International Food Research Journal 24(Suppl): S313-S319 (December 2017)
Journal homepage: *http://www.ifrj.upm.edu.my*

Gelatin-polysaccharide based materials: a review of processing and properties

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Article history

Received: 6 December 2016
Received in revised form: 9 February 2017
Accepted: 10 February 2017

Keywords

Gelatin
Polysaccharide
Hydrogel
Aerogel
Nano-particles

Abstract

The advent of green technology has flourished biomolecule applications in medical, pharmaceutical and food products. Unlike synthetic materials, gelatin-polysaccharide matrixes are generally recognized as safe (GRAS). Gelatin-polysaccharide complexes are currently being utilized for the development of nano- and micro-particles, hydrogel, aerogel and films. Gelatin-polysaccharide based materials have offered improved characteristics depending on the type and concentration of polysaccharide and crosslinking agent. Gelatin-polysaccharide based materials function as bioactive compounds entrapment and encapsulation and anti-bacteria. This review provides concise information on the theory and technological applications of gelatin-polysaccharides based materials.

Introduction

Unlike synthetic materials, proteins and polysaccharides recognized in green technology materials due to their origins. Several works have shown that proteins and polysaccharides, when combined, interact and exhibited novel properties (Lee and Lee 1995; De Kruif and Tuinier 2001; Benichou *et al*., 2002; Turgeon *et al*., 2007; Patino and Pilosof, 2011). Nutritional roles of proteins and polysaccharides are well known and the recent understandings of their functional roles as protective vehicles for bioactive compounds have attracted intense studies. Proteins and polysaccharides have the ability to form gels and emulsion thus sever as ideal materials for nutraceutical compounds encapsulations (Chen *et al*., 2006). Protein-polysaccharide complexes and coacervates are useful industrially and have been used to make micro- and nano-capsules, food stabilizers, multi-layer structures, new food gels and ingredient (Turgeon *et al*., 2007).

Gelatin is a substantially pure protein food ingredient, obtained by the thermal denaturation of collagen, which is the structural mainstay and most common protein in animal. Gelatin molecule can also be described as an unwound fragment of triple helical collagen, which is incapable of complete renaturation. Gelatin is biodegradable, nontoxic and cheap protein and it is capable of forming hydrogel (Berillo and Volkova 2014). Gelatin-polysaccharide based materials have exhibited great beneficial functionalities in food and non-food industries. Gelatin-polysaccharide systems have found useful for productions of films, hydrogels, nano- and micro-particles. We have reviewed numerous studies on gelatin combination with different polysaccharides such as gum Arabic, alginate, pectin, chitosan, konjac-glucomanan, locust bean gum, carrageenan etc.

Principles of protein-polysaccharide interaction

Individually, proteins and polysaccharides contribute greatly to the properties (structural and rheological) of foods due to their aggregation and gelation behaviors. Under thermal gelation, globular protein unfolds to polypeptide chains causing the exposure of hydrophobic amino acid residues. This leads to protein self-aggregation into a three-dimensional network that traps water by capillary forces. Also, cold- set protein-gels have also been observed when denatured protein are exposed to solutions or emulsions containing ferrous salt and calcium ions (Chen *et al*., 2006).

More so, mixture of proteins and polysaccharides

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exhibit synergistic effects that impact great improvement of many food system at reduced cost-price (Patino and Pilosof 2011). Protein-polysaccharide interactions include electrostatic and hydrophobic interactions, steric exclusion, hydrogen bonding etc. In protein-polysaccharide matrix, strong electrostatic complexes are predominant and pH dependent . Mixing of protein and polysaccharide can form three different matrixes namely complexation, cosolubility and segregation (Patino and Pilosof 2011).

Complexation occur when there are attractive interactions between protein and polysaccharide and can either result into soluble and/or insoluble complexes. Polysaccharides have negative charges (OH groups) that interact with positive charges of protein thereby exhibiting electrostatic interactions (Patino and Pilosof 2011). This eventually lead to complex coacervation separation in liquid phases into coacervate and equilibrium neutral solution. Neutral coacervate normally form complex of solid-like (precipitate) with strong polyelectrolyte (De Kruif Cornelus *et al*., 2004). Although, hydrogen bond, hydrophobic or ionic interactions are responsible for occasional coacervate stabilization, electrostatic interactions are predominant. A strong electrostatic complex arises when the mixture is made up of positive protein and negative polysaccharide.

