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Modification of plant and algal proteins through the Maillard reaction and complex coacervation: mechanisms, characteristics, and applications in encapsulating oxygen-sensitive oils

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There is an increasing demand for plant and algal proteins as alternatives to animal proteins. This demand is driven by lifestyle choices and sustainability concerns. The demand for them is particularly strong as ingredients. However, the technofunctional properties of these proteins, such as solubility, emulsifying, encapsulating, and gelling, need improvement to meet the requirements of food products. Among the approaches to modify proteins, covalent conjugation with polysaccharides through the Maillard reaction and non-covalent complexation through complex coacervation have received increased research attention. This paper provides an overview of these two approaches in terms of their mechanisms, typical process protocols, and the characteristics of modified proteins. The application of covalent conjugates and complex coacervates as emulsifiers and encapsulating shell materials for unstable and oxygen-sensitive oils is also reviewed. Recent studies that have combined these two approaches to achieve improved emulsification and encapsulation outcomes are reviewed to provide insights into the mechanism by which previously conjugated proteins undergo complex coacervation with a second polysaccharide. Ultimately, this review intends to identify the most effective technologies for creating emulsifiers and encapsulating shell materials using plant and algal proteins, particularly to stabilise and protect oxygen-sensitive oils.

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Sustainability spotlight

The continuous growth of global population has exerted an immense strain on the global food system. The increasing demand for sustenance resulting from the need of feeding the 8 billion plus population will ultimately lead to a depletion of our finite natural resources. Moreover, the climate change-induced decrease in the availability of rain and irrigation water will likely impact agricultural output. Animal proteins are unlikely to meet the protein demand of human population in the future. Also, the production of animal proteins requires large input in terms of land, water, and energy resources. It also involves higher greenhouse gas emissions. Plant and algal proteins are more sustainable to produce. The society, as a whole, is paying increasing attention to the nutritional, technofunctional and processing aspects of these alternative proteins. In this context, this manuscript provides a concise review of the differences between animal, plant and algal proteins in terms of their protein structure, amino acid composition and digestibility. Most importantly it reviews the underpinning science and technologies used for modifications of plant and algal proteins through covalent conjugation and non-covalent complexation, aiming to enhance the technofunctional properties of these proteins. This review highlights the technological pathways for converting plant and algal proteins into protein ingredients. Thus, this work meets the Sustainable Development Goals (SDGs) (goal 12) established by the UN.

1. Introduction

Protein is one of the most essential macronutrients as it is essential for the physical and physiological functions of the human body.[1,2] It provides peptides and amino acids to support and maintain the physiological functions.[3,4] Proteins are also important ingredients in food formulations as they provide important technological functions such as emulsification, gelling, and texture modification. The contemporary food industry uses dairy and animal-derived proteins such as caseins and whey protein, and gelatin as emulsifiers and encapsulants to stabilise susceptible oils for long-term storage. When an oil-in-water (O/W) emulsion is prepared, protein molecules readily adsorb at the oil–water interface to reduce the interfacial tension due to their amphiphilic nature and flexible molecular structure.[5,6] The protein layer covering the oil droplets provides electrostatic forces and steric

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hindrance among the emulsion droplets and acts against aggregation and coalescence.[7]

On the other hand, the rising global population is putting significant pressure on the natural resources required to produce food.[8] The production of animal proteins comes with sustainability constraints as it requires much higher input in terms of nutrients, water and land resources.[9,10] It has also been reported that approximately 14.5% of greenhouse gas emissions come from livestock production, thereby impeding environmental sustainability.[11,12] There is a growing ethical concern on animal welfare under current practices, which is also helping consumers choose alternative proteins.[13] Due to these reasons, there is an increasing consumer demand for food products containing non-animal proteins.[14] The fact that the population choosing vegan diet in the United States increased from 3 to 19.6 million in the last 10 years (2014–2023) underpins the future demands for non-animal proteins.[15]

Plant- and algae-based proteins are promising alternatives for this purpose. To date, various plant-based proteins derived from cereals, legumes, pulses, oilseeds, and nuts have been trialled to replace animal proteins as stabilising, foaming, thickening and gelling agents.[16,17] The contemporary food industry uses an alkaline extraction–isoelectric precipitation method to extract these (plant and algae-based) proteins, whereby the protein is solubilised in the alkali solution and precipitated around its isoelectric point, followed by separation and drying.[17] The precipitation process causes molecule aggregation and folding, producing a protein with an increased size and compact structure. In addition, plant-based proteins are commonly composed of multiple protein fractions with a high molecular weight and globular structure (e.g., albumins, globulins, glutelins, and prolamins).[18] Due to compositional and processing-related impacts, plant and algal proteins come with less than desirable functional properties (*e.g.* solubility and gelling interfacial behaviour).[19] Compared with animal proteins, plant-based ones exhibit a slow adsorption rate at the oil/water interface.[19] This ultimately compromises their application as ingredients and limits commercial application.

There is growing interest in using algae as a sustainable source of protein. The economic cost to grow algae is relatively low as they require less nutrients, water and space so long adequate sunlight is available.[20] Some of the commonly cultivated algal species are rich in protein (47–70% of dry biomass) and essential amino acids.[21] However, similar to most plant proteins, algae protein has a relatively low solubility at acidic pH values, particularly near their isoelectric points compared with the animal-based ones. Schwenzfeier *et al.*[22] reported that the acidic environment (pH of 3.0–5.0) reduced the solubility of protein extracted from green microalgae *Tetraselmis* sp. by more than 80%. In addition, the protein extracted from blue-green algae (*e.g.*, *Spirulina*) is heat sensitive. It was observed that the spirulina protein underwent denaturation upon exposure to a thermal treatment at 67 °C.[23] This temperature-sensitivity further hinders the potential application of *Spirulina* and other algal proteins in food processing when thermal treatment is involved. Hence, it is important to modify plant and algae proteins in order to improve their techno-functional properties.

To overcome the above-mentioned hurdles, proteins can be reacted with polysaccharides to modify their native structure and improve their techno-functional properties.[24,25] The resulting protein–polysaccharide assemblies are known as "conjugate" if created through covalent conjugation and "complex" if created *via* non-covalent interactions. Compared with the native proteins, the appropriately modified ones (conjugates or complexes) exhibit significantly improved emulsifying and stabilising properties.[26] Among various modification methods, the protein–polysaccharide conjugation and complexation *via* the Maillard reaction and complex coacervation (electrostatic charge driven complexation) are effective and are widely employed.[27] In the conjugation process, covalent bonds are created between protein and polysaccharide molecules. Various studies reported that the solubility and emulsifying properties of protein were substantially improved upon protein–polysaccharide conjugation induced by the Maillard reaction.[28–30] On the other hand, protein and polysaccharide molecules can form complex coacervates driven by electrostatic forces. The resulting supramolecular assembly showed superior interfacial properties compared to the native protein, making them promising emulsifiers and encapsulating wall materials.[31]

Microencapsulation technology is commonly employed in the food industry to protect and extend the shelf life of unstable oils that are prone to oxidation and developing off-flavour during food processing and storage.[32] The process of encapsulation involves the stabilisation of active compounds by entrapping them within a single or multiple substances.[33] During the encapsulation process, the active or sensitive food component is enveloped by a layer or layers of coating material. The encapsulated component is referred to as the core material, which can consist of small particles, liquids, or gases. The covalent conjugate or complex coacervate-based coating materials possess ability to form a network (shell)-like structure surrounding the active component.[32] The physicochemical stability of the encapsulated core can thus be enhanced.

There are reviews on various aspects of Maillard reaction-induced conjugation and complex coacervation-induced complexation and the application of resulting conjugates and complex coacervates where animal (*e.g.*, gelatin) and milk proteins are involved. There is no overview of knowledge (review) regarding the fundamental aspects of covalent conjugation and complex coacervation of non-animal proteins and characteristics and application of the resulting conjugates and complex coacervates. The application of plant- and algal protein- based conjugates and complex coacervates as emulsifiers and encapsulants for stabilising susceptible oils has not been adequately explored. Thus, this paper presents an overview of the mechanisms of modification of plant and algal proteins *via* their Maillard conjugations and complex coacervations with polysaccharides. The physicochemical, emulsifying and encapsulating properties of the resulting conjugates and complex coacervate will be compared with those of the unmodified proteins.

