillers have recently been considered in packaging technologies for the production of active gelatin-based films with potential antimicrobial effects since these additives are able not only to enhance barrier and mechanical properties when they are incorporated into the matrix,
but also to improve food preservation and shelf-life through their antimicrobial performance [40,53]. Silver and zinc oxide nanoparticles are examples proposed by some authors in different studies. P. Kanmani et al. introduced different amounts of silver nanoparticles (0, 10, 20, 30, and 40 mg) into gelatin using a solution casting method. The antibacterial activities of films were evaluated using the agar well diffusion and colony count methods at time intervals of 2 h during 12 h by using *E. coli*, *Listeria monocytogenes* (*L.* Monocytogenes), *Salmonella typhimurium*, *S. aureus* and *Bacillus cereus*. The results showed that all films significantly decreased the cell viability of food-borne bacteria except the control and gelatin films with a lower amount of silver nanoparticles (10 mg), where no inhibition was observed. The film containing 40 mg of silver nanoparticles solution exhibited excellent antimicrobial effects against bacteria with values lower than $10^1$ CFU/mL compared to the other films [40]. The antimicrobial mechanism suggested by several authors is supported by the morphological and structural changes found in the bacterial cells and the possibilities for silver nanoparticles to penetrate inside the bacterial structure due to their attachment to the cell membrane [54]. In line with this study, Shankar et al. prepared composite films based on gelatin incorporated with four different types of zinc oxide nanoparticles obtaining strong antibacterial activity against both Gram-positive and Gram-negative bacteria, *L. monocytogenes* and *E. coli*, respectively, for films with nanoparticles, with values of cell viability lower than $10^2$ CFU/mL after 12 h of study. These results could be related to the release of Zn$^{2+}$ ions, which could penetrate through the cell wall of bacteria and react with the cytoplasmic content, leading to microorganism death [42].

2.2. Antioxidant Agents

Nowadays, research in the field of active packaging is also focused on the development of novel food packaging materials with antioxidant agents from natural sources such as plant and spices extracts instead of synthetic antioxidants such as butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA), since synthetic antioxidants are suspected of causing some safety concerns and have been restricted in their use as food additives [55]. In this context, some studies have reported that natural antioxidants show enough capacity to control lipid oxidation inside the food package since oxidative processes can cause the degradation of proteins, pigments and lipids, which limits food shelf-life [28,56,57]. Table 1 summarizes some research studies performed to enhance the final properties and applicability of food packaging and to extend the shelf-life of food products based on gelatin films and coatings incorporated with antioxidant additives.

Extracts obtained from green tea, grape seed, ginger or gingko leaf have been studied for their excellent antioxidant properties due to the presence of some compounds in their compositions, such as polyphenolic compounds in the case of green tea extract; flavones glycosides in ginkgo leaf extract; gingerol, gingerdiol, gingerdione and other antioxidant compounds for ginger extract; or tannins and monomeric flavonoids such as catechin and epicatechin for grape seed extracts. Li et al. incorporated natural extracts into fish skin gelatin at three different concentrations, 0.01, 1.0 and 5.0 mg/mL, by using the casting technique. In this work, physical and mechanical properties of films were studied and antioxidant activity was evaluated by using three commonly methods: DPPH radical scavenging assay, reducing power and peroxide value analysis. Results showed the strongest scavenging activity against DPPH radicals (around 90%) for the formulations with 1.0 mg/mL of each extract used except for the ginger one, whose value was around 17%. In a similar way, films mixed with natural antioxidants had high absorbance values, indicating an increase in the reducing powder compared to the control except for the film added with ginger. The obtained antioxidant capacity was mainly determined by the phenolic compounds present in the extract composition. As an example, regarding green tea and grape seed extracts, the amount of epicatechin, caffeic acid and catechin was relatively high. In addition, films mixed with these extracts had greater reducing power compared to the control except for the film with ginger extract. Authors also reported a reduction of around 30% in water vapour permeability (WVP) for gelatin-based films incorporated with green tea extract. This fact can be attributed to the presence of polyphenols which could be able to form hydrogen and covalent bonds with the polar groups of polypeptide in gelatin modifying the structure [45].
Edible gelatin coating solutions enriched with orange leaf essential oil obtained from orange (Citrus sinensis (L.) Osbeck) leaves were used as a coating for shrimp. The antioxidant activity evaluation of the obtained film forming solutions showed an optimum DPPH scavenging activity around 52% with 2% of essential oil. In addition, in this study, it was demonstrated that the coating improved the quality of shrimp during the storage period in terms of chemical indices determined in shrimp meat, preserving shrimp quality during cold storage with a shelf-life extension of 10 days [32].

