

Review

Binary Hydrogels: Induction Methods and Recent Application Progress as Food Matrices for Bioactive Compounds Delivery—A Bibliometric Review

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Abstract: Food hydrogels are biopolymeric materials made from food-grade biopolymers with gelling properties (proteins and polysaccharides) and a 3D network capable of incorporating large amounts of water. They have sparked considerable interest because of their potential and broad application range in the biomedical and pharmaceutical sectors. However, hydrogel research in the field of food science is still limited. This knowledge gap provides numerous opportunities for implementing their unique properties, such as high water-holding capacity, moderated texture, compatibility with other substances, cell biocompatibility, biodegradability, and high resemblance to living tissues, for the development of novel, functional food matrices. For that reason, this article includes a bibliometric analysis characterizing research trends in food protein–polysaccharide hydrogels (over the last ten years). Additionally, it characterizes the most recent developments in hydrogel induction methods and the most recent application progress of hydrogels as food matrices as carriers for the targeted delivery of bioactive compounds. Finally, this article provides a future perspective on the need to evaluate the feasibility of using plant-based proteins and polysaccharides to develop food matrices that protect nutrients, including bioactive substances, throughout processing, storage, and digestion until they reach the specific targeted area of the digestive system.

Keywords: proteins; polysaccharides; hydrogels; functional properties; delivery systems; bioactive ingredients; plant-based food; food development



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1. Introduction

Hydrogels are viscoelastic aqueous matrices composed of crosslinked polymer chains forming a three-dimensional hydrophilic network. This three-dimensional system contains molecules, fibers, or particles, with water or an aqueous phase serving as the dispersion medium [1]. The hydrophilic character of hydrogels is caused by some hydrophilic residues (such as amino, carboxyl, and hydroxyl groups) of the polymer(s), along with the nature and density of the formed network connections. Such networks can hold large amounts of water (even 99% *w/w*) in their structure while maintaining solid-like properties [2]. The type (physical or chemical) and density (number of crosslinks) of network connections formed by these polymers help to maintain the final gel network. As a result, the structure, viscoelasticity, and water-holding capacity of hydrogels are highly dependent on the polymer source (natural or synthetic), method of preparation (induction method), ionic charge, and the size of the network [3].

Hydrogel materials are widely used, with significant applications in medical, cosmetics, textiles, agriculture, and recently in the food sector as well. Because of their broad range of applicational potential, researchers have been studying hydrogels for years. The biomedical and pharmaceutical industries have primarily implemented hydrogels as delivery systems [4,5], scaffolds for cell cultivation [6], and tissue engineering [7]. However, when it comes to the food industry, the implementation of hydrogels is constrained by restrictions

on the use of certain ingredients that need to be food-grade, generally recognized as safe (GRAS) by the Food and Drug Administration (FDA) in the USA and included in the EU list of permitted food additives laid down in Regulation EC 1333/2008. According to their origin, food-grade biopolymers are divided into proteins and polysaccharides. These biopolymers have a great potential to address today's consumer health and environmental sustainability concerns since they are renewable, affordable, biocompatible, biodegradable, and edible, as well as having a wide range of functionalities and gelation routes [8]. Proteins and polysaccharides are primary functional components in developing food colloidal systems since they can create and modify food matrix structures, textures, sensory properties, and shelf life.

Protein-based hydrogels are formed when the protein molecules unfold, revealing hydrophilic and thiol groups [9]. This unfolding of the structure (denaturation) process can be initiated by heating, pH, or salt modulation. The denaturation allows the chains to interact via covalent interactions (by hydrophobic or electrostatic interactions, hydrogen bond formation, and less frequently, disulfide bond formation). The covalent interaction between the polymer chains leads to their aggregation, forming a three-dimensional gel structure [10]. Among plant-based globular proteins, such as soy [11,12], pea [10,13], wheat [14,15], and zein [16,17] are reported in the literature to have an excellent gelling ability, similar to their animal-based proteins counterparts (whey and egg) [18,19]. The formation of polysaccharide hydrogels is less complicated than that of globular proteins. Polysaccharide hydrogel formation can be induced through various methods, among others heat and cooling, pH and salt modulation, the addition of sucrose, and freeze-thaw cycles [20]. Among the widely used in the food industry polysaccharides that have gelling abilities are carrageenan [21,22], chitosan [23,24], alginate [25,26], inulin [27,28], starch [29,30], cellulose [31,32], gum arabic [33,34], gellan gum [35,36], etc.

Binary hydrogels composed of proteins and polysaccharides were developed to avoid some of the limitations such as poor water holding capacity and weak gel strength, physical instability, etc. imposed by hydrogels prepared with a single biopolymer [37]. A different combination of proteins and polysaccharides can be used to create such binary hydrogels: protein–protein, polysaccharide–polysaccharide, and protein–polysaccharide [38]. Proteins and polysaccharides can effectively form binary hydrogels due to their ability to interact with each other via non-covalent and covalent interactions [39]. Furthermore, when the concentration of one biopolymer is insufficient to form a stable hydrogel, adding another biopolymer as a filler component can improve the physicochemical properties of the system, allowing the formation of a network structure [40,41]. A wide range of protein–polysaccharide binary hydrogels with various microstructures and physicochemical properties can be obtained based on the interaction between those two biopolymers, the individual properties of each used component, and the applied induction conditions [42]. An example would be a binary hydrogel composed of a whey protein/starch mixture, distinguished by new and intriguing properties [43]. It was discovered that the synergistic interactions between casein and carrageenan also improved hydrogel's rheological and microstructural properties [44]. Zernov et al. [45] reported that mixing chitosan and collagen makes it possible to produce a hydrogel that can act as an edible microcarrier for cultured meat. Furthermore, soy protein—a model plant-based protein mixed with polysaccharides—can form binary hydrogel and gain new properties as a food ingredient [46,47]. Combinations of soy protein gels and polysaccharides tested by other researchers are as follows: soy protein–sodium alginate hydrogel [48], soy protein–carrageenan hydrogel [49], soy protein–inulin hydrogel [50], soy protein–corn fiber gum hydrogel [51]. Other plant-based proteins and polysaccharides are also being studied regarding their ability to form binary hydrogels. Among them, the most popular in the literature are pea protein–sodium alginate hydrogel [52], pea protein–soluble soybean polysaccharide hydrogel [53], and zein protein–pectin hydrogel [54].

Since studies on the topic of food hydrogels are still minimal in comparison to biomedical or pharmaceutical hydrogels, there are immense opportunities to contribute to the

development of the food industry through a cross-integration between areas with advanced knowledge. Regarding model behavior, food biopolymer hydrogels might be more complex than synthetic hydrogels [37]. Despite this, a proper hydrogel design based on a thorough understanding of the mechanisms in food matrices can improve the final food matrix's quality, nutrition, and nutrient bioavailability [41,55]. Therefore, using a bibliometric analysis as a research performance investigation tool for detailed databases can reveal trends and patterns in scientific research areas worldwide. This statistical tool has raised researchers' considerable interest in providing an in-depth view of the advancements in binary hydrogels' food processing and applications [41,56,57]. The purpose of conducting a bibliometric analysis is not to discuss the findings of the identified papers but to characterize research trends in a chosen field of knowledge [58,59]. The significance of this manuscript is to provide a mixed review that combines bibliometric analysis and a literature review of the latest developments in hydrogel induction methods and the present research findings on the topic of protein–polysaccharide hydrogels as a food matrix.

2. Methodological Procedures

The article presents a literature review emphasizing protein–polysaccharide hydrogel induction methods and the application progress of protein–polysaccharide hydrogels as food matrices to supplement the information provided by the bibliometric analysis.

In this study, a mixed methodology was carried out, including a bibliometric analysis of papers obtained from the Scopus database (<https://www.scopus.com/search/form.uri?display=advanced>, accessed on 1 December 2022) and a literature review emphasizing the induction methods and the application progress of protein–polysaccharide hydrogels as food matrices. A survey was carried out in the Scopus database (in October 2022) to access the papers used to perform the bibliometric analysis. The methodological procedure adopted for the bibliometric analysis was divided into two general phases, the data collection phase, and the data mapping/visualization phase.