2. Relationship between proteins' composition and structure and digestibility

The main difference among the animal, plant and algal proteins is presented in Table 1, focusing on the composition, essential amino acid content, structure and digestibility. Proteins from different sources come with unique structures, which can be attributed to distinct polypeptide sequences, the nature of protein fractions and the secondary and higher order structure.[34] The proteins derived from plant and algae sources exhibit a domination of globular conformation, with hydrophobic regions buried within the proteins' interior while hydrophilic regions are positioned on the exterior.[18] The diversity and difference observed in the globular structures of proteins are determined by the nature of protein fractions and the configuration of secondary and tertiary structures.[34] The nature of the globular structure and nature of the protein fractions exert significant impact on the technofunctional properties of the proteins. In addition to the protein structure, the nutritional value of a dietary protein source is also an important factor to consider when assessing its application. Essential amino acids (EAAs) are a group of amino acids that cannot be synthesized by the human body and must be acquired through diet. Consequently, proteins rich in EAAs can be classified as 'complete' proteins, and the content of EAAs is used to evaluate protein quality.[35] The EAA content of plant and algal proteins is comparatively lower when compared to milk proteins. However, some plant and algal proteins such as soy, pea, lupin, flaxseed, sunflower, *Spirulina* and *Chlorella vulgaris* can meet the WHO/FAO/UNU requirements of essential amino acids.[36]

The digestibility of proteins is also an importance factor in determining the nutritional value, as it governs the extent to which they can be digested and peptides and amino acids can be absorbed by the human body.[34] The Digestible Indispensable Amino Acid Score (DIAAS) is commonly used to evaluate the bioavailability of amino acids, and a higher DIAAS value indicates a better digestibility of protein. Milk proteins exhibit superior digestibility, characterised by relatively higher DIAAS values compared to plant and algal proteins (Table 1). Recent studies by Zeng *et al.*[37] showed that, based on the DIAAS values, algal proteins show better digestibility compared to wheat and bean proteins. Although there is a lack of published data on the DIAAS value of *Spirulina* protein due to limited adoption of this method, their relatively high digestibility is shown through the protein digestibility corrected amino acid score (PDCAAS). Notably, *Spirulina* protein exhibits a higher PDCAAS value compared to pulse proteins.[38]

3. Maillard reaction driven conjugation and electrostatic charge driven complex coacervation between proteins and polysaccharides

The modification of proteins through these two methods is aimed to improve the techno-functional properties of proteins

Table 1 Protein structure, fractions, essential amino acid content and digestible indispensable amino acid score (DIAAS) of some protein sources

Protein source	Protein	Protein structure	Protein fractions	Essential amino acid content (% of total protein)	DIAAS[a]	References
Milk	Whey protein	Globular protein	β-Lactoglobulin α-Lactalbumin	46.9	100	39 and 40
	Casein	Disordered; flexible and unfolded protein	α_{s1}-Casein α_{s2}-Casein β-Casein κ-Casein	54.8	117	41
Legume	Soybean protein	Globular protein	β-Conglycinin (7S) Glycinin (11S)	37.2	89	42
	Pea protein	Globular protein	Vicilin (7S) Legumin (11S)	30	70	43 and 44
	Lupin protein	Globular protein	α-Conglutin (11S) β-Conglutin (7S) γ-Conglutin (7S) δ-Conglutin (2S)	33.2	68	45 and 46
Oilseed	Flaxseed protein	Globular protein	11–12S globulins 1.6–2S globulins	35.2	22	47–49
	Sunflower protein	Globular protein	11S globulin 2S albumin	38.7	67	50 and 51
Cereal	Wheat protein	Globular protein	Gliadin Glutenin	22	45	52
Microalgae	*Spirulina* protein	Globular protein	Phycoerythrin Allophycocyanin Phycocyanin	33.3	NA	53
	Chlorella vulgaris protein	Globular protein	N/A	39.4	73	54 and 55

[a] DIAAS values were obtained according to the 6 month-to-3 year-old reference pattern score.

and expand their applications in the food industry.[25] The covalent bonding and electrostatic interaction between proteins and polysaccharides have been extensively investigated for improving the functional properties of proteins, as both methods enable modification of proteins' structure and function.[56]

3.1 Maillard reaction between proteins and polysaccharides

3.1.1 Mechanism of the Maillard reaction. The Maillard reaction is a nonenzymic reaction between free amine groups of proteins and carbonyl groups of reducing carbohydrates. This reaction generates various Maillard reaction products.[57,58] The Maillard reaction is divided into initial, intermediate and advanced stages, as presented in Hodge's three-stage scheme in Fig. 1.[59]

The initial stage of the Maillard reaction involves a condensation reaction between the carbonyl group of reducing sugar and an amino compound of protein (especially the ε-amino group of lysine). The Schiff base is formed through the release of a water molecule. This compound is inherently unstable and undergoes cyclisation to yield an *N*-substituted glycosylated product. Subsequently, this compound rearranges irreversibly *via* Amadori rearrangement to form 1-amino-1-deoxy-2-ketose, commonly known as Amadori rearrangement products (ARPs). ARPs are colourless and an indicator of the initial stage Maillard reaction, with great potential for applications in food products as a modified protein.[60]

In the intermediate stage of the Maillard reaction, ARPs degrade through several simultaneous reactions. The pH of the sample significantly affects these reactions. The 2,3 enolisation is the predominant Amadori product degradation process under alkaline conditions (pH > 7). Reductones such as 4-hydroxy-5-methyl-2,3-dihydrofuran-3-one, and various fission products, including acetol, diacetyl, and pyruvaldehyde, are formed. These reductones are highly reactive and form dehydroreductones. In the presence of amino acids, the Strecker degradation occurs to form aldehydes and α-aminoketones.[58] The Strecker degradation products are responsible for the development of flavour and aroma. Under neutral and acidic conditions (pH ≤ 7), ARPs predominantly undergo 1,2 enolisation and form hydroxymethylfurfural (HMF) (when hexoses are involved) or furfural (when pentoses are involved).

When the Maillard reaction progresses to the advanced stage, melanoidins are formed due to polymerisation of the highly reactive intermediates and condensation reaction of furfurals or dehydroreductones. These nitrogenous brown compounds are commonly used as the indicator of the advanced stage of the Maillard reaction.[61]

Some physicochemical including organoleptic characteristics of food proteins can be improved when they undergo the Maillard reaction. Briefly, the initial and intermediate Maillard reaction products (MRPs) not only contribute to the aromatic and flavour characteristics of food but also exhibit antioxidative, anti-inflammatory and antimutagenic properties.[62] However, an uncontrolled Maillard reaction can lead to compromised food quality and generate food safety issues. Advanced glycation end products (AGEs) are formed in the advanced stage. Many clinical trials have shown correlation between AGEs and the occurrence of various chronic diseases, such as diabetes, kidney disease, cardiovascular complications, and Alzheimer's disease.[63] Therefore, it is important to control the Maillard reaction by adjusting reaction conditions

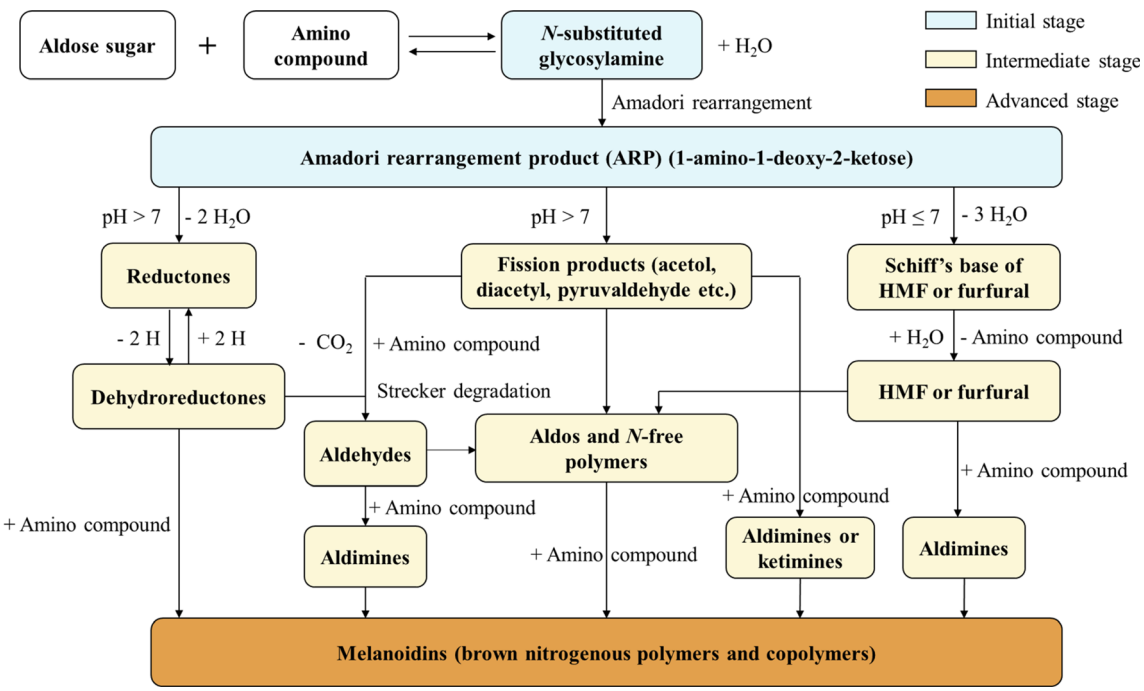

Fig. 1 Stages of the Maillard reaction adapted from Hodge (1953).

(temperature, pH, time, water activity, and mass ratio of the amine groups and the carbonyl groups) to avoid the formation of AGEs.[64,65] Due to the complex reaction pathway and formation of a wide range of reaction products, the Maillard reaction between every set of proteins and polysaccharides needs to be optimised.

3.1.2 Methods to initiate the Maillard reaction. The Maillard reaction can be initiated through either dry-heating or wet-heating routes. Dry-heating employs a dry blend, while wet-heating utilizes an aqueous blend of protein and polysaccharides. The dry-heating method appears to be used more often in laboratory settings.[66–68] In this method, proteins and polysaccharides are dissolved in water at a desired ratio and total solid concentration to produce mixed powder. This mixed powder is incubated under controlled temperature and relative humidity conditions for a specific duration to induce the Maillard reaction. The reaction time can range from hours to weeks depending on the nature of the protein and polysaccharide used and reaction conditions (temperature and relative humidity). After the set period, the reaction is terminated by rapidly cooling the reactants to ambient temperature. Although this dry-heating method has proven effective at a laboratory scale, its commercial application is challenging due to its small scale and long reaction time.[64] In addition, it is hard to control the reaction and to avoid it proceeding to the advanced stage.