The mechanisms of action of these natural antioxidants in contact with food are related to lipid oxidation reactions. In addition, they are focused on phenols and other compounds with hydroxyl groups present in the essential oils composition. Hydrogen atoms from phenol hydroxyl groups could react with peroxyl radicals produced in the early stages of the oxidation mechanisms to yield stable phenoxy radicals and, consequently, resulting in the termination of the lipid peroxidation chain reactions. However, understanding the antioxidant activity mechanisms of these phenolic compounds is a hard task since this activity depends on the electronic and steric effects of their ring substituents, the strength of hydrogen-bonding interactions between the phenol and the solvent in the essential oil, and the interactions with matrix and food [56].

In order to protect additives from temperature or light, encapsulation is a promising technique that can be used during film processing [58,59]. Liu et al. investigated the applicability of gelatin-based films packaged with sunflower oil with different free/encapsulated tea polyphenol ratios through the synthesis of chitosan nanoparticles at 3 different encapsulation efficiencies (50%, 80% and 100%). The results showed a reduction in the oxidation of sunflower oil obtaining lower peroxide (PV) and thiobarbituric acid reactive substance (TBARS) values for oils exposed to the new films. In addition, an improvement in antioxidant activity when using an optimum partition of free and encapsulated (20:80, respectively) additives was demonstrated over a long period of storage (6 weeks) as well as the preservation of the functional properties of the new films [49].

2.3. Other Agents

As it has been mentioned before, as a consequence of gelatin’s highly hygroscopic nature, it tends to swell or dissolve easily in contact with food despite its good barrier properties to oxygen and carbon dioxide [11]. Also, gelatin films show lower mechanical strength compared to synthetic ones [60]. To avoid these drawbacks, gelatin can be blended with different substances and/or polymers to obtain bio-composite films and coatings that combine the advantages of each component [11].