The entered query string included the terms “protein polysaccharide food hydrogels”, “food biopolymer hydrogel”, and “food hydrogel” as search words in the publication's titles and abstracts. The publication timeframe was set from 2012 to 2022, and the types of documents were considered: articles and reviews. Some words were excluded (e.g., aerogels, oleogels, male, female), as well as some research areas (e.g., economics and finance, computer science, business management and accounting, mathematics, social sciences, energy, planetary sciences, neuroscience, nursing, and health professions) to refine the study. A result of 297 documents was obtained, of which 239 were articles and 58 were reviews, all in the final publication stage.

The data mapping/visualization phase was accomplished using a state-of-art scientometric mapping tool provided by VOSviewer software (version 1.6.18, CWTS, Leiden, The Netherlands). The data, including all the details regarding the 297 documents found by the search engine in the Scopus database, were exported, and a performance analysis was carried out to discover the general patterns of research on protein–polysaccharide hydrogels. A cluster analysis was carried out based on the keywords co-occurrence and the bibliographic coupling of in-country collaborations [60–64].

3. Bibliometric Analysis

A total of 297 documents were analyzed, of which 80.5% were articles and 19.5% were review papers. Figure 1 shows the evaluation of the scientific publication on protein–polysaccharide food hydrogels registered in the Scopus database in 2012–2022. Figure 1A shows the number of publications, and Figure 1B represents the main subject area of the publications.

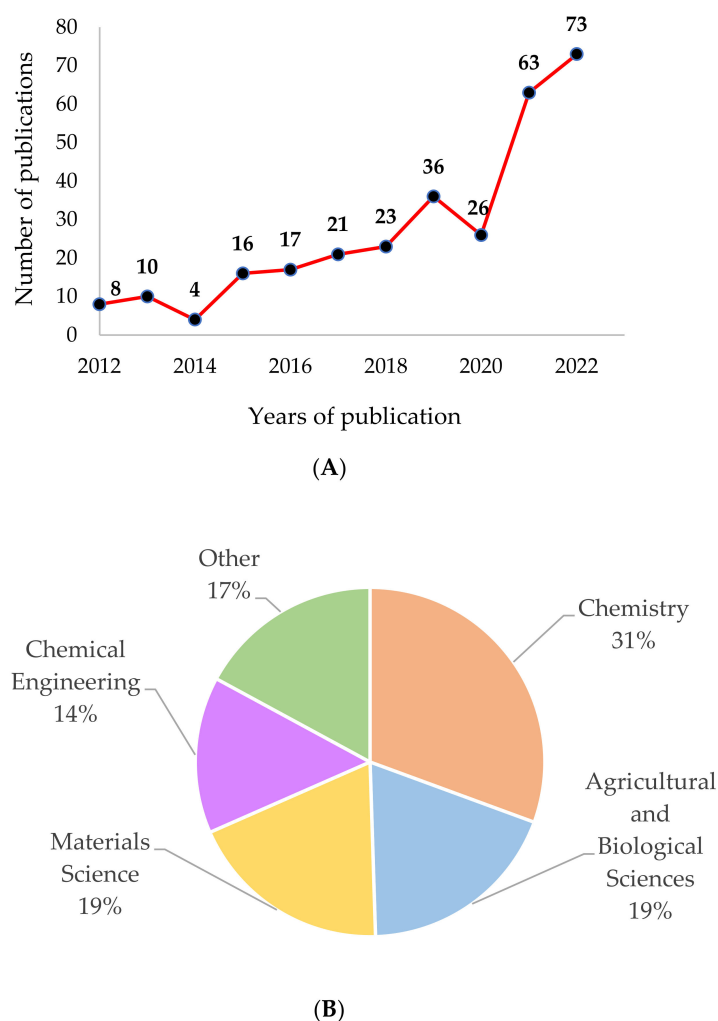


Figure 1. Evaluation of the scientific publications. **(A)** The number of publications registered on the topic of protein–polysaccharide food hydrogels over the last ten years; **(B)** the main subject area in which the publications registered on the topic of protein–polysaccharide food hydrogels over the last ten years (research carried out in the Scopus database in October 2022).

By analyzing the data presented in Figure 1A, a slow but systematic growth of the number of publications, it in years 2014–2019, can be observed. Currently, since 2020, there has been a dynamic increase in the number of published articles on protein–polysaccharide food hydrogels. In 2020, the number of publications on this topic reached 63, and in 2022—73. The growth in the number of published documents reflects the awareness of the potential uses of hydrogels in the food sector. This growth could be caused by the food industry’s growing concern about providing enough nutritious food for everyone while protecting natural resources. This growing concern has resulted in the faster development of plant-based foods and hybrid food products (from animal and plant sources), which have emerged as a new growing trend that can help the sustainability challenge [65]. The growing interest in the development of plant-based foods (including hybrid foods) has increased the number of studies on food hydrogels, which have the potential to improve the appearance, texture, flavor, mouthfeel, and functionality of these new products [20,37,66–68].

The scientific papers that addressed the topic of protein–polysaccharide food hydrogels were published mainly in four subject areas (Figure 1B): chemistry (31% of published documents), agriculture and biological sciences (19%, which include food science), materials science (19%), and chemical engineering area (14%). The other areas in which the analyzed scientific documents were published were physics and astronomy (7.7%); biochemistry, genetics, and molecular biology (5.9%); pharmacology, toxicology, and pharmaceuticals

(1.6%); immunology and microbiology (1.5%); medicine (0.5%). These research areas prove the interdisciplinary aspect of protein–polysaccharide food hydrogels [1,8].

Citation is one of the most critical indicators of a publication’s relevance [69]. Table 1 provides the most cited publications during the ten years. Moreover, to determine the current trends in scientific research on the topic of protein–polysaccharide food hydrogels, the keywords’ co-occurrence in the studied documents was performed. It can be observed that the most recent publications are related to the topic of polysaccharide hydrogels and hydrogels properties, such as self-healing, self-assembly, and mechanical properties (Figure 2).

Table 1. The 10 most cited original research papers on the topic of binary hydrogels from 2012 to 2022.

Sr. No.	Material	Induction Method	Key Findings	Applications	Ref.
1	Xanthan gum/ β -lactoglobulin	pH (4.4)	The complexation between the polymers resulted in a functional hydrogel, in which the structure strength mainly depended on xanthan gum.	Encapsulation of bioactive molecules	[70]
2	Whey protein/ pectin Whey protein/ alginate Whey protein/xanthan	Heat (90 °C)	The study provided information on the release mechanism of the obtained emulsion-filled hydrogels. Whey protein/pectin hydrogel had the highest release exponent.	Bioactive compounds delivery matrices	[71]
3	Gelatin/glucan	Heat (45–120 °C)	In comparison with pure gelatin the gelatin/glucan hydrogel exhibited improved mechanical properties.	Food and pharmaceutical	[72]
4	Konjac glucomannan/gum tragacanth	Heat (60 °C)	The obtained hydrogel was formed mainly by hydrogen bonding. The hydrogel exhibited a significant thermosensitive behavior between 35–45 °C.	Thermosensitive delivery system	[73]
5	Gelatin/tara gum	pH (3.5–11.0) and ion (salt: 0–300 mmol/L)	A synergistic effect of tara gum of the gelatin gel structure was observed. The hydrogel formation was not affected by the pH. The addition of salt (50 mmol/L) had the most significant on the mechanical attributes.	Food rheology modulation and delivery system	[74]
6	Caseinate/pectin	Enzymatic (transglutaminase)	The hydrogels obtained using enzymatic crosslinking exhibited significant integrity under pH ranging from 6 to 8. Both enzymatically crosslinked and not crosslinked network displayed a high stability to heating and low pH.	Delivery matrices for lipophilic bioactives	[75]
7	Gellan gum/collagen	Heat (90 °C)	A new process of gelation was proposed, which is based on dripping the gellan-anthocyanin dispersion into the cold (10 °C) collagen dispersion. The obtained network exhibited high anthocyanin retention (>84%).	Encapsulation of bioactive molecules	[76]
8	Starch/alginate	Ion (CaCl ₂)	The retention of insulin was >80%. The obtained hydrogel exhibited promising properties in terms of safe delivery of insulin via oral pathway.	Insulin oral delivery system	[77]
9	Soy protein/ κ -carrageenan	Heat (80 °C)	The hydrogel with the addition of 0.6% κ -carrageenan displayed the most dense and uniform structure. Additionally, κ -carrageenan protected the soy protein and the embedded flax lignans from erosion caused by digestive enzymes.	Carriers for water-soluble bioactive compounds	[78]
10	Alginate/inulin Chitosan/inulin	Heat (80–90 °C)	The addition of alginate or chitosan had no significant impact on the gelling ability of inulin. Chitosan (0.5 g/100 g) addition improved the stability of the obtained hydrogels.	Functional ingredient for developing new health-promoting food products	[29]