The wet-heating method can be easily and effectively performed and is also amenable to scaling up.[69] In this method, the proteins and polysaccharides are dissolved in an aqueous solution and the pH is adjusted as required. Then, the aqueous mixture is heated at the desired temperature for a certain duration to facilitate the reaction. The reaction occurring in the aqueous phase maximises the contact between protein and polysaccharide molecules. Therefore, the required reaction time is significantly reduced to a few hours.[64] This method enables the control of the degree of glycation by precisely controlling reaction conditions and prevents the formation of AGEs.[57] Zhang *et al.*[70] conducted the Maillard reaction between pea protein isolate (PPI) and maltodextrin (MD) using the wet-heating method. By performing the reaction at the initial reaction pH of 7.5 and 8.0 and reaction time of up to 5 h, the authors successfully controlled the Maillard reaction at the initial stage without significant accumulation of melanoidins.[70] If the dry-heating method is used, it requires a much longer reaction time to satisfactorily carry out the Maillard reaction between PPI and MD. For example, Chen *et al.*[71] reported that the implementation of dry heating for PPI and MD at 60 °C and 79% relatively humidity required 3 days and achieved a glycation degree of 38%.

3.2 Mechanism of complex coacervation

The non-covalent interaction between proteins and polysaccharides occurs through various mechanisms, as illustrated in Fig. 2. Briefly, the repulsive or attractive interactions between proteins and polysaccharides can be driven by multiple forces, including electrostatic and hydrophobic forces, hydrogen bonding, and steric exclusion.[72]

The interaction between a protein and a polysaccharide in their mixture is affected by their characteristics (*e.g.*, molecular

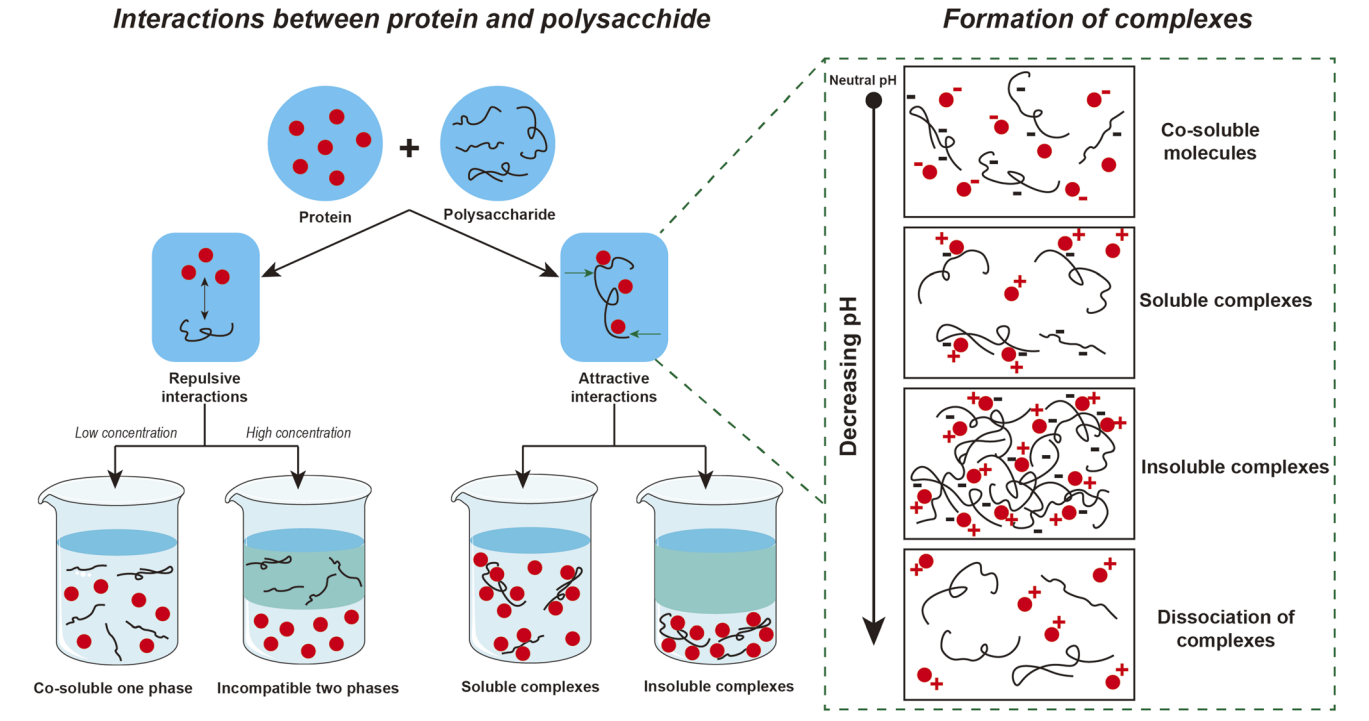

Fig. 2 The interaction between a protein and polysaccharide in aqueous systems during the process of formation of complexes when pH is adjusted.

structure, charge density and distribution) and environmental factors (pH, ionic strength, temperature, biopolymer concentration and ratio, and pressure).[65] At a relatively low polymer concentration, proteins and polysaccharides may co-solubilise in a single phase (Fig. 2). However, thermodynamic incompatibility between proteins and polysaccharides arises as the biopolymer concentration increases. It causes segregative phase separation into one phase enriched with proteins and the other with polysaccharides (Fig. 2).

In contrast, when proteins and polysaccharides carry opposite charges, their electrostatic attraction results in associative phase separation (complex coacervation). The aqueous phase is separated into a lower phase enriched with biopolymers and an upper phase enriched with water (Fig. 2). When the pH value of the system is adjusted to a value slightly below the protein isoelectric point (pI) but above the acidic dissociation constant (pK_a) of polysaccharides,[73] the "co-soluble one phase" and "incompatible two phases" in Fig. 2 can transform into soluble complexes. In this scenario, the initially negatively charged protein molecules become positively charged. It results in relatively weak electrostatic interactions between proteins and anionic polysaccharides and soluble protein–polysaccharide complexes are formed. With the progression of complex coacervation and subsequent pH reduction, more positively charged protein molecules are associated with negatively charged polysaccharides. It leads to electrical equivalence between biopolymers at a specific pH value. At this point, electrostatically neutral insoluble complexes are formed through strong electrostatic interactions and exhibit the lowest overall charge.[72] The precipitation of insoluble complexes creates a dispersed complex coacervate phase and significantly affects turbidity.[74] This is the stage at which "insoluble complexes" are formed. If the pH is further reduced, the complexes can dissociate due to the extensive protonation of reactive groups along the polysaccharide backbone.[75]

4. Maillard reaction between proteins and polysaccharides

Maillard reaction induced conjugation affects the structure of proteins and the resulting conjugates usually exhibit better techno-functional properties, making them highly effective emulsifiers and encapsulating materials.[76] The Maillard reaction between proteins and polysaccharides is influenced by various factors, including the nature of the reacting protein and polysaccharide, their mixing ratios, and reaction conditions such as heating methods, temperature, pH and reaction time.[77] The combined effect of these factors determines the final techno-functional properties of these conjugates as shown by examples listed in Table 2 and Fig. 3a.

4.1 Determination of protein–polysaccharide conjugates after the Maillard reaction

4.1.1 Colour change during the Maillard reaction. The measurement of change of the reacting mixture due to the formation of pigmented products is one of the most straightforward approaches to monitor the progress of the Maillard reaction. As discussed, once the reaction is beyond the initial stage, the degradation of Amadori rearrangement compounds leads to the formation of unsaturated brown nitrogen-free products and other copolymers. This process significantly contributes to colour development.[89]

The colour profile of protein–polysaccharide conjugates during the Maillard reaction is colorimetrically measured in terms of L^*, a^* and b^* values. Here, L^*, a^* and b^* represent the lightness, greenness-to-redness, and blueness-to-yellowness indices, respectively.[93] Among these parameters, the L^* and b^* value changes indicate a colour change towards the yellow-brown range, which is closely associated with accumulation of pigments and the extent of conjugation. For example, Zhang *et al.*[87] reported that prolonged reaction time reduced the L^* and increased the b^* values of the *Spirulina* protein-MD during the Maillard reaction.

The colour change during the conjugate formation process of the Maillard reaction can also be measured through a spectrometric method. Briefly, Amadori compounds are formed in the initial stage of the Maillard reaction. These compounds exhibit a distinct absorption at 304 nm and can be accordingly quantified using a spectrophotometer. On the other hand, melanoidins, which are responsible for the brown colour of the conjugates in the advanced stages, can be detected at 420 nm.[92] In Zha *et al.*'s[78] study, a gradual increase of absorbance of pea protein hydrolysate-gum Arabic conjugate was observed at 304 and 420 nm with the increased reaction time. The increase of absorbance indicated the formation of Amadori compounds and melanoidins in the process. Similarly, Zhang *et al.*[70] conducted the Maillard reaction between a pea protein isolate and maltodextrin using the wet-heating method and used the absorbance data obtained at these two wavelengths to control the Maillard reaction within the initial stage.

4.1.2 Glycation degree. As mentioned above, the Maillard reaction initiates a condensation reaction between the carbonyl group of reducing sugar and an amino group of a protein.[86] Thus, the free amino acids can be measured and used as an indirect measure of the degree of glycation. The *o*-phthalidialdehyde (OPA) method is commonly used based on its fluorogenic reaction with free amino acid groups. The glycation degree is calculated by comparing the difference in free amino group content before and after reaction.[80,92] In Yan *et al.*'s[86] study, the Maillard reaction was carried out between *Cinnamomum camphora* seed kernel protein and dextran. The degree of glycation increased gradually when the reaction time was increased up to 3 h, beyond which it remained unchanged, possibly due to the complete consumption of reactants.