Hydrophobic substances such as lipids and oils have been used to improve the water vapour barrier properties of gelatin films. Limpisophon et al. [61] introduced stearic and oleic fatty acids into edible films based on blue shark skin gelatin by the casting technique. Stearic and oleic acid content in film solution were 25%, 50% and 100% (w/w) of the protein content. As stearic acid content increased, a reduction in WVP from 1.04 ± 0.09 to 0.70 ± 0.06 × 10\(^{-10}\) g m\(^{-1}\) Pa\(^{-1}\) s\(^{-1}\) was reported, which was higher than that obtained for oleic acid at the same concentration (from 1.02 ± 0.06 to 0.91 ± 0.06 × 10\(^{-10}\) g m\(^{-1}\) Pa\(^{-1}\) s\(^{-1}\)). Tongnuanchan et al. recently studied the physical, barrier, structural and thermal properties of fish skin gelatin films containing palm oil at 25%, 50%, 75% and 100% (based on protein) showing a reduction in WVP of 35.83%, 53.54%, 56.30% and 72.52%, respectively [62]. In other study, Bertan et al. evaluated the incorporation of Brazilian elemi oil (1%, 2.5%, 5%, 10%, 15% and 20%, w/w of dry gelatin) into bovine hide type A gelatin to obtain films by the casting technique, using a blend of palmitic and stearic acids (1:1 stearic/palmitic acid, 10%, w/w of dry gelatin) [63]. As a result, the addition of 10% elemi oil reduced WVP by about 57% compared to the film containing only plasticizer and the fatty acid blend. Similarly, Ma et al. developed composite films from bovine hide gelatin type B with olive oil (olive oil/ protein weight ratios of 5%, 10%, 15% and 20%) by the microfluidic emulsification technique [64]. A decrease in WVP from 5.610 ± 0.068 to 4.194 ± 0.044 × 10\(^{-10}\) g m\(^{-1}\) Pa\(^{-1}\) s\(^{-1}\) when 20% of oil was incorporated was obtained. Xiao et al. reported the development of new bio-films by the casting technique based on the addition of palm oil at different degrees (8°, 18°, 24°, 33° and 44°) with a significant reduction in WVP at 36 wt % of gelatin content compared to the control gelatin film [65]. In particular, the lowest WVP value was achieved.
for the film containing the palm oil with $24^\circ$ (2.19 ± 0.07 × 10$^{-11}$ g·m$^{-1}$·s$^{-1}$·Pa$^{-1}$) in contrast to the control (1.55 ± 0.05 × 10$^{-11}$ g·m$^{-1}$·s$^{-1}$·Pa$^{-1}$). Finally, Nilsuwan et al. investigated the influence of palm oil concentration (250, 500, 750 and 1000 g·kg$^{-1}$ protein) and soy lecithin surfactant (500 g·kg$^{-1}$ palm oil) on the stability of film-forming dispersion and properties of fish tilapia skin gelatin films obtained by the casting technique [66]. In general, films showed an improvement in WVP properties with increasing palm oil concentration, obtaining values of WVP of 24.52 ± 0.51 for the control and 6.37 ± 0.30 × 10$^{-11}$ g·m$^{-1}$·s$^{-1}$·Pa$^{-1}$ for 1000 g·kg$^{-1}$ for the films added with palm oil. As a conclusion, from these studies it could be suggested that oils added into a gelatin matrix could act as hydrophobic and nonpolar substances, increasing hydrophobicity with a decrease in the permeation of moisture through the films [65].

Oils have also been used to enhance the structural and mechanical properties of gelatin. Tongnuanchan et al. developed new bio-films based on fish gelatin obtained from tilapia skin and 25% (w/w) of basil and citronella essential oils at a ratio of 1:1 (w/w) by the casting technique [67]. In general, higher opaqueness, lower $T_d$ and thermal degradation temperatures were reported with essential oils incorporation. Wang et al. evaluated the effect of pH and corn oil addition on the mechanical properties of porcine skin gelatin films [68], obtaining an optimum film-forming solution with 55.18% of corn oil with a pH of 10.54 and a predictive value of tensile strength of 17.58 MPa, elongation at break of 305.90% and WVP of 44.21 g mm kPa$^{-1}$·d$^{-1}$·m$^{-2}$. These results suggest the interruption of protein–protein interactions with an increase in chain mobility in gelatin. A simplified illustration of film matrix interactions with and without oil incorporation after the casting technique is showed in Figure 3. When oil is incorporated into the gelatin matrix, the protein–protein interactions by hydrogen bonds are reduced and a different orientation of the gelatine matrix takes place. Then, two different phases coexist in the matrix, the hydrophobic phase of the oil and the hydrophilic phase, characteristic of the protein which is stabilized by hydrogen and hydrophobic interactions among them.