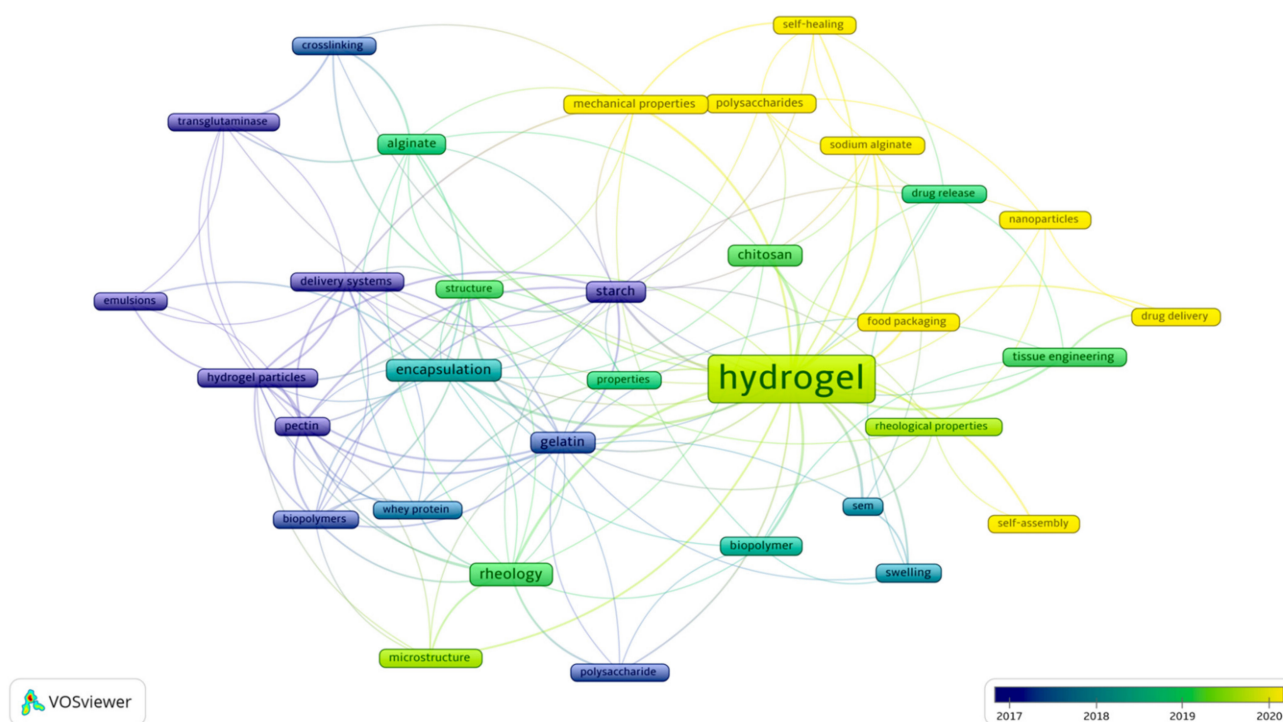


Figure 2. Visualization of the keywords network based on their co-occurrence. The frame size represents the frequency of the keyword's co-occurrence. The color scale represents the average number of document publications per year.

Most of the studies concerned the application of hydrogels in food packaging and drug delivery. Auriemma et al. [79] stated that polysaccharide hydrogels have a promising potential in developing drug delivery systems aimed at controlling and targeting the delivery of many drugs. Despite their potential, many breakthroughs in clinical studies of the release mechanisms are needed to use these hydrogels as drug carriers while also focusing on the SbD (safe-by-design) approach. The standardization of the analysis regarding the release mechanisms of hydrogel delivery systems is a crucial topic in the meaningful, intelligent delivery systems design [80,81]. Protein–polysaccharide food hydrogels have received significant attention because of the growing need to replace plastic packaging with new, safe, and biodegradable materials. Additionally, researchers are trying to implement the knowledge from disciplines, such as the pharmaceutical one, to develop hydrogel-based packaging materials with the ability to release bioactive compounds that could prevent the growth of harmful microorganisms while protecting the food product from moisture and nutrient loss [82,83]. The study of co-occurring keywords helped isolate two main interlinked clusters. The first and most significant cluster included observations of hydrogels from the perspective of self-assembly, swelling, and rheological properties, with the word hydrogel the most highlighted. The second cluster focused on encapsulation from the perspective of biopolymers, hydrogel particles, emulsions, and delivery systems. These two clusters showcase the transition from studies concerning the model properties of such hydrogels (cluster 2, before 2018) to the application of these hydrogels in tissue engineering, drug release and delivery, and the current application of self-assembly and self-healing hydrogels in food packaging (cluster 1, after 2019).

4. Hydrogel's Induction Methods

Two factors need to be met to form a food hydrogel. The initial one is that the used biopolymer has hydrophilic groups, whereas the second one is the presence of crosslinking strength between the particles and molecules to initiate the aggregation process and the final formation of the network [1]. Figure 3 illustrates the main mechanisms of polysaccha-

rides and proteins hydrogel formation. Based on the crosslinking mechanism of gelling, hydrogels can be divided into physically-, chemically-, enzymatically-, or multi-crosslinked. Physically crosslinked hydrogels are systems in which noncovalent interactions between the polymers are the precursor interactions that lead to the development of the structural network. Such physical mechanisms include electrostatic interactions [84], hydrogen bonds [85], crystallization [86], metal-ligand coordination [87], stereocomplex crystallization [88], hydrophobic interactions [89], conformation transformation [90], host-guest interaction [91], molecular specific binding [92], and π - π stacking [93]. Chemically crosslinked hydrogels are also known as “true gels”. They are obtained through the formation of covalent bonds between two polymers (Figure 3C). These kinds of junctions are usually non-reversible, permanent, and highly stable. Chemically crosslinked hydrogels can be obtained by free radical polymerization (pathway via monomers) [94] or by using crosslinkers, high-energy radiation, and the chemical reaction–pathway via polymers [95,96]. Enzymatically crosslinked hydrogels are obtained using enzymes such as trans-glutaminase [97], tyrosinase [98], laccase [99], horseradish peroxidase [100], etc. Notably, many hydrogels are obtained through multi-crosslinking mechanisms, using at least two described mechanisms depending on their structural complexity [101–103].