4.1.3 Change of the molecular weight of proteins. The Maillard reaction changes the molecular weight of proteins. The resulting conjugates usually have a higher molecular weight than the native protein. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is most commonly used to detect the change of molecular weight. This method identifies the molecular weight fractions (bands) of proteins making use of their electrophoretic mobility and determines their respective molar masses. Yang *et al.*[82] investigated the conjugation of

Table 2 Physicochemical properties of typical protein–carbohydrate conjugates and methods used for analysis

Reactant system	Reaction conditions	Analysis method	Physicochemical properties	References
Dry-heating method				
Pea protein hydrolysate and gum Arabic	(1) Pea protein hydrolysate and gum Arabic were mixed at a ratio of 1 : 4 (w/w) and dissolved in water and lyophilised	(1) Amadori compounds and melanoidins formation measurements at 304 and 420 nm, respectively	The solubility and emulsifying properties of the conjugate obtained after 1 day of reaction exhibited significant improvement	78
	(2) The lyophilised mixture was incubated at 60 °C and 79% relative humidity for 1, 3 and 5 days	(2) Free amino group quantification by the OPA method (3) SDS-PAGE electrophoresis and FTIR (4) Solubility test (5) Emulsion stability against pH change and oxidation	Emulsions stabilised by the conjugate reacted for 1 day showed superior stability against lipid oxidation	
Soy protein isolate and glucose	(1) Soy protein isolate and glucose were mixed and dissolved in water at specific ratios (4 : 1, 2 : 1, 1 : 1, and 1 : 1, w/w). The pH of the mixture was adjusted to 8.0 and the solution was lyophilised	(1) SDS-PAGE electrophoresis	The Maillard reaction conjugate exhibited improved solubility within the pH range of 3–10, making it suitable for producing an emulsion with higher physical stability against thermal treatment and high ionic strength	79
	(2) The lyophilised mixture was incubated at 50 °C and 65% relative humidity for 6 h and 1–14 days	(2) Amino acid analysis (3) Measurement of brown colour using a white meter (4) Solubility test (5) Emulsifying properties		
Peanut protein isolate and dextran	(1) Peanut protein isolate was extracted and mixed with dextran in water at 1 : 1 (w/w) and then the mixture was freeze dried	(1) SDS-PAGE electrophoresis	Solubility and thermal stability of the protein were improved after conjugation	77
	(2) The dried mixture was incubated at 60 °C and 79% relative humidity for 1, 4 and 7 days	(2) Secondary structure analysis by circular dichroism (3) Thermodynamic profile obtained using DSC (4) Protein solubility test (5) Emulsion activity and stability	The conjugation of protein with dextran for a duration exceeding one day resulted in alterations in its tertiary conformation, leading to a reduction in emulsion stability	
Sweet potato protein and dextran	(1) The sweet potato protein was extracted and mixed with dextran at a 1 : 1 weight ratio and then the mixture was lyophilised	(1) Browning intensity measurement at 420 nm	The secondary structure of the protein was changed after the Maillard reaction	80
	(2) The lyophilised mixture was incubated at 60 °C and 79% relative humidity for 1, 3, 5 and 7 days	(2) Free amino group measurement by the OPA method (3) Secondary structure characterisation by circular dichroism (4) Thermodynamic profile obtained using DSC (5) Gel mechanical property measurement	The denaturation temperature of the protein decreased after conjugation The resilience and hardness of gel formed by the conjugated protein were significantly improved	

Table 2 (*Contd.*)

Reactant system	Reaction conditions	Analysis method	Physicochemical properties	References
Soy protein isolate and different pectin compounds (citrus pectin and apple pectin)	(1) Soy protein isolate was extracted using the acid precipitation method	(1) Grafting degree measurement by the OPA method	Conjugation of soy protein isolate with both citrus pectin and apple pectin formed products with higher molecular weight	81
	(2) The extracted soy protein was mixed with citrus pectin and apple pectin, respectively, at a ratio of 1 : 1 (w/w), and freeze dried and ground into powders	(2) SDS-PAGE electrophoresis	The solubility and emulsifying properties of soy protein were significantly improved	
	(3) The freeze dried samples were stored at 60 °C and 79% relative humidity for 1–7 days	(3) FTIR analysis (4) Surface hydrophobicity measurement (5) Solubility test (6) Emulsifying properties		
Soy protein isolate and soy soluble polysaccharide	(1) The soy soluble polysaccharide and soy protein isolate were mixed dissolved in water at specific ratios (3 : 8, 1 : 2, 3 : 5 and 4 : 5, w/w) and lyophilised	(1) SDS-PAGE electrophoresis	The formed conjugate exhibited improved emulsification capacity	82
	(2) The dried powder was stored at 55, 60 and 65 °C for 36–96 h, respectively with a saturated sodium chloride solution	(2) FTIR analysis (3) Emulsifying properties (4) Emulsion stability against simulated gastric digestion	Emulsions stabilised by the conjugates obtained under optimised conditions showed superior physical stability during heat treatment and simulated gastric digestion	
Soy protein isolate and citrus pectin	(1) The soy protein isolate and citrus pectin was mixed and dissolved in water at a ratio of 1 : 1 (w/w) and lyophilised	(1) FTIR analysis	The microwave treatment changed the conformational structure of soy protein and induced conjugation with a higher glycation degree. The conjugate exhibited improved antioxidative and emulsifying properties. The microwave-assisted dry heating method can shorten the conjugation time	83
	(2) Traditional dry heating method was used: the lyophilised mixture was incubated at 60 °C and a relative humidity of 79% for 1–7 days	(2) Glycosylation degree measurement by the OPA method		
	(3) Microwave assisted dry heating method was applied: the lyophilised powder was stored at 25 °C and 80% relative humidity for 11 h. The powder was then heated using a microwave processor at the power densities of 0.5, 1, 1.5, 2.0 and 2.5 W g^{-1} by controlling the highest working temperature with 60 °C	(3) Browning index measurement at 420 nm (4) Emulsifying properties (5) Antioxidant activity		
Black bean protein and different carbohydrates (dextran, chitosan and sodium alginate)	(1) The black bean protein was extracted using the acid precipitation method	(1) Free amino group quantification by the OPA method	Secondary structure of the protein was altered by the Maillard reaction and a less compact structure was formed. Solubility, antioxidant activity and emulsifying properties were improved after conjugation. The conjugates formed with sodium alginate exhibited higher thermal stability than other samples	84
	(2) The protein and carbohydrates were mixed and dissolved in water at a ratio of 1 : 1 (w/w) and then lyophilised	(2) Surface hydrophobicity measurement		
	(3) The lyophilised mixture was incubated at 70 °C and a relative humidity of 79% for 24 h	(3) FTIR analysis (4) Solubility and antioxidant activity test (5) Emulsifying properties (6) Thermal stability analysis		

Table 2 (*Contd.*)

Reactant system	Reaction conditions	Analysis method	Physicochemical properties	References
Wet-heating method				
Pea protein isolate and maltodextrin	Pea protein isolate and maltodextrin were mixed and dissolved in water at a ratio of 1 : 1 (w/w), and the pH of the mixture was adjusted to 7.5, 8.0 and 8.5, respectively. The mixture was heated at 90 °C for 5 h	(1) Amadori compounds formation measurement at 304 nm (2) Melanoidins formation measurement at 420 nm (3) SDS-PAGE electrophoresis (4) Secondary structure analysis by FTIR (5) Solubility test by Bradford method (6) Emulsifying property	The conjugation conducted at pH 7.5 and 8.0 for 5 h was effectively controlled within the initial stage. The conjugates exhibited higher molecular weight and increased random coil content. Solubility of pea protein significantly increased within the pH range of 3–8. The controlled wet-heating method can be used for the development of effective protein-based emulsifiers	70
Soy protein isolate and D-galactose	(1) Soy protein isolate and D-galactose were mixed and dissolved in water at different protein/D-galactose ratios (1 : 1, 2 : 1, and 4 : 1, w/w), and pH was adjusted to 9.0 (2) The aqueous mixture was heated at 80 °C for 13 h	(1) Degree of glycation measurement by the OPA method (2) SDS-PAGE electrophoresis (3) FTIR analysis (4) Solubility and surface hydrophobicity tests (5) Emulsifying properties (6) Rheological properties	The solubility of soy protein was significantly increased within the pH range of 4–9. The emulsifying properties of the conjugates improved with increased content of D-galactose involved in the conjugation. The covalent bonding between the protein and polysaccharide induced the enhancement of viscosity of soy protein isolate	85
Cinnamomum camphora seed kernel protein isolate and dextran	The *Cinnamomum camphora* seed kernel protein isolate and dextran were mixed and dissolved in water at a ratio of 1 : 1 (w/w). The mixture was incubated at different temperatures (70, 80, 90 and 100 °C) for 2 h and at 90 °C for 1–4 h	(1) Browning intensity measurement at 420 nm (2) Glycation degree measurement by the OPA method (3) SDS-PAGE electrophoresis (4) Secondary structure analysis by FTIR (5) Tertiary structure analysis by hydrophobicity and tryptophan related fluorescence measurement (6) Solubility and emulsifying capacity measurement	Based on the conjugation with dextran, the secondary and tertiary structures of *Cinnamomum camphora* seed kernel protein isolate changed significantly After the conjugation, the protein solubility and emulsion stability decreased; the protein aggregated during the heat treatment The conjugate exhibited improved thermal stability and $ABTS^{+}$ radical scavenging activity	86
Spirulina protein concentrate and maltodextrin	(1) The *Spirulina* protein concentrate was extracted using the acid precipitation method (2) The extracted protein was mixed with maltodextrin and dissolved in water at a ratio of 1 : 1 (w/w). The mixture was pH adjusted to 10.0, and heated at 60 °C for 3, 6, 12, and 24 h	(1) Glycation degree by the OPA method (2) Amadori compound formation measurement at 304 nm (3) Melanoidin formation measurement at 420 nm (4) Molecular weight change measurement by MALDI-TOF-MS (5) Secondary structure analysis by FTIR and CD (6) Solubility and antioxidant activity tests	The solubility of *Spirulina* protein was improved after 6 hours conjugation, whereas it exhibited a decline upon further heat treatment The *Spirulina* protein–maltodextrin conjugates exhibited an increase of molecular weight and a more flexible structure, suggesting the potential of this product as a novel emulsifier and encapsulating material	87