![Figure 3. Scheme of gelatin matrix interactions with and without oil incorporation.](image)

Regarding polymer blends, shellac, a special natural polymer obtained from purified resinous secretion of lac insects, *Laccifer Lacca*, has been widely studied as an edible film coating. Puncture strength and percentage elongation of composite films based on shellac (6% w/w) and gelatin type A (10%, 20%, 30%, 40% and 50% w/w into the shellac solution), obtained by the casting technique, increased from 3.61 to 15.58 MPa and from 3.80% to 32.47% as the gelatin concentration increased to 50% w/w, respectively, indicating an enhancement in strength and flexibility of the shellac film [69]. Regarding other blends, sago starch and fish gelatin at different ratios (1:0, 2:1, 3:1, 4:1, and 5:1) plasticized with glycerol or sorbitol (25%, w/w) were developed by the casting technique [70]. In this study, fish gelatin was extracted from fish waste provided by a local surimi processing plant.
By varying the ratio of the two polymers, the strength and extensibility of the composite films can be modified. Starch/gelatin solutions at 4:1 ratio formed good flexible films with tensile strength values of $9.87 \pm 0.64$ MPa for the control film containing only gelatin matrix compared to $18.06 \pm 0.55$ MPa for the 4:1 film. Also, elongation at break and Young modulus decreased from $17.11\% \pm 6.11\%$ and $6.17 \pm 0.01$ N·m$^{-2} \times 10^7$ for the control sample to $5.53\% \pm 0.42\%$ and $1.71 \pm 0.05$ N·m$^{-2} \times 10^7$ for the 4:1 film, respectively. In addition, Tao et al. studied the effect of pH (3, 7 and 10) on the physical properties of surimi-gelatin composite films at different blending ratios (10:0, 8:2, 6:4, 5:5, 4:6, 2:8 and 0:10) by the casting technique [71]. The composite films of surimi and gelatin could be formed irrespective of pH, and they became stronger under acidic or alkaline conditions. In general, as a higher content of gelatin was used in the blend, higher values of tensile strength and lower elongation at break were obtained.

Other biopolymers have been blended with gelatin with an improvement in gelatin film properties such as chitosan [72,73], lignin [74], lignosulphonates isolated from spent sulphite liquors [75] or fish protein isolate [76].

Nowadays, agricultural by-products are normally incinerated or dumped, causing environmental problems such as air pollution, soil erosion and decreasing soil biological activity [77]. The incorporation of agricultural residues into polymer matrices is currently a trending topic in research due to the relatively high strength, stiffness and low density of natural fibres present in these residues [78–80]. Coconut husk, the fibrous external portion of the fruit of coconut palms, is a by-product of the copra extraction process and is generally considered waste [81]. To revalorize this by-product, the effect of Ethanolic extracts from coconut husks (0%–0.4% w/w, on protein basis) on properties of tilapia skin gelatin films obtained by casting were reported [82]. Gelatin film with 0.05% of Ethanolic extract from coconut husk showed an improvement in mechanical properties with Young modulus, tensile strength and elongation at break of 1048.03 $\pm$ 31.40 MPa, 41.93 $\pm$ 0.49 MPa and 7.90 $\pm$ 0.03% for the control sample compared to 1129.63 $\pm$ 25.58 MPa, 43.65 $\pm$ 0.68 MPa and 7.63 $\pm$ 0.01% for the formulation with 0.05%. These positive results could be explained due to higher interactions between functional groups of gelatin and phenolic compounds.

Soy protein isolate is an abundant, inexpensive, biodegradable, and nutritious raw material, whereas microcrystalline cellulose is a commercially available material prepared by acid hydrolysis of wood fiber, back-neutralization with alkali, and spray-drying [83]. The effect of these two compounds in a gelatin matrix was studied after casting preparation of films. In particular, microcrystalline cellulose content of 2.5% in soy protein isolate and gelatin matrix significantly improved mechanical and barrier to water properties with values of Young modulus of 45.32 $\pm$ 3.28 MPa for the control to 107.35 $\pm$ 6.13 MPa for the formulation at 2.5%. Also, moisture content decreased from 20.28% $\pm$ 9.07% for control to 16.81% $\pm$ 8.75% for the 2.5% film.