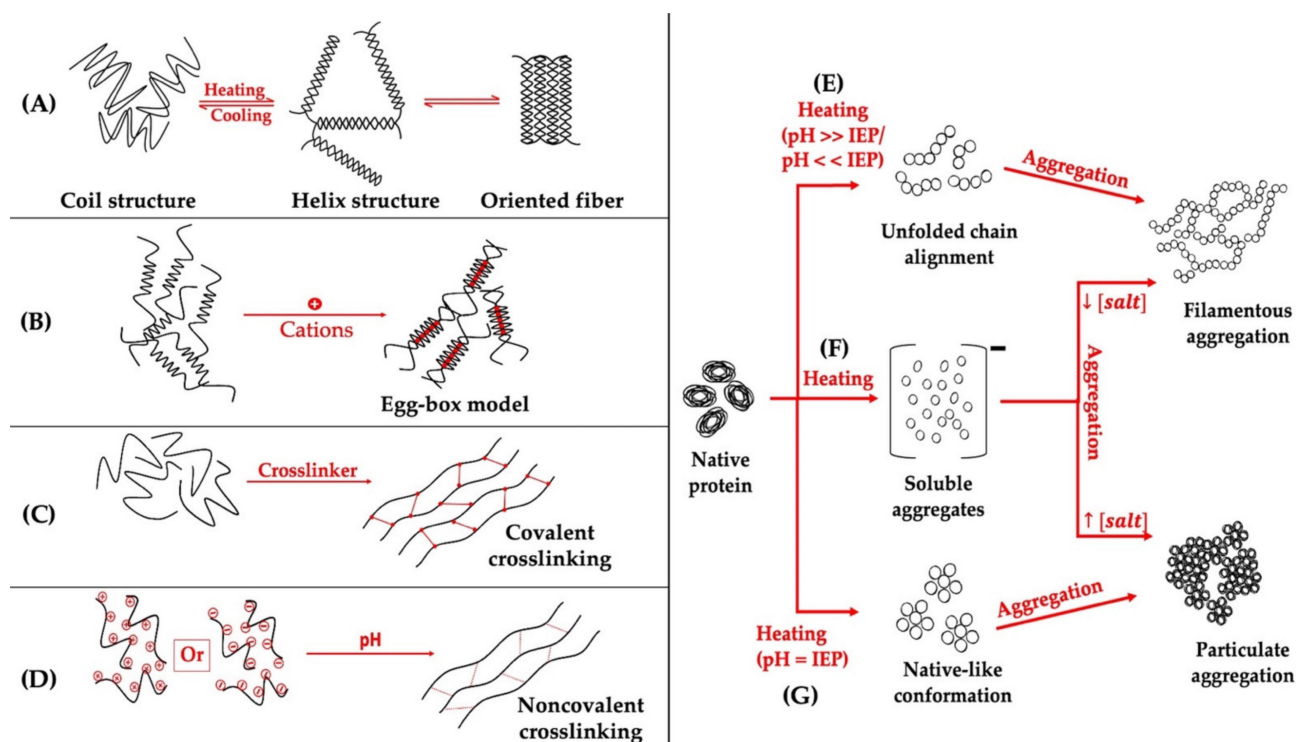


Figure 3. Illustration of the main mechanisms of formation of polysaccharides (A–D) and globular proteins hydrogel (E–G). (A) temperature-induced gelation of coil structure polysaccharides (e.g., κ -carrageenan), (B) ion-induced egg-box gelation of alginate, (C) covalent crosslinking-induced gelation (e.g., epichlorohydrin for cellulose hydrogel induction, glutaraldehyde for chitosan hydrogel induction), (D) pH-induced gelation (e.g., induction of pectin hydrogels), (E,G) temperature- and pH-induced globular protein gelation, (F) temperature- and ion-induced globular protein gelation [8,79,104].

Through the years, many food hydrogel induction methods have been developed and applied in the food sectors [8,39,105]. The most conventional, well-studied methods of inducing proteins and polysaccharides gelation are pH, temperature, ion modulation (physical crosslinking methods), and enzymatic crosslinking. The recent development in the field of hydrogels brings new, unconventional induction methods, such as high-pressure and pulsed electric field [106,107]. The most crucial induction methods are discussed further below.

4.1. pH Induction

The pH induction is a cost-effective, simple, safe, and widely used food hydrogel induction method. By modulating the pH of the protein and/or polysaccharide dispersion, it is possible to affect the solubility, molecular conformation, and charge, as well as the zeta potential of the used biopolymers, altering the attractive and repulsive forces between particles, allowing the formation of intermolecular and intramolecular interactions that lead to the formation of the gel structure. Moreover, the conformational changes in the structure of proteins may occur [108,109]. Hydrogels obtained by pH induction can be utilized, among others, in the encapsulation of bioactive compounds. Zhan et al. [110] reported that it is possible to encapsulate curcumin in a zein-whey binary system using the pH-induced method. In other report, the pH-induced method was used to obtain an economical and environmentally friendly chitosan colloidal gel system with the potential for food or pharmaceutical formulations [111].

4.2. Heat Induction

This induction method is a “green” and environment-friendly method widely applied in food hydrogels. In the case of protein (globular proteins) hydrogels, the heat induction method involves two stages: protein unfolding (denaturation) or dissociate, and then the interaction and aggregation of the unfolded molecules caused by the interaction between their functional groups, allowing for the for the preparation of higher molecular weight complexes [112]. Lui et al. [113] reported in their study that they obtained a pectin-whey protein hydrogel with high structural strength and storage modulus by heat induction. Furthermore, Fu et al. [114] studied the heated-induced gelation of soy protein isolate at the subunit level. Depending on the polysaccharide structure and their source, a gel structure via heat induction can be produced, and examples may be cellulose (and its derivatives) [115], curdlan [116], glucomannan [117], starch [118].

4.3. Ions Induction

The ions induction method, in some cases also known as cold induction (esp. in case of pre-denatured protein gel induction), is the addition of a salt ion (e.g., Na^+ , K^+ , Fe^{3+}) to induce the formation of gel structure, which is also a very widely used method. The gelation process of the protein and polysaccharides can occur when the electrostatic repulsive interaction between the polymers is decreased or removed [119,120]. Recently, Zhou et al. [121] have reported that adding Na^+ to a low-methoxyl pectin and soy protein dispersion affected the texture and viscoelastic properties of the cold-induced hydrogel. Additionally, they reported that only the addition of a low concentration of Na^+ positively affected the studied properties. On the other hand, k-carrageenan gelation can be induced by adding K^+ ions, as was studied by Chen et al. [122]. Additionally, it was demonstrated that it is possible to produce a composite hydrogel using chitosan and oxidized tannic acid by adding Fe^{3+} [123].

4.4. Enzymatic Induction

By adding enzymes to biopolymers, it is possible to induce the formation of a hydrogel through a biochemical path in which the enzymes play the leading role in constructing the gel structure. Enzyme-induced gelation is based on the insertion of covalent crosslinks. The use of transglutaminase, which can induce protein gelation by promoting intramolecular and intermolecular crosslinking of the peptide chains (Figure 4), is one example of such an enzymatic induction [124].

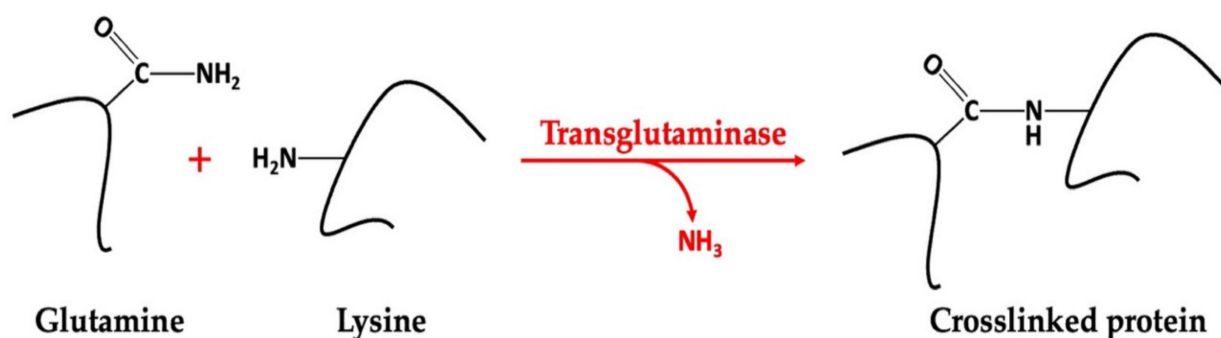


Figure 4. Schematic representation of protein crosslinking mechanism induced by transglutaminase [125,126].

The characteristic of transglutaminase-induced hydrogel crosslinking is related to the composition and conformation of the protein [127]. Transglutaminase has been effectively used in the induction of different types of proteins, such as soy protein [128], Bambara protein [129], as well as in the induction of binary-protein hydrogels composed of gelatin and carrageenan [130]. The other example can be protease, e.g., produced by *Bacillus licheniformis*, that can be used to induce the hydrolyzes α -Lactalbumin, which can then be used for the preparation of an amphiphilic peptide hydrogel used among others in the encapsulation of curcumin [131].

4.5. Freeze-Thaw Induction

This method involves freeze-thaw cycles, leading to phase separation and crystallization that affect the polysaccharide chain, allowing for the interaction between the chains by microcrystalline junction zones. This method is based on a repeated freezing process, storing in subzero temperatures, and thawing the dispersion in high temperatures [132]. Figure 5 represents the freeze-thaw induction method of cellulose nanocrystals. Xu et al. [133] studied β -glucan freeze-thaw gels as the carrier for the encapsulation of curcumin. They reported that these gels have great potential in developing natural drug delivery carriers. This induction method proved to be effective when it comes to thermolabile bioactive substances.