Table 2 (*Contd.*)

Reactant system	Reaction conditions	Analysis method	Physicochemical properties	References
		(7) Emulsifying properties		
Canola protein isolate and gum Arabic	The system containing 2% canola protein isolate and 1% gum Arabic was heated at 90 °C for 15 min	(1) Protein solubility (2) Zeta-potential (3) Oil-in-water emulsion characterisation (4) Rheological behaviour	Decreased droplet size and improved stability in the conjugate-stabilised oil-in-water emulsion; canola protein isolate-gum Arabic conjugate exhibited increased viscosity	88
Soybean protein isolate, chitosan oligosaccharide and glucose	(1) Pea protein was mixed with chitosan oligosaccharide or glucose at a ratio of 4 : 1 (w/w) and dissolved in water	(1) Colour measurement with a colorimeter	Intermediate products (HMF and furosine) were generated during the conjugation	89 and 90
	(2) The aqueous mixture was pH adjusted to 8.0 and incubated at 80 °C for 3, 6, 12, 24 and 48 h	(2) Hydroxymethyl furfural (HMF) content measured using HPLC	The reaction between soy protein and chitosan oligosaccharide exhibited a milder profile compared to the conjugation involving glucose, resulting in a slower accumulation of intermediates	
		(3) Secondary structure by circular dichroism (4) Thermal stability analysed using TGA (5) Solubility test (6) Emulsifying properties	Solubility and emulsifying properties of the protein improved after the conjugation with the chitosan oligosaccharide	
Pea protein isolate and inulin	The system containing 2% pea protein isolate and 1% inulin was pH adjusted to 10.0 and sonicated at 400 W for 25 min. During the sonication process, the temperature was controlled at 80 °C	(1) Particle size of emulsion	The attachment of hydrophilic inulin to pea protein molecules promoted the adsorption onto the interfacial layer and increased the thickness	91
		(2) Zeta-potential (3) Emulsion stability (4) Interfacial layer thickness (5) Oxidative stability	Ultrasonic induced conjugation improved the emulsifying properties of the protein and the emulsions stabilised by the conjugates exhibited enhanced stability against thermal treatment and oxidation	
Pea protein isolate and xylo-oligosaccharide	(1) Pea protein was dissolved in water and homogenised at 80 MPa 3 times	(1) Determination of grafting degree by OPA method	The solubility, thermal stability, emulsifying properties of the protein were improved using a combination of homogenisation and sonication method	92
	(2) The homogenised pea protein solution was mixed with xylo-oligosaccharide to obtain a protein-to-carbohydrate ratio of 1 : 3 (w/w), and the pH of the mixed solution was adjusted to 9.0	(2) Browning intensity measurement at 420 nm		
	(3) Ultra-sonification was used to induce the Maillard reaction with an ultrasound power of 400 W, at 70 °C for 20 min	(3) SDS-PAGE electrophoresis (4) Protein secondary structure measurement by circular dichroism (5) Solubility measurement (6) Thermodynamic profile using DSC (7) Emulsifying properties and foamability		

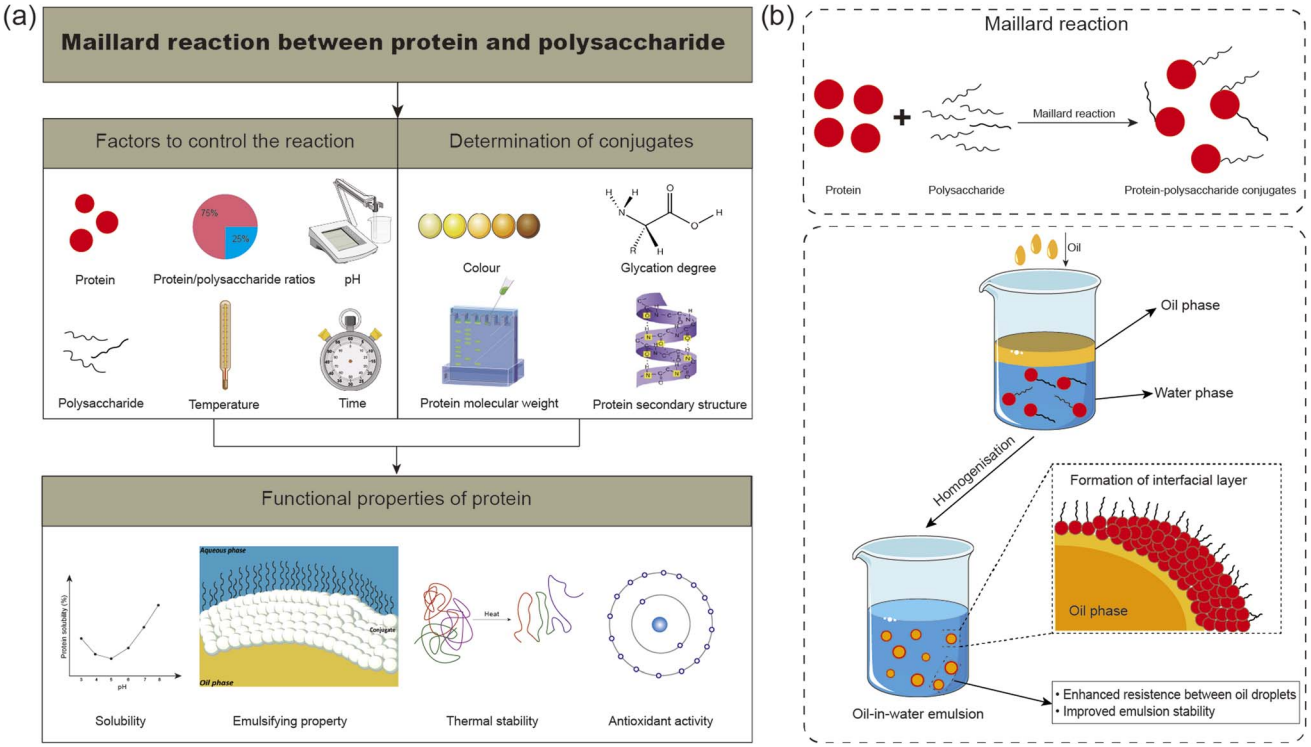

Fig. 3 Maillard reaction between a protein and polysaccharide: (a) effect of the Maillard reaction on the functional properties of the protein; (b) formation of protein–polysaccharide conjugates and mechanism of emulsion formation by the conjugates.

a soy protein isolate with soy soluble polysaccharide *via* the Maillard reaction and used SDS-PAGE to measure the change of molecular weight. The results showed that the original characteristic bands of the soy protein isolate observed around 20, 50 and 80 kDa disappeared due to the Maillard reaction and new bands were observed at bands above 150 kDa. The information obtained from SDS-PAGE results can also be used to indicate the extent of progress of the Maillard reaction. Yan *et al.*[86] produced conjugates of *C. camphora* seed kernel protein isolate and dextran and observed an increase of band intensity when the reaction time and temperature increased.

Matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF-MS) is another experimental method which can provide information on the molecular weight distribution of proteins more comprehensively. The detection of new peaks with increased molecular weight indicates the confirmation of the protein–polysaccharide conjugate. The formation of *Spirulina* protein concentrate (SPC)-maltodextrin (MD) conjugates was investigated by Zhang *et al.*[87] using MALDI-TOF analysis. The Maillard reaction significantly shifted the molecular weight peaks of the major SPC proteins from 5.4–9.9 to 11.7–16.7 kDa. The increase was positively correlated with the reaction time, indicating successful conjugation between SPC and MD. Pham *et al.*[94] also reported that MALDI-TOF was a much more sensitive method to provide an increase of the molecular weight of flaxseed protein when conjugated with polyphenol.

4.1.4 Change of the secondary structure of proteins. The change of the secondary structure of proteins after the Maillard reaction is commonly determined using Fourier-transform infrared spectroscopy (FTIR) and circular dichroism (CD). The composition of protein secondary structures (α-helix, β-sheet, β-turn, and random coil) are calculated using the spectra of FTIR and CD.[77,95] The Maillard reaction induced protein-carbohydrate conjugation and thermal denaturation affect the secondary structure of proteins. Zhang *et al.*[85] observed a decrease of α-helix content of soy protein isolate after conjugation with D-galactose. It was attributed to the reaction between the amino groups in or near the α-helix protein region and the carbonyl group in D-galactose. The thermal treatment caused the protein to unfold and led to the formation of a more relaxed protein structure, as evidenced by the increase in random coil content.[85] These conjugates with an altered protein secondary structure showed improved flexibility, which enhanced their functional properties. However, the changes in a protein's secondary structure highly depend on its original structural feature. Yan *et al.*[86] observed that the conjugation between *C. camphora* seed kernel protein isolate and dextran increased α-helix and decreased random coil contents of the protein. This result suggested that a more ordered protein structure was formed.