In recent years, innovative food packaging technologies using biopolymer-based nanocomposites have emerged in response to increasing global waste disposal problems caused by non-biodegradable petroleum-based plastic packaging materials [84]. In this context, chitosan nanoparticles have been used as reinforcement agents in gelatin matrix [85]. The application of chitosan nanoparticles synthesized by the ionic cross-linking method modified the crystalline structure of gelatin mainly due to the nucleating effect of nanoparticles as detected by X-ray diffraction assays. This addition also decreased the $T_g$ of gelatin increasing chains mobility. Finally, the thermal stability of the nanocomposite films increased up to 7 $^\circ$C for the onset degradation temperature. Other authors reported a remarkable increase in tensile strength caused by the addition of chitosan nanoparticles from 7.44 $\pm$ 0.17 MPa for the control sample to 11.28 $\pm$ 1.02 MPa for films with a chitosan nanoparticles content of 8% [86]. Also, an increase in elastic modulus with additive content from 287.03 $\pm$ 14.25 MPa for control to 467.2 $\pm$ 49.63 MPa for the addition of 8% of the additive was reported.

Recent trends in the use of nanoclays as reinforcement agents in gelatin have been reported in the literature, such as montmorillonites [87–89] and laponites [90]. However, there is still some controversy regarding the current legislation about the use of these compounds for food packaging applications despite the fact that the research in this area is becoming more and more relevant.
For food packaging applications, it is necessary to maintain the gelatin structure to guarantee the film stability under humid conditions. Up to now, glutaraldehyde has been used as a crosslinker agent, but it has one great disadvantage, since it is a systemic and cell toxic compound [4]. To avoid the use of this substance, Biscarat et al. developed alternative matrices based on gelatin type A with three different crosslinkers: N-hydroxysuccinimide, Bis(succinimidyl)nona(ethylene glycol) and ferulic acid to prepare films by dry-casting [4]. Among them, the use of ferulic acid allowed flexible films to be obtained without using toxic agents. Also, it was shown that gelatin films with ferulic acid supported humid conditions (98% RH at 20 °C) for 15 days without breaking, making them a promising, environmentally friendly packaging system for food applications.

Other agents have been used in gelatin matrices to develop innovative food packaging technologies that include smart materials to extend the safety and quality of food products during their shelf-life. As an example, Musso et al. developed bio-films by casting based on bovine gelatin, glycerol and three acid-base indicators (methyl orange, neutral red and bromocresol green) capable of sense pH changes [91]. Results showed that colour was reversibly modified in all samples when they were put in contact with liquid, semisolid and gaseous media at different pHs, making them an environmentally friendly alternative to replace synthetic indicators.

3. Edible Film and Coating Applications

As has been mentioned in the present review, gelatin has several advantages when used as edible film and coating in different food products. The present section is focused on current applications of gelatin in food packaging reported in recent years (Table 2).