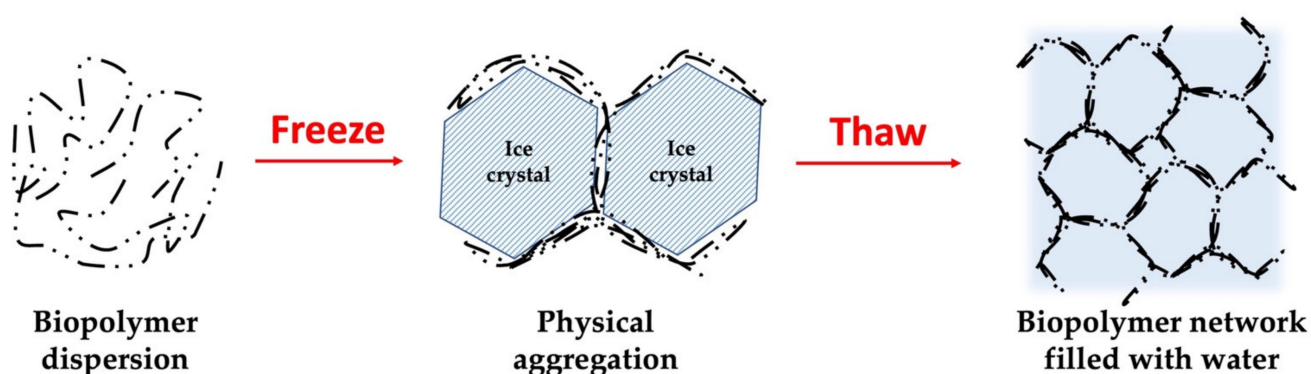


Figure 5. Schematic representation of freeze-thaw induction effect on cellulose nanocrystals hydrogel network formation [134,135].

The freeze-thaw induction method proved very effective in regulating hydrogel's textural properties while not negatively affecting its stability, even when two polymers were used in structure formation. This induction method was also demonstrated in research conducted by Shang et al. [136], where the effect of starch addition and freeze-thaw conditions on the water retention and texture properties of konjac glucomannan hydrogels was studied.

4.6. High Hydrostatic Pressure Induction

High hydrostatic pressure (HHP) induction is a novel method that has been extensively studied in terms of its ability to modify the physical properties of the protein and polysaccharide hydrogels. HHP provides the structural modification, aggregation, fragmentation that leads to gelatin production [137]. HHP can also transform protein structures by destroying the hydrophobic and electrostatic interactions, which influences denaturation, aggregation, and gelation. This induction technique can be used by itself or in combination with other induction methods (Figure 6), such as temperature induction [138].

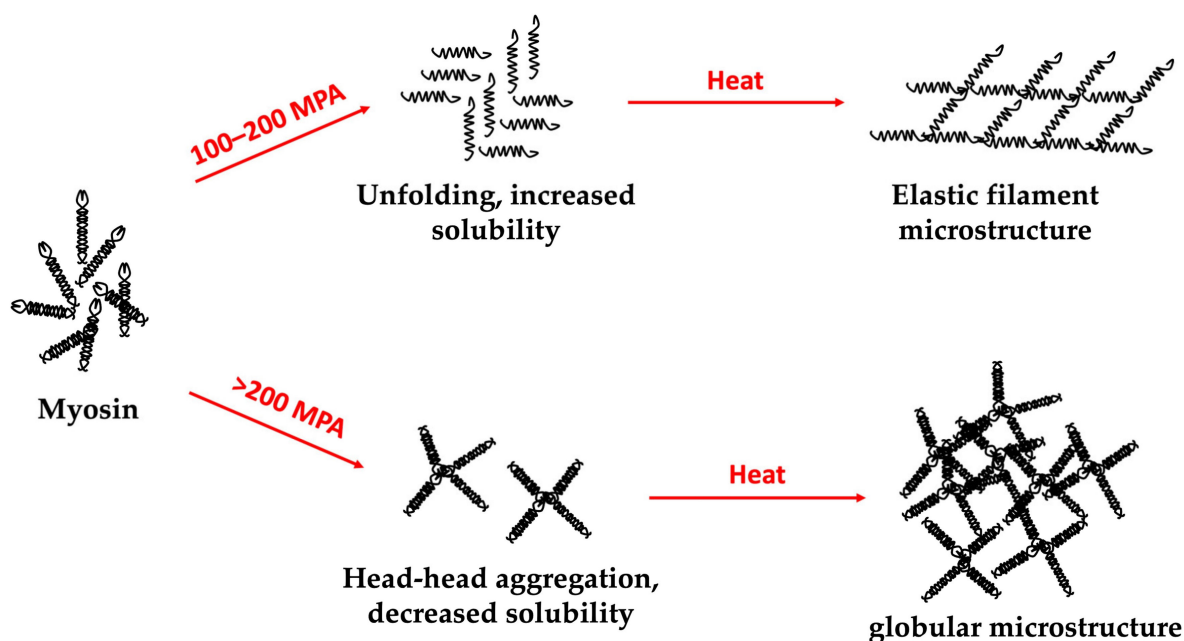


Figure 6. Schematic representation of HHP- and heat-induction effect on myosin hydrogel microstructure [139,140].

Luo et al. [141] have studied the effect of HHP on the gelation behavior and microstructure of quinoa protein isolate dispersions. They found that using HHP induction allowed them to obtain hydrogels similar to the ones induced using heat treatment. Moreover, when using HHP, it is possible to obtain hydrogels at lower induction temperatures, which has excellent potential in incorporating thermolabile food compounds and nutraceuticals into the quinoa protein gel matrix. In a study conducted by Florowska et al. [28] regarding the effects of pressure level and time treatment of HHP on inulin gelation and properties of obtained hydrogels, the use of HHP pressure (higher than 300 MPa) was reported. The obtained hydrogels had higher stability and a more compressed and changed structure, which resulted in higher yield stress, lower spreadability, and more rigid and adhesive hydrogels. On the other hand, Liu et al. [142] stated that the induction of starch hydrogels using high pressure resulted in starch gels with different functional properties compared to those obtained by heat induction. The authors also reported that such a starch induction method might be of interest for food processing.

4.7. Pulsed Electric Field Induction

Pulsed electric field (PEF) is a new physical method used to improve processes such as extraction, fermentation, dehydration, decontamination, etc. [143,144]. Figure 7 represents the effect of PEF on globular proteins. In addition, according to Giteru et al. [145], PEF treatment has the potential to be used to alter the functional properties of proteins and polysaccharides by inducing structural or conformational changes [146,147].

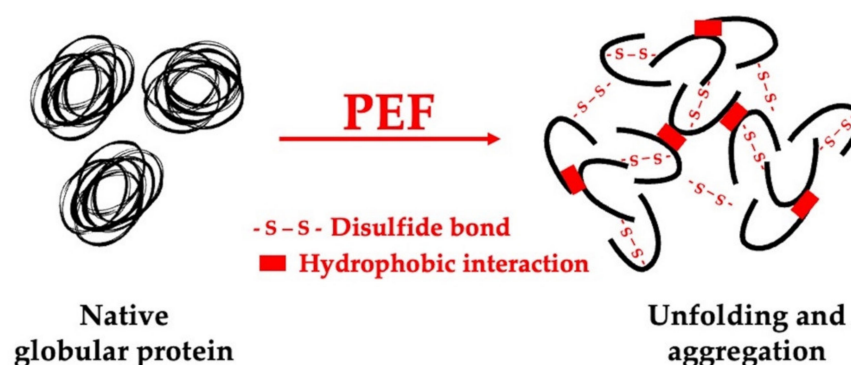


Figure 7. Schematic representation of PEF induction effect on globular protein [148,149].

The use of a moderate pulsed electric field caused the structural unfolding of the myofibrillar protein of the porcine muscle, which resulted in the formation of a uniform and compact gel structure [150]. PEF treatment can also change myofibrillar protein hydrogels' water distribution and mobility [151]. Moreover, the study conducted by Zhu et al. [152] on the use of the distributed electric field to induce the orientation of nanosheets resulted in the formation of complex anisotropic structures. These findings can be applied in the formation of hydrogels with biomimetic functionalities. PEF can be coupled with other induction techniques to design more complex hydrogels with specific functions [153].

5. Application Progress of Hydrogels as Food Matrices

Hydrogels present a wide range of properties (including high water content, flexibility, softness, and compatibility), making their application highly tunable for different food systems. Protein–polysaccharide composites have been so far successfully used only in the food packaging industry as they possess an oil barrier, water solubility, and tastelessness [154]. The commercially used edible films are produced mostly from cellulose and whey protein biopolymers [155], or alginate and collagen [156].

However, one of the critical characteristics of hydrogels is their similarity to living tissues, which can open new avenues for their use in food, particularly in the production of meat analogs [3]. Hydrogels can be used as base structures (matrices) when designing new food products since they can play a crucial role in achieving structure stability, sensory attributes, and nutritional aspects, such as being carriers for a wide range of nutrients and nutraceuticals [157].