4.2 The effects of the Maillard reaction on the functional properties of proteins

The functional properties of proteins play an important role in the characteristics of food products in which they are part of. These properties can be modified through the Maillard

reaction.[57] Solubility, emulsifying capacity, thermal stability, and antioxidative potential are the most important functional properties of proteins as well as their conjugates.[78,92]

4.2.1 Solubility. Solubility in water is a fundamental functional property of proteins that significantly affects other functional properties, including emulsifying, foaming, gelling, viscosity, and rheological behaviour.[57] Compared with animal-based proteins, plant and algal proteins usually exhibit poor solubility under acidic conditions, particularly near their isoelectric point. This greatly hinders their application in food products.

During the Maillard reaction, the attachment of hydrophilic carbohydrate groups on the protein molecule chain enhances its solubility. Additionally, the overall electrostatic charge of protein–polysaccharide conjugates also becomes different. In many cases, the isoelectric point of the conjugate is lower than that of the original protein.[96,97] Liu *et al.*[77] reported the improved solubility of peanut protein isolate–dextrin conjugate in the pH range of 2–10, compared with the original protein. It was due to the enhanced affinity between the protein and water molecules due to the attachment of hydrophilic carbohydrates. The study conducted by Pirestani *et al.*[88] also demonstrated that the solubility of canola protein isolate increased significantly when conjugated with gum Arabic. However, it should be noted that the solubility of the conjugate can be compromised due to the thermal denaturation of the protein due to excessively long reaction time. For example, in Zhang *et al.*'s[87] study, the solubility of *Spirulina* protein concentrate–maltodextrin conjugate increased during the reaction time of 0–6 h. However, it started to decrease when reaction was carried out for longer than 6 h. The reduced protein solubility was attributed to the protein self-association and thermal denaturation during the reaction.[96] The prolonged heating caused unfolding of the protein structure and exposed protein hydrophobic groups, which increased surface hydrophobicity and promoted the aggregation of the protein.[87]

4.2.2 Emulsifying properties. There has been an extensive investigation of the emulsifying properties of protein–carbohydrate conjugates produced through the Maillard reaction. The amphiphilic structure of proteins enables them to function as natural emulsifiers, facilitating their rapid adsorption at the oil/water interface and effectively stabilising the emulsion by forming a viscoelastic layer.[64] However, the emulsifying properties of proteins are commonly compromised by acidic pH, high temperature, and elevated salt concentration. These factors may lead to the destabilisation of protein-stabilised emulsions during storage. Although the surface activity of carbohydrate polymers is inferior to that of proteins, their adsorption at the oil/water interface thickens and enhances the mechanical strength of the layer.[57] It is now commonly accepted that the Maillard reaction improves the emulsifying properties of proteins, as the conjugates formed through this reaction possess better emulsifying activity and stability compared to the native proteins and physical mixture of proteins and carbohydrates. Ma *et al.*[81] conducted a comparative analysis of emulsions stabilised by a mixture of soy protein isolate and apple pectin and their conjugates. The results revealed that the emulsion stabilised by the conjugates had much higher emulsifying activity and emulsion stability. In addition, Pirestani *et al.*[88] investigated the effect of storage temperature and pH on the stability of canola protein isolate-gum Arabic conjugate-stabilised emulsions. Their results showed that compared to emulsions stabilised by native proteins, the conjugate-stabilised emulsions had smaller droplet size and higher stability when subjected to heating from 30 to 90 °C under acidic pH (4.0 and 5.0).

The mechanism explaining the enhanced emulsifying properties of Maillard reaction conjugates is elucidated in Fig. 3b. Their enhanced emulsifying properties can be attributed to the increased solubility (Section 4.5.1). The protein structure in conjugates is more flexible which facilitates their rapid adsorption at the oil/water interface (Section 4.5.2).[92] The covalent bonding with polysaccharides can also alter the surface charge of proteins. The protein–polysaccharide conjugates form a thicker interfacial layer around the oil droplets, thereby inhibiting droplet coalescence or flocculation and ultimately enhancing the stability of the emulsion.[76] The steric hindrance offered by the carbohydrate component of the conjugates also minimizes the coalescence and flocculation of oil droplets.[98]

4.2.3 Thermal stability. Thermal stability is an important quality attribute of proteins as heat treatment impacts their structural integrity and functional properties and diminishes their nutritional value. The Maillard reaction has been reported to enhance the thermal stability of conjugated proteins compared to the native proteins.[82,84,97] For instance, the peanut protein isolate–dextran conjugate had an improved thermal stability compared to the native protein.[77] It might be due to the higher thermal stability of the polysaccharide component compared to the protein. The covalently bonded polysaccharide helps to minimize the aggregation of protein molecules.[77] The thermal denaturation temperature of *Spirulina* protein concentrate was increased from 66.1 to 87.8 °C after its Maillard reaction-induced conjugation with maltodextrin indicating an increased thermal stability.[99]

4.2.4 Antioxidant activity. Several studies have reported the improved antioxidant activity of protein–polysaccharide conjugates compared to native proteins and their application to mitigate oxidative stress and inhibit lipid oxidation.[66,86] Han *et al.*[84] investigated the antioxidant activity of black bean protein after its Maillard reaction with different carbohydrates, including dextran, chitosan, and sodium alginate. Compared with native proteins, the resulting conjugates exhibited enhanced radical scavenging activity against both 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). It was because the intermediate compounds produced during the Maillard reaction could break the free radical chain reaction by supplying hydrogen atoms, thereby providing an antioxidant effect.[84] Similarly, Zhang *et al.*[87] also reported the improved antioxidant activity of *Spirulina* protein–maltodextrin conjugate, compared with the native protein.

Table 3 Optimum conditions, methods used for analysis and main findings of typical protein–carbohydrate complex coacervates

Biopolymers		Complex coacervation conditions				
Protein	Polysaccharide	Optimum pH	Optimum biopolymer ratio	Characterisation techniques	Main findings	References
Soybean protein isolate	Chitosan	6.0–6.5	4 : 1	(1) Zeta-potential (2) Turbidity measurement (3) Coacervate yield (4) FTIR and microstructure analysis (5) Thermodynamic profile obtained using DSC	The optimal conditions resulted in the maximum yield of coacervates. The complex coacervate exhibited enhanced thermal stability	101
Soy protein isolate	*Flammulina velutipes* polysaccharide	4.5	10 : 1 and 15 : 1	(1) Zeta-potential (2) Turbidity measurement (3) FTIR analysis (4) Viscoelastic measurement	The incorporation of *Flammulina velutipes* polysaccharide increased the β-sheet content within soy protein. In a low concentration of NaCl environment, coacervates with a compact structure were induced	102
Pea protein isolate	Alginate	2.75	4 : 1 and 8 : 1	(1) Zeta-potential (2) Turbidity measurement (3) Secondary structure analysis by Raman spectroscopy	The increase of biopolymer mixing ratios led to a corresponding increase in optimal pH values for coacervation. No significant change was observed in the secondary structure of pea protein following complexation with alginate	103
Pea protein isolate	Beet pectin	3.0	1 : 1	(1) Zeta-potential (2) Turbidity measurement (3) Viscoelastic measurement (4) Microstructure analysis by SEM (5) FTIR analysis	The increase in the biopolymer ratio from 1 : 1 to 20 : 1 led to a corresponding increment in the optimal pH value from 3 to 5.5. A more robust and compact coacervate structure was formed near the optimal pH value	104
Canola protein isolate	Chitosan	5.8–6.2	16 : 1	(1) Zeta-potential (2) Turbidity measurement (3) Coacervate yield (4) FTIR analysis (5) Thermodynamic profile obtained using DSC	The complex coacervates formed under optimal conditions exhibited higher thermal denaturation temperature and denaturation enthalpy compared to the native protein and polysaccharide	105
Chia seed protein isolate	Chia seed gum	2.7	6 : 1	(1) Zeta-potential (2) Turbidity measurement (3) Coacervate yield (4) FTIR analysis (5) Microstructure analysis by SEM (6) Thermodynamic profile obtained using DSC	The thermal stability of the complex coacervate was enhanced, and this enhancement was further augmented through cross-linking with transglutaminase	106

Table 3 (*Contd.*)

Biopolymers		Complex coacervation conditions				
Protein	Polysaccharide	Optimum pH	Optimum biopolymer ratio	Characterisation techniques	Main findings	References
Oak protein isolate	Gum Arabic	3.2	4 : 1	(1) Zeta-potential (2) Turbidity measurement (3) Thermal behaviour by DSC (4) Protein solubility test (5) Emulsifying properties	The solubility of oak protein isolate was improved after the complex coacervation process. The emulsions stabilised by the coacervates exhibited decreased droplet size and increased stability	107
Hemp protein isolate	Gun Arabic	3.5	2 : 1	(1) Zeta-potential (2) Turbidity measurement (3) Particle size measurement (4) Coacervate yield (5) FTIR analysis (6) Microstructure analysis by SEM	The optimal conditions for complex coacervation led to the highest coacervate yield and maximum mean particle size. The FTIR result substantiated the presence of electrostatic interactions between the protein and polysaccharide	108
Hemp protein isolate	Gum Arabic	3.5	4 : 1	(1) Zeta-potential (2) Turbidity measurement (3) Emulsifying properties (4) Emulsion stability	Decreased droplet size and improved stability against storage in the coacervate-stabilised oil-in-water emulsion. Hemp protein isolate-gum Arabic complex coacervate can be used as a novel emulsifier	109
Spirulina protein concentrate-maltodextrin mixture (1 : 1)	Carrageenan	3.0	24 : 1	(1) Zeta-potential (2) Turbidity measurement (3) Viscoelastic measurement (4) Thermal behaviour determined by DSC	The complex coacervate exhibited a shear-thinning behaviour and the highest viscosity under the optimal conditions	99

5. The effects of complex coacervation on the physicochemical properties of proteins

5.1 Optimisation of the complex coacervation process

As mentioned in Section 3.2, complex coacervation occurs between oppositely charged proteins and polysaccharides. The interaction is driven by electrostatic attraction to form soluble and insoluble complexes. The extent of complex coacervation is affected by several intrinsic factors, such as pH, mixing ratio, and ionic strength. These factors ultimately play an important role in the formation, structure, stability and rheological properties of the coacervates.[31,100] The complex coacervation process is optimised using the zeta-potential and turbidity data of the protein–polysaccharide mixture as a function of pH and the biopolymer mixing ratio (Table 3).