| Table 2. Different gelatin matrices used in edible films and coatings for meat and fishery products. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Meat products**               |                 |                 |                 |                 |                 |
| Beef steaks                     | Bovine gelatin type B mixed with chitosan | Dipping into matrix solution | Coating | [92]           |
| Pork sausages                   | Gelatin, pectin and sodium alginate blends | Extrusion film |         |                 |
| Pork loin                       | Porcine gelatin  | Dipping into gelatin matrix | Coating | [94]           |
| Kabanosy dry sausages           | Pork gelatin, kappa-carrageenan and glycerol | Dipping into matrix solution | Coating | [95]           |
| Chicken tenderloin              | Skate skin gelatin with thyme essential oil | Casting film |         |                 |
| Raw beef                        | Gelatin, Tween 80 and essential oils of *Thymus vulgaris* and *Rosmarinus officinalis* | Dipping into matrix solution | Coating | [97]           |
| Turkey bologna                  | Gelatin, glycerol and Nisaplin and Guardian CS1-50 antimicrobial additives | Casting film |         | [98]           |
| Bacon                           | Gelatin         | Casting film |         | [99]           |
| Rainbow trout                   | Cold water fish skin, chitosan and glycerol | Casting and dipping into matrix solution | Film and coating | [100]         |
| Cod fillets                     | Bovine hide gelatin, chitosan, sorbitol and glycerol with clove essential oil | Casting film |         | [99]           |
| Minced trout fillets            | Cold water fish skin gelatin, chitosan, glycerol, red grape seed extract and *Ziziphora linopodioides* essential oil | Casting film |         | [101]         |
| Rainbow trout fillets           | Food grade gelatin, glycerol, sorbitol, Tween 20 and laurel leaf essential oil | Casting film |         | [102]         |
| Tuna meat                       | Gelatin, red pepper seed meal protein and several plasticizers (glycerol, sorbitol, fructose and sucrose) | Casting film |         | [96]           |
| Fish sausages                   | Warm-water fish gelatin, chitosan, shrimp concentrate, Tween 80 and glycerol | Casting film |         | [19]           |
| Atlantic Salmon                 | Warm-water fish gelatin, lignin, sorbitol and glycerol | Casting film |         | [103]         |
| Salmon                          | Porcine skin gelatin, barley bran protein, sorbitol and grapefruit seed extract | Casting film |         | [104]         |
| Cold smoked Salmon              | Pork gelatin, chicken feather protein, sorbitol and clove oil | Casting film |         | [105]         |
| Shrimps                         | Gelatin, glycerol, sorbitol, Tween 20 and orange leaf extract | Dipping into matrix solution | Coating | [32]           |
3.1. Meat Products

Regarding meat products, gelatin has been used blended with chitosan as a coating to reduce colour deterioration from red to brown as a consequence of a gradual accumulation of metmyoglobin in the meat’s surface, mainly due to oxygen exposition and lipid oxidation of beef steaks [92]. The gelatin-chitosan coating successfully maintained the organoleptic properties of beef steaks during 5 days of retail display increasing their shelf-life. In other work, the potential use of blend films from pectin, gelatin and sodium alginate for breakfast pork sausages was reported [93].

According to Davis and Lin [106], approximately 50% of the worldwide daily protein intake is from pork. The application of a gelatin coating (0%, 10% and 20%) led to an improvement in preserving the quality and shelf-life of refrigerated pork meat during a storage period of 7 days. No significant colour changes due to the retard of metamyoglobin formation and lipid oxidation were observed, underlying the potential of gelatin as a bio-based material to be used as a coating to extend the shelf-life of meat products. Similarly, Tyburcy and Kozyra studied the effect of coating dry sausages with pork gelatin, kappa-carrageenan and glycerol as an alternative to vacuum packaging to avoid weight loss which was directly related to profit loss [95]. As a result, coating meat reduced its weight loss and, therefore, financial benefits could be achieved by the application of this type of packaging. However, according to the authors, more studies are necessary to reduce coating thickness.

Gelatin extracted from natural sources such as skate skin was used with thyme essential oil to prepare antimicrobial edible films for chicken tenderloin packaging [96]. The film containing 1% thyme oil reduced the population of \textit{L. monocytogenes} and \textit{E. coli} on chicken tenderloin during storage. The contamination of meat products with \textit{L. monocytogenes} has been considered a serious public health problem [107]. Oliveira et al. studied the antimicrobial effect of the addition of \textit{Thymus vulgaris} and \textit{Rosmarinus officinalis} essential oils to gelatin solution in raw bovine meat pieces [97]. The effectiveness and viability of this coating were proven with a reduction in \textit{L. monocytogenes} proliferation accompanied by acceptable sensory properties of the packaged meat. The use of antimicrobial coatings based on gelatin to reduce \textit{L. monocytogenes} growth was also reported in other ready-to-eat poultry meats such as turkey bologna [98]. In this case, two commercial antimicrobial agents were added (Nisaplin and Guardian CS1-50) into gelatin to obtain films using glycerol as a plasticizer by the casting technique. The incorporation of the antimicrobial additives reduced the tensile strength of films whereas increased the elongation at break. Despite these structural changes, active films effectively inhibited \textit{L. monocytogenes} on bologna at 4°C up to 8 weeks.