Hydrogels have also been used successfully as fat mimetics in different food systems. Paglarini et al. [158] in their research demonstrated the potential of soy protein emulsion-filled hydrogel as a fat mimetic in frankfurter sausages. They reported that the sausages prepared using this emulsion-filled hydrogel exhibited the same hardness as traditional frankfurters. Moreover, Domínguez et al. [159] reported that the correctly chosen hydrogel formulation does not modify the sensory characteristics of meat products and allows for the reduction of both total fat and saturated fatty acids. Furthermore, the latest studies on hybrid gel prepared using canola oil/candelilla wax oleogel and gelatinized corn starch hydrogel also demonstrated the potential of hybrid hydrogels to be used as an alternative to commercial shortening to produce cookies with low-saturated fat content [160].

Recent research advances have recognized the utilization of bio-based biodegradable materials for food packaging to address the growing problem of the widespread use and misuse of petroleum-based polymeric materials [161]. Hydrogels prepared using biopolymers have great potential in manufacturing traditional, active, and intelligent food packaging. Hence, by embedding antimicrobial compounds (e.g., silver nanoparticles) into a hydrogel matrix, such a hydrogel can find use in the manufacturing of active packaging, which can reduce or inhibit the growth of harmful microorganisms [162]. Hydrogels can

also be used to develop biosensors for intelligent food packaging, conveying information about a product's freshness or the presence of contaminants [163–165].

The most recent trend in using hydrogels is the development of matrices that can replace animal-based food products in terms of texture and nutritional aspects. The food sector is increasingly becoming more concerned with providing enough nutritious food for everyone while protecting natural resources. That is why plant-based foods and hybrid food products (from animal and plant sources) are a new growing trend that can help with this sustainability challenge [65]. While developing new healthier foods using plant-based ingredients, the goal is to achieve the desired appearance, texture, flavor, mouthfeel, and functionality using healthy and sustainable plant-derived ingredients, such as lipids, proteins, and carbohydrates [65,166]. Additionally, plant-based products are often deficient in essential nutrients, such as vitamins (B₁₂, D, etc.) and minerals (iron, zinc, etc.). As a result, there is a growing interest in fortifying such food systems with these nutrients. This fortification can be taken a step further by adding nutraceuticals such as carotenoids, curcuminoids, and polyphenols to improve the healthiness of these plant-based food systems. It is critical to comprehend how these ingredients can be integrated to form complex matrices resembling those found in animal-derived foods, as well as how the properties of these matrices affect the physicochemical and organoleptic properties of the final product [167]. Therefore, in this paper, the advancements in using hydrogels as bioactive substances carrying food matrices will be further discussed.

5.1. Encapsulation and Delivery Systems of Bioactive Compounds

Hydrogels are increasingly used as encapsulating and delivery agents because of their high encapsulation efficiency, biocompatibility, low cost, and environmentally friendly properties. These properties can be achieved due to their porous nature caused by the three-dimensional structures in which crosslinked polymers form large interstitial spaces that are densely packed with water. These interstitial spaces can also incorporate various nutrients and bioactive compounds [3]. That is why these spaces can be utilized to overcome some challenges related to adding health-beneficial substances to food products; for example, low thermal and chemical stability, poor solubility, and undesirable flavor organoleptic profile. Encapsulating the bioactive substances in hydrogels makes it possible to protect them from external environmental factors during production, storage, and even after consumption. Such factors include oxygen, heat, light, pH, enzymes, etc. [168–170].

Moreover, by mixing proteins and polysaccharides, it is possible to obtain improved structural and functional properties, which can be explained by the formation of protein–polysaccharide complexes via covalent and noncovalent interactions. These binary protein–polysaccharide hydrogels can be used as a matrix for embedding hydrophilic and hydrophobic compounds [171]. Hydrophobic compounds can be embedded into a hydrogel by first preparing an emulsion containing these bioactive substances and then introducing the biopolymers to the emulsion, resulting in an emulsion-filled hydrogel [172]. Both hydrophilic and hydrophobic compounds can either form the gel network, contributing to the strength and stability of the final hydrogel—such compounds are called active fillers (Figure 8C,D). However, the embedded compound might not interact or can interact minimally with the gel network—such compounds are called inactive fillers (Figure 8A,B).

Protein and polysaccharide hydrogels can be used as delivery systems for polyphenols, a group of compounds (over 8000 phenolic compounds) with a range of physiological functions, including antioxidant, anti-inflammatory, anti-virus, antibacterial, and immunity enhancement. These functional properties are mainly related to the phenolic groups and the conjugated double bonds [173]. Polyphenols are widely used in the food industry, but their bioavailability still imposes challenges because of their poor solubility and stability [174]. That is why many researchers are involved in designing a food-grade hydrogel carrier that can protect those compounds from oxygen, heat, light, and pH degradation. The latest finding regarding the use of hydrogels as delivery systems for phenolic compounds and vitamins are mentioned below.

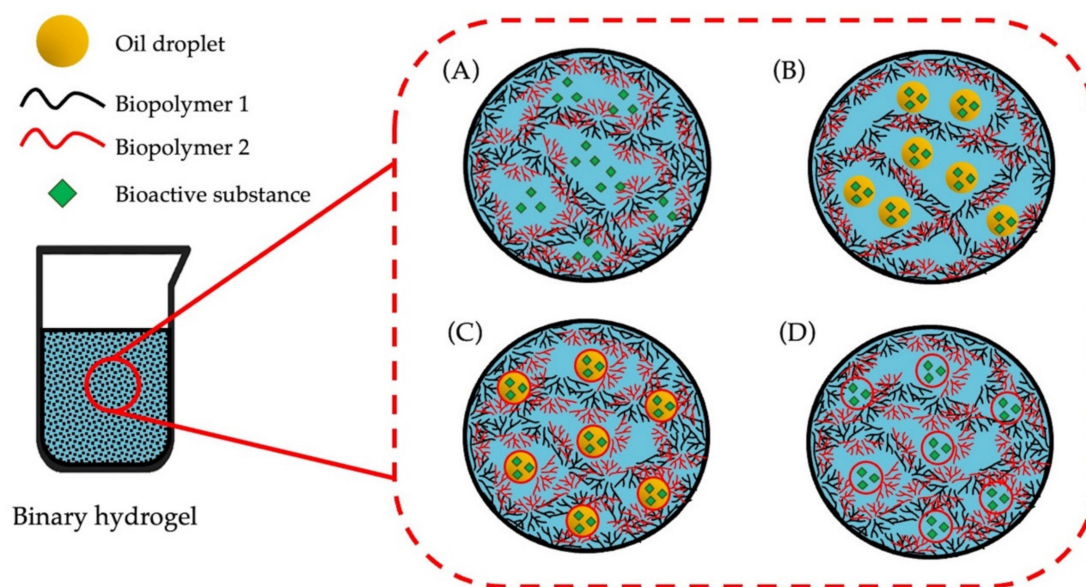


Figure 8. Schematic representation of the way in which bioactive substances can be embedded into a hydrogel matrix. (A) The hydrophilic bioactive substance is an inactive filler; (B) the hydrophobic bioactive substance is encapsulated in oil droplets and the oil droplets are inactive fillers; (C) the hydrophobic bioactive substance is encapsulated in oil droplets and the oil droplets are active fillers; (D) the hydrophilic bioactive substance is an active filler; Based on Farjami et al. [172], Liu et al. [41] and Li et al. [1].

Curcumin, a phenolic compound extracted from turmeric (*Curcuma longa* Linn.), has been well known for its health-promoting properties (antimicrobial, anti-inflammatory, antirheumatic, immunomodulatory, anti-carcinogenic). However, it exhibits poor water solubility and low bioavailability after ingestion [175]. Recently, proteins and polysaccharides-based hydrogels were developed to improve curcumin's stability and bioavailability. George et al. [176], in their research on cellulose-chitosan-zinc oxide composite hydrogels for the encapsulation of curcumin, reported that the loading efficiency reached 89.68%. In addition, the obtained hydrogel exhibited an antimicrobial effect on *Trichophyton rubrum* and *Staphylococcus aureus* and a controlled release at pH 7.4. In another study, curcumin was embedded in a chitosan/lotus root pectin hydrogel with an efficiency of 90.3% and improved solubility and stability [173]. Moreover, a nanoparticles-in-microparticles hydrogel system was fabricated by electrospray technology for curcumin colon-targeting oral delivery, which enabled curcumin release and entry to the macrophages [177]. Kour et al. [178] studied the effect of nanoemulsion-loaded hybrid biopolymeric hydrogel beads on the release kinetics, antioxidant potential, and antibacterial activity of encapsulated curcumin. They found that the high structural stability of the obtained carriers and their effective delivery of curcumin can provide a novel and tailored formulation out of polymers for oral drug delivery.