Protein and polysaccharide molecules can be linked together by covalent bond resulting into specific, strong and essentially permanent conjugate (Patino and Pilosof 2011). The Maillard reaction is a type of non-enzymatic browning which involves the reaction of simple sugars (carbonyl groups) and amino acids (free amino groups). They begin to occur at lower temperatures and at higher dilutions than caramelization. Maillard-type conjugation of protein and polysaccharides result into formation of products with improved functionalities. Therefore, Maillard reaction has been very useful for development of protein-polysaccharide based materials mostly films. Also, the Maillard reaction influences the colour, flavor and texture of food system (Gerrard *et al*., 2003). Numbers of research have shown that Maillard reaction is result in protein crosslinking and consequently affect the functional properties of food systems (Gerrard, 2002; Gerrard *et al*., 2002, 2003; Sun *et al*., 2004; Oliver *et al*., 2006; Gu *et al*., 2009; Choi *et al.*, 2010; Jing *et al*., 2011; Li *et al.*, 2013).

Crosslinking of proteins and polysaccharides

Crosslink of proteins and carbohydrates plays an important role in the functional properties of food systems. Control of the degree of food polymer crosslinking during food processing offers the opportunities for manipulation of the functional properties of polymers (Hennink and Van Nostrum, 2012). Crosslinking of food polymers result in change in properties like polymer conformation, flexibility and biological roles. Crosslinking provides an opportunity to create gel, films and particles structures from protein-polysaccharide solutions, colloidal, dispersion systems. Crosslinking of food polymers can be achieved through physical, chemical and biological crosslinking (Gerrard Juliet, 2002).

Chemical crosslinking

Chemical crosslinking of food polymers usually occurs immediately when food is harvest and during processing. The chemical crosslinking approaches involves formation of covalent bonds between food polymers (Back *et al*., 2003, Gerrard *et al.*, 2003). Many crosslinking agents that have found useful in biopolymer applications. The mechanism of crosslinking –polymer interaction depends on the polymer structural relationship. Generally, crosslinking agents are double-headed reagents that interact with functional groups of polymers (Gerrard *et al*., 2002). Synthetic crosslinking agents are not GRAS, therefore, plant phenolics have currently been explored for gelatin-polysaccharide crosslinking for green chemistry achievement. Coacervated gelatin-pectin micro particles became more lipophilic and thermal resistant (up to 200^{o}C) when cross-linked with grape juice or coffee (Strauss and Gibson 2004). Natural crosslinking agents (ferulic acid and tannin acid) exhibited crosslinking effect on gelatin film (Cao *et al.*, 2007).

Biological crosslinking

Biological crosslinking involves the use of enzymes to modify the polymers functional properties. This has attracted pronounced interest due to its bio-mimicking nature. There are many protein crosslinking enzymes, however, easily available transglutaminase has been mostly used. Transglutaminase catalyses the acyl-transfer reaction between the γ-carboxyamide group of peptide-bond glutamin reside and various primary amines. Numerous works have shown the potential of tranglutaminase for crosslinking of gelatin (Motoki and Seguro 1998; Nonaka *et al*., 1989; Babin and Dickinson 2001; De Carvalho and Grosso 2004; Zhu and Tramper 2008; Fuchs *et al*., 2010) and gelatin-gum Arabic coacervate (Dong *et al*., 2008).

Physical crosslinking

Radiation process has been recognized as one of

the physical method for crosslinking of biopolymer most proteins. Radiation can cause protein depolymerization or polymerization depending on the dose and exposure time (Kuan *et al*., 2013). Irradiated biopolymers often develop novel functional properties (Khan *et al.*, 2013). Ultraviolet irradiation resulted into improvement of gel strength of fish gelatin (Bhat and Karim 2009). Gamma-irradiation was reported to cause increase in molecular weight of calcium caseinate (Vachon *et al*., 2000). Gamma irradiated whey proteins not only displayed crosslinking effects but also dose dependent development of antioxidative fluorescence Maillard reaction products (Chawla *et al*., 2009). Ultraviolet-B radiation was reported to induce cross-linking effect and cause improvement of physical properties of cold- and warm-water fish gelatin gels and films (Otoni *et al.*, 2012).

In contrary, the effect of irradiation on polysaccharide was detrimental. Irradiation caused degradation and increase in radical formation of polysaccharides including cassava starch (Bertolini *et al*., 2001), sodium alginate (Nagasawa *et al.*, 2000; Wasikiewicz *et al*., 2005) and carrageenan (Relleve *et al*., 2005; Abad *et al*., 2009). Thermal stability of gelatin was increased by UV-induced cross-linking with glucose. However, irradiation caused on cellulose crosslinking rather than degradation (Wang and Chen 2005). Presence of glucose significantly increase the degree of crosslinking in gelatin, suggesting that glucose has a role in crosslink formation (Masutani *et al*., 2014). Hence, it is possible that irradiation cause crosslinking effect on polysaccharide and protein mixture.