The development of edible films and coatings and their applications on meat food products have been subject of a great number of scientific publications during the last decade, but several patents have also been commercialized. As an example, the development of gelatin and carboxymethylcellulose films with potassium sorbate were proven to be effective in extending the shelf-life of bacon (CN 102487988B patent) [108].

3.2. Fishery Products

Fish is one of the most perishable food products mainly due to chemical reactions, enzymatic response, and microbial spoilage [109]. As a consequence of its reduced shelf-life, the freshness and quality of fish have always gained the attention of Food Regulatory Agencies and Food Processing Industries. Proper handling, pre-treatment and preservation techniques can improve the quality of fish products. Much research in this field has been focused on the development of edible films and coatings to increase fish products shelf-life maintaining their quality parameters. Coating and films based on cold water fish-skin gelatin and chitosan blends were reported for rainbow trout fillet packaging, showing antioxidant properties. However, higher protective effect against lipid oxidation was obtained for coatings compared to films, due to higher chitosan migration in solution as an active additive [99]. Gelatin and chitosan blends were also used to obtain antimicrobial films for cod fillet packaging by adding clove essential oil to the matrix, resulting in a drastic reduction in microorganism growth for gram-negative bacteria [100]. In a different study, the development of chitosan-cold water fish skin
gelatin films incorporated with grape seed extract (1% and 2%) and Ziziphora clinopodioides essential oil (1% and 2%), separately and in combination, led to a decrease of *L. monocytogenes* and shelf-life extension of minced trout fillets during refrigerated storage at 4 °C over a period of 11 days [101]. Also, trout fillets wrapped with 8% gelatin films containing laurel essential oil (0%, 0.1% and 1%, w/w) and vacuum packaged were evaluated to study the quality of fish during refrigerated storage at 4 °C over a period of 26 days [102].

In order to revalorize a form of agricultural food waste, fatty tuna meat was packaged into blend films of gelatin and red pepper seed meal isolated protein by the casting technique [96]. The results showed the potential of this material as an antimicrobial and antioxidant packaging, being the optimal formulation that containing 1% of gelatin and 4% of red pepper seed protein. As a result, tensile strength and elongation at break were improved and *L. monocytogenes* and *Salmonella Typhimurium* growths were reduced on tuna meat compared to the control. In a different work, the effect of a formulation obtained from a shrimp concentrate waste from the seafood industry, as a coating and film, on the shelf-life and characteristics of fish sausages was studied [19]. An extension of the shelf-life of fish sausages to 15 days was observed with harder texture, lower pH and greater microbiological control.

Regarding oxidative and organoleptic degradation, salmon is probably the most studied fish since it is one of the most sensitive food products [110]. In fact, various studies have applied gelatin in combination with other biopolymers or active additives such as lignin [103], and barley bran protein and grapefruit seed extract [104] to protect salmon against cooking processes. In addition, since cold-smoked salmon is generally consumed without cooking, it can cause serious health problems in consumers due to contamination with pathogenic bacteria, mainly *E. coli* and *L. monocytogenes* [111]. To avoid this problem, chicken feathers, a by-product of the poultry industry, were successfully used as a film base material after extraction of chicken feather protein in combination of gelatin and clove oil as an antioxidant and antimicrobial active agent to package smoked salmon [105].