Epigallocatechin gallate (EGGG) is a catechin phenolic active compound with several health-beneficial properties, such as antioxidant, anti-tumor, antiviral, antibacterial, and cardio cerebral vessel protective. The polyhydroxy structure of catechins makes them unstable in neutral and alkaline pH. Additionally, they can be glucosylated or methylated by gastrointestinal tract enzymes, making them highly unstable and biologically unavailable [179]. To improve the stability and release of EGGG, Wang et al. [180] prepared a composite protein–polysaccharide hydrogel using carboxymethyl konjac glucomannan and gelatin. Authors reported that obtained hydrogels had better pH-sensitive properties, which enhanced the encapsulation and the bioavailability of EGGG. Furthermore, Yu et al. [181] reported that EGGG added to collagen hydrogels acted as an active filler by narrowing the pore size and strengthening the collagen fiber network. This effect

was due to the formation of covalent bonds between lysine and EGCG. What is more, the incorporation of nanofiber particles coated with epigallocatechin-gallate (EGCG) into gelatin methacryloyl hydrogel reduced the free-radical-derived cellular damage when using 3D tissue fabrication (ex vivo) [182]. Wu et al. [183] demonstrated that using konjac galactomannan with the addition of oxidized hyaluronic acid enhances the stability and control release of EGCG. Other studies also reported the positive effect of EGCG on the structural remodeling of soy protein-derived amyloid fibrils hydrogel [184].

Resveratrol is another poorly water-soluble polyphenolic compound that exhibits various physiological properties (e.g., oxidative stress, anti-inflammatory, anti-obesity, anti-cancer, etc.) [185]. Additionally, to its poor water solubility, resveratrol is characterized by a fast metabolism in the gastrointestinal environment, which affects bioavailability. Fan et al. [186] prepared pea protein particles with calcium-induced cross-linking in which they encapsulated resveratrol. This encapsulation led to enhancing the physicochemical stability of the compounds, as well as led to a better antioxidant ability. Other studies on the improvement of resveratrol stability included the preparation of a resveratrol-loaded nanostructured lipid carrier hydrogel that significantly enhanced anti-UV irradiation and anti-oxidative activity in vitro and in vivo [187]. Currently, Pickering emulsion presents a high potential in the encapsulation of resveratrol. Based on Wu et al.'s [188] reports, it is possible to conclude that Pickering emulsion prepared using sodium alginate and pectin has a promising potential in developing low-calorie food products while contributing to the delivery of resveratrol to the gastrointestinal tract.

Anthocyanins are water-soluble flavonoids with high antioxidant activity. Their use in the food industry is limited due to their rapid degradation triggered by the pH value. They also have a low bioavailability and recovery rate after ingestion because of their low resistance to environmental changes [189]. Additionally, Jin et al. [190], in their study, prepared a konjac glucomannan and xanthan gum hydrogel in which they embedded anthocyanins. They reported that this synergistic hydrogel enhanced the thermal stability of anthocyanins at various pH values (3.0, 6.0, and 9.0). Ćorković et al. [191] also reported that the use of carboxymethylcellulose hydrogel as polyphenol carriers, specifically anthocyanins, helped preserve their antioxidant capacity. These findings showcased that proper formulation of food hydrogel, including the proper selection of biopolymers, can significantly maximize the retention of anthocyanins. In the current study conducted by Liu et al. [192], it was reported that the efficiency of anthocyanin encapsulation in gelatin/gellan hydrogel was high because of the high density of the formed structure. Moreover, the gelatin/gellan hydrogel protected the embedded anthocyanins during digestion, increasing its bioavailability in the small intestine. However, the proper selection of hydrogel building components is critical because anthocyanins may be degraded rather than protected, as observed in the studies of Kopjar et al. [193], in which the fortification of anthocyanins-loaded pectin hydrogel with apple fibers caused a substantial degradation in the retention of the anthocyanins. Furthermore, hydrogel loaded with anthocyanins can also be utilized as a colorimetric pH indicator to monitor, for example, the freshness of food products [166,194,195].

Quercetin, a flavonoid with beneficial properties, such as exhibited antioxidant, anti-inflammatory, anticancer, and cardioprotective, also exhibits low solubility and physicochemical instability, making it hard to be absorbed and utilized by the human body [196]. Several hydrogel systems have been recently prepared to protect this compound from the environment and raise its bioavailability. Quercetin-loaded pH-sensitive gellan gum hydrogels were induced using an ionotropic gelation method, and it was found that the obtained hydrogel beads had a pH-responsive release behavior. This release behavior improved the intestinal stability of this bioactive substance [35]. Moreover, Liu et al. [197] developed a lotus root amylopectin-coated whey protein hydrogel to protect quercetin. They reported that the obtained hydrogel enhanced the stability of quercetin while improving its bioavailability (in mice). In another study, linseed oil and quercetin were co-loaded to liposome-chitosan hydrogel beads. Based on the obtained results, the authors found that the chemical stability of quercetin could be improved by loading liposomes into hydrogel

beads [198]. Moreover, Hu et al. [199] studied the co-encapsulation of epigallocatechin and quercetin in double-emulsion hydrogel beads and reported that obtained hydrogel beads inhibited oil digestion while increasing quercetin bioavailability.

Hydrogels obtained using food-grade biopolymers (proteins and polysaccharides) have been utilized for vitamin protection and delivery. The complexation of vitamin A and milk protein has been proven to increase the water-solubility and the light and heat stability of this vitamin [200]. Moreover, Rana et al. [201] also reported that vitamin A-loaded caseinate complexes improved vitamin A bioavailability. Similarly, Kaur et al. [202] highlighted the potential of chitosan and gelatin-based hydrogel to deliver vitamin B₁. A chemically crosslinked cellulose–hemicellulose-based vitamin B₁₂-loaded hydrogel was also reported to be effective in releasing this vitamin when the *in vitro* release is performed in successive buffers (from pH 1.2 to 7.4) [203]. Furthermore, β -cyclodextrin-soy soluble polysaccharide-based hydrogel was used to encapsulate and deliver vitamin E, showcasing the tunability of the swelling release properties of this vitamin both *in-vitro* and *in-vivo* [204]. Moreover, Martinez et al. [205] reported that the incorporation of vitamin E into a bigel (a combination of a hydrogel and an organogel) increased the diameter of the inner phase and the strength of the obtained structure. Mir et al. [206], in their research on glycerol-crosslinked guar gum monoaldehyde-based superabsorbent hydrogels for vitamin B₆, concluded that the release of vitamin B₆ depended on the pH of the medium (at pH 7, the concentration of the released vitamin was 79.2%).

5.2. Bioactive Substances Targeted Transport and Controlled Release

Because of the ability of hydrogels to hold large amounts of water or biological fluids, they can be used as carriers for bioactive substances, which can be embedded in the 3D hydrogel's structure. Hydrogels have significant potential in developing targeted release systems, which can release the embedded substances into the digestive tract. When choosing biopolymers such as building blocks, what needs to be taken into consideration is their digestibility [207–209]. Proteins are known to be very efficiently digestible because of multiple peptidases in the digestive system. Additionally, denatured proteins in hydrogels obtained using heat induction are even more digestible [210]. On the other hand, polysaccharides have diverse digestion pathways, which depend on their type. For example, starch digestibility varies from rapidly digestible to indigestible. Some starches can be rapidly hydrolyzed by amylase in the mouth or the small intestine [211]. However, some polysaccharides, such as inulin, pectin, alginate, etc., can only be fermented by the microbiota in the colon [212,213].