Gelatin-polysaccharides conjugates

Previous works on gelatin-polysaccharide interaction have shown tremendous results. The mechanical properties of Gelatin-based films were improved by adding gellan, k-carrageenan, glucomannan or pectin (Chambi and Grosso 2011). Figure 1 shows the types of materials that have been developed from gelatin-polysaccharide matrixes/ gels.

Films

Films are flexible leave-like materials that display light, vapor, O_2 and CO_2 barrier functionalities. Films are produced from biological materials like protein and/or polysaccharides with varying mechanical properties (strength and flexibility), water solubility and barrier properties (permeability to water vapor) (da Silva *et al*., 2009). Increase in consumers' interest in health, nutrition, food safety and environmental issues spur the development of

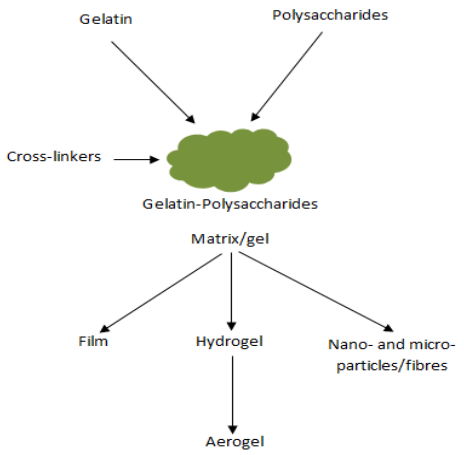

Figure 1: Types of gelatin-polysaccharide materials

films as packaging and protective materials in food systems (González-Estrada *et al*., 2015). In addition, protective functionalities of film are easily enhanced by addition of other adjuncts such as antimicrobials, antioxidant, nutrients, colorant etc (Ou *et al*., 2005).

Hydrogel and Aerogels

Protein and polysaccharide hydrogels are hydrophilic products of network of polymers chains with ability to swell and retain significant amount of water within their three-dimensional networks structure. Hydrogel structure is maintained by cross-linking of polymer chain through covalent bonds, hydrogen bonding, van der Waals interactions, or physical entanglements. Their interaction with water is as a result of their polymeric backbone and hydrophilic functional groups for water absorption and water resistance (Ahmed 2015). More recently, natural hydrogels are one- or multi-component of the similar or different substances. They are found useful in human health care for drug delivery, wound care and tissue engineering. Natural hydrogel are non-toxic, biocompatible and biodegradable (Gupta *et al.*, 2014).

Aerogels are byproduct of dehydrated hydrogel without with intact internal dispersed space. They are lightweight materials and highly porous capable of active release and sorption of particles (Quignard *et al*., 2008). Aerogel has large surface area with diverse accessible surface functionalities suitable for heterogeneous catalysts, adsorbents and supports (Quignard *et al*., 2008).

Micro- and nano-particles

Incorporation of protein and polysaccharide hydrogels in non-solid and semi-solid foods requires reduction of matrix sizes in order to avoid alteration of food sensory qualities (Chen *et al.*, 2006). This led to development of Nano- and micro-particles.

Nano- and micro-particles are attractive due to their large surface area, thus, found very useful as efficient delivery system of bioactive compounds. Several biomolecules have been explored for production of nano- and micro-particles and have shown interesting properties most especially in drug delivery study. Nano- and micro-particles has great potentials for target and controlled delivery during drug administration of therapeutic molecules to the diseased lesions (Kumar 2000). Nano- and micro-particles of biological origin have equally been found useful for formulation and stabilization of food emulsions (Dickinson 2012). These particles have even been recognized as food ingredient as a result of their nutritional roles – encapsulation and entrapment for delivery of bioactive compounds – to address the delivery challenges in food system (Augustin and Hemar 2009).

Micro- and Nano-fibers

Nano- and microfibers are thread-like materials that are inter-twine but non-woven. The chemistry of nano-fibers showed that they are of characteristics and functionalities capable of impacting novel features for advanced applications. Due to their high surface-to-mass (or volume) ratio and porous structure with great pore-interconnectivity, nano-fibers are found very useful in a wide range of applications including filtration, affinity membranes and recovery of metal ions, wound healing, release control, tissue engineering scaffolds, sensors, energy storage, catalyst and enzyme carriers, food packaging, food matrix improvement etc. (Fang *et al*., 2008).

Properties of gelatin-polysaccharide based materials

Interaction between protein and polysaccharide complexes enables designing novel microstructures. The properties of resulting protein-polysaccharide complex depend on initial environmental conditions, kinetic of interaction/phase separation, intrinsic properties of the macromolecules and application of physical treatments. Macromolecules concentration dictate microstructure formation to be either bulk or coacevate phase with novel rheological properties. Also, presence of another phase in water system may lead to specific air/water or oil/water interfacial properties due to protein-polysaccharide complexation synergistic effects (Turgeon *et al*., 2007).