### 3.3. Other Food Products

Aside from meat and fishery products, other food products are also susceptible to be coated or packaged into gelatin-based solutions or films. Potential applications of gelatin edible films in the food industry may include the transport of gases (O₂ and CO₂), water vapour, and flavours for fruits and vegetables [112]. As an example, refrigerated Red Crimson grapes were coated with gelatin type A, starch and glicerol films obtained by the casting technique [2]. As a result, an increase in gelatin concentration in the mixture provided an increase in thickness, WVP and mechanical resistance reducing the total weight loss without influencing consumers acceptance. Also, the incorporation of red bean powder as colorant and flavouring agent into gelatin films was studied for use in candies and brewing food as it was reported in CN 103589173A patent [113]. In another patent, gelatin and glucomannan films with garlic juice were described as antimicrobial and favouring agents (CN 103589168A patent) [114]. Other vegetables and fruits recently reported to be coated or wrapped with gelatin-based films and coatings are carrots [115], cherry tomatoes [116], calyx from physalis [117], oranges [118], banana and eggplant epicarps [119], fresh-cut melons [120], peppers [121], strawberries [122], blueberry fruit [123], pineapple fruit [124] and minimally processed persimmon [125].

Gelatin films prepared from cold-water fish show significantly lower WVP values than those from warm-water fish, due to their higher hydrophobicity directly related to lower amounts of two aminoacids, proline and hydroxyproline. As a consequence, cold-water fish gelatin films are particularly useful for applications related to reducing water loss from refrigerated or frozen food systems [126].

Residues generated by fruit and vegetable processing are well-studied sources of antioxidants, bio-polymers and dietary fibres [127]. Indeed, large amounts of oil nutraceutical capsule waste from coconut, chia, safflower and linseed, composed mainly of gelatin, are being generated with high waste
treatment costs for industry. A reduction in the oxidative rancidity of sunflower oil exposed to films developed from chia oil nutraceutical capsule wastes by the casting technique after storage at 40 °C in the presence of light for 13 days was obtained [128]. Gelatin was extracted from capsule wastes composed of gelatin (48%), water (30%) and glycerol (22%). Blueberry pomace fibre and extract wastes were used as active additives. All gelatin films retarded oil lipid oxidation during the studied period. However, gelatin films added with fibre and extract significantly reduced lipid oxidation compared to the control film without antioxidants addition after 8 days of the oxidative treatment. Similarly, the effect of films based on beet root residue powder, obtained from peels, stalks, and shavings wastes derived from the production of linseed oil nutraceutical capsules, on the retardation of sunflower oil oxidation was recently reported [47]. Sunflower oil containing no artificial antioxidants was stored for 35 days at 35 °C and 54% RH and exposed to fluorescent light with an intensity of 900–1000 lux. Peroxide values were determined at different times. Films with antioxidants had a positive effect on the stability of sunflower oil during the entire storage period and, also, at the end of the experiment, presenting the packed oil peroxide values under the recommended limit of Codex Alimentarius (10 milliequivalent per oil kilogram). As a result, biodegradable films based on residues of beet root and gelatin capsules could be a potential tool to control and retard rancidity of different oils.

4. Conclusions

The use of gelatin-based edible films and coatings represents a stimulating route for creating new food packaging materials. Due to the hygroscopic properties of gelatin, some research studies have been conducted to evaluate the overall effect of the addition of different substances such as crosslinkers, strengthening agents, plasticizers or additives with antimicrobial or antioxidant properties to gelatin-based products to improve the functional properties of gelatin-based edible films and the shelf-life of food products. An increasing number of publications have reported the development of gelatin-based films for meat applications as coatings to reduce the colour deterioration from red to brown as a consequence of lipid oxidation. Regarding fish products, different studies have been focused on the application of gelatin in combination with other biopolymers or active additives to protect fresh fish against cooking processes and microbial/oxidation deterioration. In addition to fish and meat, some other food products such as fruits and vegetables can be coated with gelatin-based films in order to retard degradation processes due to the transport of gases (O₂ and CO₂) and water vapour. Extensive research is still needed on new methods for gelatin-based film formation to improve the final properties and potential applications.

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