Binary protein–polysaccharide hydrogels that deliver bioactive compounds to specific areas of the digestive tract can be developed based on the properties of the biopolymers used as hydrogel building blocks. These hydrogels can be designed to deliver the bioactive substance in the right place and time under the influence of factors such as pH, temperature, enzyme, or microbiota. These factors affect the hydrogel's 3D structure, leading to its swelling or shrinkage and the release of the compound [214,215]. Based on the physiological conditions in different parts of the human digestive tract, it is possible to design a suitable hydrogel to deliver the bioactive compound to the targeted delivery site. The embedded bioactive substances can be released (Figure 9A) via swelling (change in volume), disintegration (dissociation of electrostatic coacervates), change in the molecular interactions (e.g., change in the electrostatic interaction between the bioactive compound and the polymeric building blocks), erosion (fermentation by the microbiota, digestion by enzymes) of the hydrogel's carriers [216]. For the hydrogels to deliver the embedded compound to the oral cavity, stomach, or small intestine, they should be pH- and enzyme-sensitive (Figure 9B). When the targeted site is the colon, the used hydrogel should be pH-sensitive and fermentable by the microbiota [208].

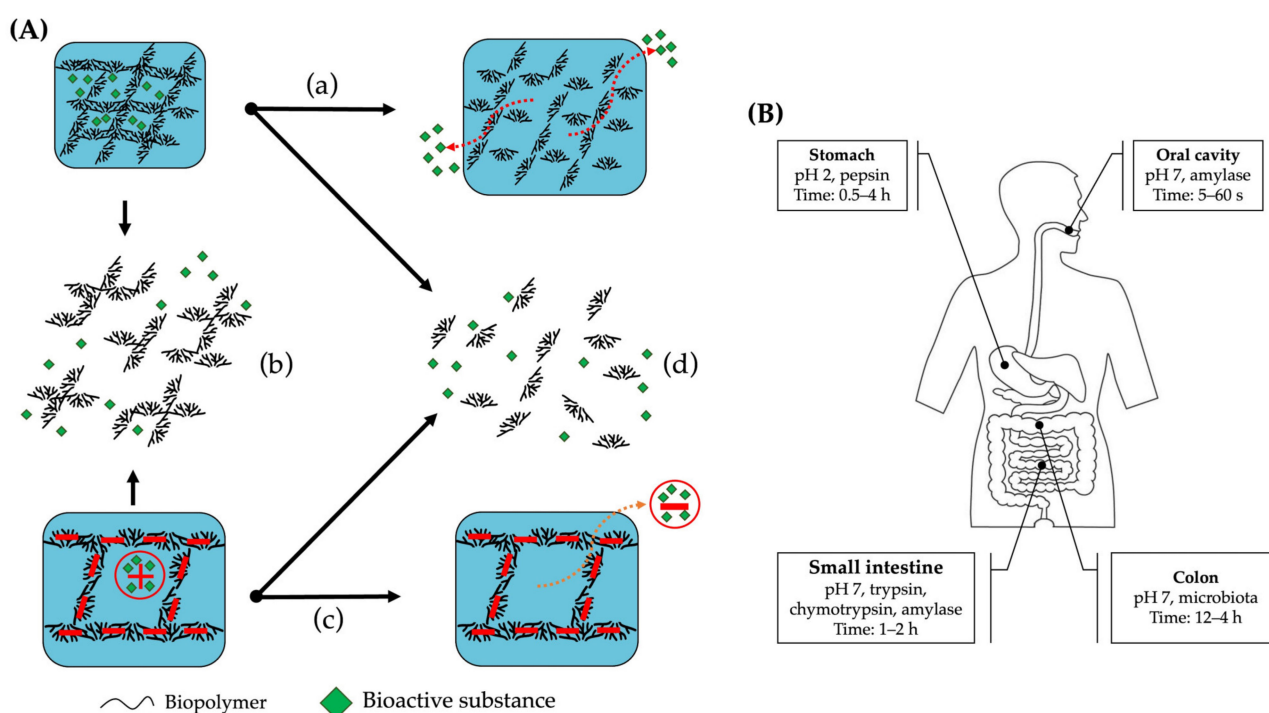


Figure 9. Food hydrogel and the digestive system interaction. (A) Potential pathways for targeted compound release from hydrogels: (a) swelling; (b) disintegration; (c) molecular interaction; (d) erosion. (B) Schematic representations of physiological conditions (pH, enzyme, and retention time) of the gastrointestinal tract [207,216,217].

Certain hydrogels can respond to chemical changes in the pH and ionic composition in the environment surrounding them. This response leads to changes in the structure of the polymer network. Such hydrogels are called pH- and ion-responsive [218]. Xie et al. [219] reported that they synthesized a hydrogel using Chinese quince seed gum, which has promising potential for the oral delivery of drugs. Furthermore, Sariyer et al. [220] developed pH-responsive alginate and κ -carrageenan hydrogels for the targeted release of bovine serum albumin. The targeted delivery of albumin to the intestines was achieved through diffusion and polymer structure relaxation. Temperature-responsive hydrogels are another type of carrier that respond to the changes in the temperature of the environment they are in by swelling or shrinking, which allows for the bioactive compounds to be released from the gel structure [221]. Temperature-responsive hydrogels might not be used to deliver bioactive substances to the stomach, small intestine, and colon but instead for oral (buccal) delivery. The such hydrogel can be developed to release the embedded substance at a temperature of 37 °C. Baus et al. [222] assessed in-vitro methods for the characterization of mucoadhesive hydrogels prepared using biopolymers, such as hydroxyethyl cellulose, carboxymethyl cellulose, xanthan gum, hyaluronic acid, and sodium alginate. They found out that xanthan gum had the highest resistance to the removal by artificial saliva. They also reported that based on the residence time of hydrogels, it is possible to develop a formulation with the best mucoadhesive properties for the delivery of bioactive compounds to the buccal area. Another type of hydrogel undergoes changes in its structure because of the activity of a specific enzyme. These hydrogels are enzyme-responsive and can be used to deliver a compound to a specific region of the digestive tract—where the concentration of enzymes, such as proteases or amylases, are the highest. The microbiota can also release the embedded compounds since it also produces enzymes that are not produced by the human gastrointestinal tract and can hydrolyze specific bonds of the biopolymers present in the 3D structure of the hydrogel. Wang et al. [223] developed an intestine enzyme-responsive polysaccharide-based hydrogel using carboxymethyl chitosan embedded with

an antitumor-selective kinase inhibitor. They reported that the obtained hydrogel was able to enhance the therapeutic efficiency.

Because of the wide range of possibilities in developing protein–polysaccharide hydrogels, it is possible to design hydrogels that can be responsive to multiple stimuli depending on the targeted delivery area. Zhao and Li [224] obtained pH- and temperature-responsive hydrogels using Tremella polysaccharides, carboxymethyl cellulose, and nonionic surfactants as the main hydrogel building blocks. Whereas Liao and Huang [225] obtained a pH- and magnetic-responsive hydrogel using carboxymethyl chitin, for which the swelling structure degree can be regulated depending on the concentration levels of Fe_3O_4 , the release mechanism is triggered by pH modulation.

6. Concluding Remarks and Future Perspectives

Protein–polysaccharide hydrogels have great potential for overcoming the limitations of hydrogels prepared with a single biopolymer, such as poor water-holding capacity and gel strength, as well as physical instability. In this review, we conducted a bibliometric analysis to characterize research trends in food protein–polysaccharide hydrogels (over the last ten years). We also discussed the latest development in conventional methods of inducing proteins and polysaccharides gelation (pH, temperature, ions modulation, and enzymatic crosslinking) and the new, unconventional induction methods, such as high-pressure and pulsed electric field treatment. Additionally, the newest developments regarding the application of hydrogels as food matrices, specifically as carriers for the targeted delivery of bioactive compounds, were discussed.

The studies regarding protein–polysaccharide hydrogels in food science are still minimal. This knowledge gap allows for new findings to be implemented in developing novel hydrogels for food applications. This hydrogel development can be achieved through a cross-integrated multidisciplinary approach between the food industry and other industry areas with advanced hydrogel knowledge (pharmaceutical, biomedical).

Protein–polysaccharide hydrogels have a promising potential in food applications by improving the stability and increasing the nutritious value of food systems while building a structural matrix that can be utilized as non-invasive bioactive compounds-targeted delivery systems. These highly tunable hydrogel properties can allow for the development of new, health-promoting plant-based or hybrid food systems that provide consumers with all the necessary nutrients based on their physiological needs. Therefore, there is considerable room for further research in a wide range of food hydrogel applications. There is a particular need to assess the possibility of using building blocks, such as plant-based proteins and polysaccharides, to develop a food hydrogel matrix that will protect the bioactive compound during processing, storage, and digestion, while increasing the bioavailability of these bioactive substances in the specific targeted area of the digestive system.

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