The pH of the solution is the most significant factor in the complex coacervation process, as it determines the degree of ionisation of functional groups (amino and carboxyl groups) present in biopolymers. Ultimately, their zeta-potential values of proteins, polysaccharides and their mixture as a function of pH can serve as indicators at what pH the formation of complex coacervates is at its peak. Complex coacervation occurs when the solution pH value decreases below the isoelectric point (pI) of proteins and above the acidic dissociation constant (pK_a) of polysaccharides. The optimal complex coacervation is achieved when the complex becomes electrically neutral. The pH value corresponding to this state is pH_{opt}.[105,106] During complex coacervation, the zeta-potential of the protein–polysaccharide mixture is monitored by carefully adjusting its pH values until pH_{opt} is detected.[25] Besides the zeta-potential of the mixture, its turbidity is another indicator to evaluate the extent of complex

coacervation. The turbidity value of the mixture increases when insoluble complexes are formed during pH adjustment. Published literature on this theme reports that turbidity keeps on increasing as the pH value moves closer to the pH_{opt} and is highest at this pH.[103,110]

The equilibrium of the electrostatic charge between proteins and polysaccharides also depends on their mixing ratio. Some examples of the optimum pH and biopolymer mixing ratio used to produce protein–polysaccharide complex coacervates are presented in Table 3.

Klemmer *et al.*[103] studied the effect of the protein-to-polysaccharide ratio on complex coacervation between pea protein isolate (PPI) and alginate (AL). The pH_{opt} for complex coacervation ranged from 2.1 to 3.3 with the increased PPI-to-AL ratio from 1 : 1 to 20 : 1. In Zhang *et al.*'s[99] work, a similar trend was observed when a *Spirulina* protein–maltodextrin mixture was complexed with carrageenan. An increase of the protein component in the system caused excess positively charged molecules. The excessive protein interacts with the relatively low polysaccharide content at higher mixing ratios and facilitates complex precipitation at relatively high pH values.[104] In other words, achieving the charge neutralisation becomes easier at higher pH values due to the relatively lower presence of negatively charged groups that need to be neutralised by the positively charged groups.[111]

Apart from the pH and biopolymer ratio, the ionic strength of the solution also affects the complex coacervation process *via* charge screening effects.[74] The aqueous phase with high ionic strength usually hinders the formation of complex coacervates between proteins and polysaccharides, which ultimately reduces yield and turbidity. However, low ionic strength sometimes promotes the formation of complex coacervates. Zhang *et al.*[102] investigated the effect of ionic strength on the complex coacervation between soy protein isolate and *Flammulina velutipes* polysaccharide. The authors reported that the complex coacervation process was accelerated in the presence of 10 and 50 mM NaCl. A further increase of NaCl concentration inhibited the interaction between polymers. The structure of the resulting complex coacervates was more robust and compact in the presence of NaCl.[102]

5.2 Characteristics of complex coacervates

5.2.1 Characterisation of complex coacervation using FTIR. FTIR enables one to gain insights into the characteristic interactions such as electrostatic interactions and hydrogen bonding occurring between proteins and polysaccharides during complex coacervation. The characteristic IR bands of proteins can be observed in three distinct regions of the FTIR spectrum, namely 1700–1600 cm^{-1} corresponding to amide I, 1550–1450 cm^{-1} corresponding to amide II, and 1229–1301 cm^{-1} for amide III. Lan *et al.*[104] reported that the characteristic peaks of amides I, II and III in pea protein isolate shifted towards the high wavelength range after the protein formed complex coacervates with sugar beet pectin. This was attributed to the electrostatic interactions between the amino groups of proteins and carboxylic groups of polysaccharides.[104] Naderi *et al.*[107] observed an enhanced hydrogen bonding in the complex coacervates, evidenced by a shift towards higher wavenumbers in the O–H stretching band in oak protein isolate-gum Arabic complex coacervates.

The secondary structure of proteins during complex coacervation can be measured using FTIR. In Zhang *et al.*'s[102] study, complex coacervation between soy protein isolate and *Flammulina velutipes* polysaccharide was conducted. The authors observed a decreased α-helix and an increased β-sheet content at pH_{opt}. It was due to the electrostatic interaction and the enhanced hydrogen bonding.

5.2.2 Solubility and thermal stability. The charge equilibrium and hydrophilic/hydrophobic balance of a protein are modified when it undergoes complex coacervation with a polysaccharide. Thus, it changes the protein solubility. Naderi *et al.*[107] observed a significant increase of solubility of oak protein around its isoelectric point when it underwent complex coacervation with gum Arabic. This increased solubility was attributed to the presence of a polysaccharide which hindered protein–protein aggregation and, thus, improved the protein's solubility. Carpentier *et al.*[112] made similar observations in pea protein isolate–tragacanth gum complex coacervates. These results suggest that complex coacervates could be used across a wide pH range. However, Klemmer *et al.*[103] observed a decrease of protein solubility in pea protein-alginate complex coacervates compared to that of the native protein around its isoelectric point due to the formation of precipitates. Depending on the strength of protein–polysaccharide interaction, the solid-dense phase can contain either coacervate- or precipitate-type structures.[113] In this particular system, the interaction between highly charged pea protein and alginate resulted in the formation of precipitate structures which promptly precipitated out of the solution and did not entrap solvent as much as the coacervate-type structure did.[114] The strong biopolymer (protein–polysaccharide) interaction promoted the precipitation of the polymer and consequently reduced the solubility.[115]

The thermal stability of protein is commonly assessed in terms of thermal denaturation temperature (T_d) and enthalpy change upon denaturation (ΔH), measured by differential scanning calorimetry (DSC). In general, complex coacervation increases the stability of proteins against thermal denaturation.[101,107] Chang *et al.*[105] reported that the T_d of canola protein isolate increased from 77.0 to 92.3 °C after its complexation with chitosan. Compared with native proteins, the complex coacervates usually have an increased ΔH. Timilsena *et al.*[106] reported similar results regarding forming complex coacervates using chia seed protein isolate and chia seed gum. The increased T_d and ΔH indicated the enhanced thermal stability of the protein and an elevated energy requirement for protein denaturation, potentially attributed to the formation of the compact internal structure within the coacervate through electrostatic interactions between proteins and polysaccharides.[116] The authors suggested that the enhanced thermal stability exhibited by these complex coacervates provides valuable insights for their potential application as shell materials in encapsulating thermally sensitive substances. As polysaccharides are relatively more stable against thermal

treatment, the covalent conjugation with proteins is expected to increase the latter's thermal stability.

5.2.3 Rheological properties. Change in the viscosity and viscoelastic behaviour of a protein–polysaccharide mixture after the formation of complex coacervates can indicate the progress of the complex coacervation process. Electrostatic interactions and hydrogen bonding drive the formation of structures and networks of complex coacervates. In turn, these supra-structures induce internal friction and offer greater resistance to motion in the aqueous medium.[31] Zhang *et al.*[99] measured the viscosity of *Spirulina* protein concentrate (SPC)-maltodextrin (MD) mixture/carrageenan (CG) coacervates as a function of pH and mixing ratio. The authors found that the coacervates exhibited shear-thinning behaviour due to the disruption of hydrogen bonding during shearing. In addition, a positive correlation between the viscoelasticity of complex coacervates and the strength of electrostatic interaction between the protein and polysaccharide was also observed. The highest viscosity, storage (G') and loss (G'') modulus were detected at the optimal pH and mixing ratio where charge neutralisation occurred. The strongest electrostatic interactions between the SPC–maltodextrin mixture and carrageenan were observed under optimum conditions of complex coacervation.[99] Lan *et al.*[104] investigated the dynamic rheological properties of pea protein isolate-sugar beet pectin coacervate. They observed that the elastic modulus (G') consistently exceeded the viscous modulus (G'') in the entire frequency range tested, indicating the formation of a highly interconnected gel-like network through electrostatic interactions between biopolymers.

5.2.4 Emulsifying properties. The protein–polysaccharide complex coacervates combine the surface-active nature of proteins and structure stabilizing (high viscosity and steric hindrance) nature of polysaccharides, marking them promising emulsifiers for oil stabilisation.[109,117,118] The complex coacervates can be applied *in situ* or *ex situ* as emulsifiers to stabilise emulsions (Fig. 4).