Thermal stability of proteins in protein-polysaccharide complexes can either be improved or impaired. Previous studies showed increased thermal stability of proteins in flaxseed gum-meat protein and carboxymethylcellulose-α-lactalbulmin or –β-lactoglobulin systems. However, complex of β-lactoglobulin and chitosan cause increased protein thermal aggregation and thermal stability of lysozyme was reduced in complex with heparin (Turgeon *et al*., 2007).

Compressibility and gelling strength

The gel strength of gelatin-polysaccharide systems is usually measure using texture analyzer that determine the young modulus during compressibility test. Compared to gelatin only, possible change in young modulus in gelatin-polysaccharide systems might occur depending on the composition and type of the polysaccharide added. Although inclusion of 10% inulin lacks noticeable difference, 20% inulin gave higher Young's modulus compared to 5.0% gelatin only. This increase was attributed to formation of inulin crystals. Increase in Young's modulus of gelatin-polysaccharide system depends on the formation of gelatin triple helix structure and gelation of the polysaccharide counterpart. Variation in interaction of gelatin and different type of polysaccharides might affect the formation of triple helix gelatin structure. Increase in gelatin concentration in gelatin- HPMC (Harrington and Morris 2009), -κ-carrageenan (Haug *et al*., 2004) and –agar (Somboon 2013) led to increase in Young's modulus. Addition of KCl, that trigger gelation of κ-carrageenan, to gelatin-κ-carrageenan system result into multifold increase in Young's modulus (Haug *et al*., 2004). Similar result was observed with addition of calcium to gelatin-gellan system (Lau *et al.*, 2000).

Air/water behavior and foaming properties

Protein-polysaccharide complexes are useful for modification of adsorption kinetics of proteins at the air/water and modification of microstructure of adsorbed layer thus control its stability. This is possible because excess polysaccharide limit thermodynamic of protein adsorption due to electrostatic barrier or creation of charge neutralization by protein-polysaccharide complexes limit its adsorption diffusion. Studies of several protein-polysaccharide complexes showed that neutral complexes build dense viscoelastic interfacial network at the air/water interface. The gas permeability and high foam stabilization properties were also reduced (Turgeon *et al.*, 2007).

Oil in water behavior and emulsion properties

Unlike foam stabilization, charged protein-polysaccharide complexes is preferred for emulsion stabilization. The possible reason for this is that uneven charge distribution in protein-polysaccharide

complexes giving rise to electrostatic repulsion forces between droplets were dominating the interaction potential than van der Waals attraction forces. Therefore, emulsion stabilization with protein-polysaccharide complexes confer salt stability than protein alone. Also, uneven charge compensation was reported to play important stabilization role against flocculation. This is found useful for designing micro- or nano-capsules for the delivery of lipophilic materials (Turgeon *et al.*, 2007).

Turbidity

Increase in turbidity of gelatin and polysaccharide system indicate the occurrence of possible association between the polymers (Haug *et al*., 2004). The turbidity of gelatin-polysaccharide system varies depending on the type of polysaccharides. Polarimeter was used to study of the effect of different soluble polysaccharides (carboxymethylcellulose (CMC), hydroxypropylmethylcellulose (HPMC), guar gum, gum arabic, dextran and inulin) on optical rotation – turbidity – of gelatin-polysaccharide systems showed that gelatin-HPMC and -guar gum were highly turbid compared to others even at 45°C. Upon cooling, the change in turbidity of gelatin-dextran and gelatin-CMC systems were similar while that of gelatin-inulin and –gum Arabic were similar (Harrington and Morris 2009). Reduction in turbidity of gelatin-polysaccharide systems during gelation possibly suggests occurrence of bi-continuous networks. gelatin-polysaccharide bio-continuous networks develop when the polysaccharide gels first before gelatin (Haug *et al*., 2004). This phenomenon is termed segregative phase separation. However, it is difficult to compare the results of gelatin-polysaccharide systems across literatures because most turbidity measurements were done visually.

Conclusion

The renewed interest in utilization of biomolecules in medical, pharmaceutical and food area is on the rise due to their biocompatibility, biodegradability and sustainability. The development of environmental friendly materials has called for emergence of biomaterials to replace the synthetic ones. Nutritional roles of proteins and polysaccharides are well known, recent understandings of their functional roles as protective vehicles for bioactive compounds have attracted intense studies.

Conflict of Interest

There is not conflict of interest.

Acknowledgments

This is to acknowledge that the funding of this projects is from Research Initiative Grant Scheme (RIGS16-396-0533).

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