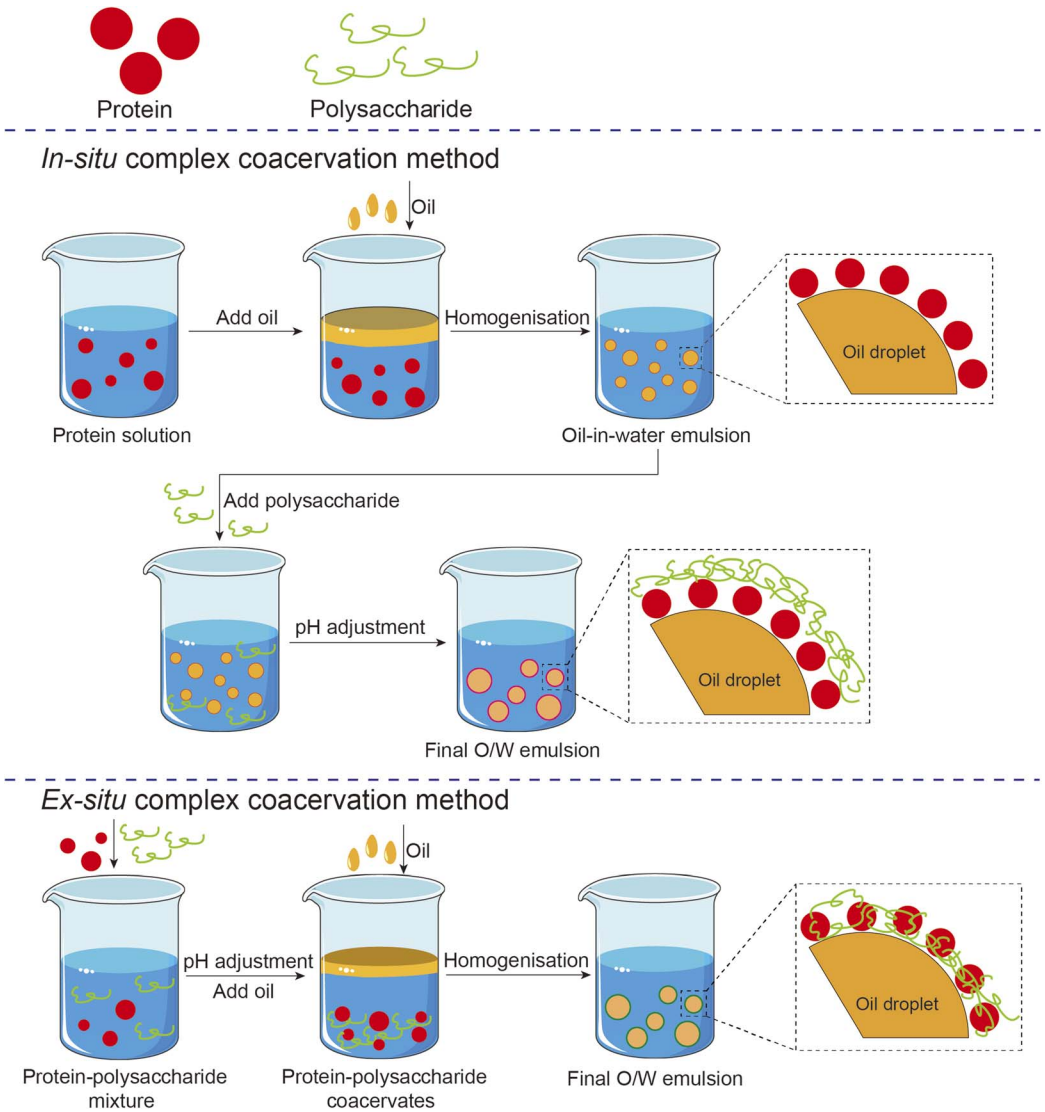

Fig. 4 Schematic diagram of *in situ* and *ex situ* methods of using complex coacervates to produce oil-in-water (O/W) emulsions.

Table 4 Application of some complex coacervates for microencapsulation of unstable and oxygen-sensitive oils

Core material	Wall material	pH	Polymer mixing ratio (w/w)	Core/wall ratio (w/w)	Encapsulation efficiency (%)	Conclusion	References
Chia seed oil	Chia seed protein isolate and chia seed gum	2.7	6 : 1	1 : 2	93.9	Microcapsules produced using a complex coacervate as the wall material exhibited high encapsulation efficiency and low surface oil content. Improved oxidative stability of entrapped oil during thermal treatment and storage	121
Chia seed oil	Soy protein isolate and gum Arabic	3.15	1 : 1 and 2 : 1	2 : 1	83.1 and 87	Increased encapsulation efficiency with the increase of the polymer mixing ratio from 1 : 1 to 2 : 1. Improved the thermal stability	122
Hemp seed oil	Pea protein isolate and sugar beet pectin	2.5	5 : 1	1 : 1, 1 : 2, and 1 : 4	79.6–95.8	The highest encapsulation efficiency was observed at a core/wall ratio of 1 : 4. The decrease in the core/wall ratio resulted in reduced oxidative stability of the core material	123
Flaxseed oil	Flaxseed protein and flaxseed gum	3.1	3 : 1	1 : 4	87.6	The spray-dried microcapsules exhibited low surface oil content, superior encapsulation efficiency and enhanced oxidative stability compared to the freeze-dried method	124
Flaxseed oil	Soya proteins and gum Arabic	3.15	1 : 1	N/A	81.5	The increase in oil load resulted in a decrease in both mean encapsulation efficiency and total yield	125
Fish oil	Soy protein isolate and inulin	4.0	2.5 : 1	N/A	69.3	The microcapsules exhibited resistance to release within the pH range of 5.5–6.5 and at temperatures ranging from 50 to 100 °C after the cross-linking with transglutaminase	126
Sweet orange oil	Soybean protein isolate and gum Arabic	4.0	1 : 1	1 : 10	83	The incorporation of sucrose during complex coacervation significantly enhanced the encapsulation efficiency. The microcapsules demonstrated excellent capacity for retaining flavour	127
Rose essential oil	Mung bean protein isolate and apricot peel pectin	4.1	4 : 1	1.25 : 1	89.9	Improved oxidation stability and sustained release during *in vitro* digestion	128
Microencapsulation using a Maillard reaction conjugate as an encapsulant through complex coacervation							
Stearidonic acid soybean oil	Gelatin and gum Arabic	4.4	1 : 1	3 : 1	99.8	Smaller particle size, improved antioxidant capacity of MRPs and improved oxidative stability. Enhanced rigidity of the capsule wall and improved colloidal stability	129 and 130

Table 4 (*Contd.*)

Core material	Wall material	pH	Polymer mixing ratio (w/w)	Core/wall ratio (w/w)	Encapsulation efficiency (%)	Conclusion	References
Cinnamaldehyde	Gelatin and low methoxyl pectin	4.3	6 : 1	2 : 1	91.4	Controlled glycation during the Maillard reaction resulted in improved encapsulation efficiency and decreased particle size. Improved pH and ionic strength stability	131
Vitamin E	Soy protein isolate and chitosan	6.3	4 : 1	1 : 2	67.3–78.6	The occurrence of the Maillard reaction when operating the complex coacervation at high temperatures. More compact microcapsule structure and improved storage stability	132 and 133
Canola oil	*Spirulina* protein concentrate–maltodextrin conjugate and carrageenan	3.0	24 : 1	1 : 3	92.5	Decreased microcapsule particle size, improved encapsulation efficiency. Improved thermal stability and oxidative stability. Controlled release during *in vitro* digestion	134

In the *in situ* complex coacervation method, an aqueous phase with the protein is first used to stabilise an oil-in-water (O/W) emulsion. The polysaccharide is then added to the emulsion and the pH is adjusted to induce complex coacervation. In this method, the protein component is usually oriented towards oil while the polysaccharide is oriented towards the water (Fig. 4). On the other hand, the *ex situ* complex coacervation method involves producing protein–polysaccharide coacervates first and using them as emulsifiers. Naderi *et al.*[107] conducted a comparative analysis of these two methods using oak protein isolate-gum Arabic complex coacervate for oil stabilisation. The authors observed that the emulsion stabilised using the *ex situ* method had smaller droplet size and better emulsion activity than the emulsions created using the *in situ* method. The complex coacervates stabilise the emulsions by adsorbing to and stabilization of the oil–water interface, irrespective of whether they are applied using *in situ* or *ex situ* methods. In contrast, Liu *et al.*[109] showed that the emulsion prepared using the *in situ* method exhibited better stability during storage. It was attributed to the exterior layer formed by polysaccharides, which enhanced electrostatic repulsion and steric stabilisation effects.[109]

6. Application of protein–polysaccharide conjugates and complex coacervates to encapsulate oxygen-sensitive oils

Several methods or processes are used to microencapsulate unstable and oxygen-sensitive oils including spray and freeze drying, and fluidized bed coating using coacervates and conjugates as shell materials.[119] The encapsulation efficiency of encapsulated oil can be significantly influenced by the properties of the wall material used.[120] This section briefly outlines the application of protein–polysaccharide conjugates, their complex coacervates, and conjugate-based coacervates as wall materials for encapsulating susceptible oils (Table 4).

6.1 Microencapsulation of susceptible oils using protein–polysaccharide conjugates

Proteins are widely used to stabilise susceptible oils by the contemporary food industry. However, proteins as encapsulating shell materials are susceptible to acidic pH near the proteins' isoelectric point, high temperature, and ionic strength. As in Section 4.5, protein–polysaccharide conjugates formed *via* the Maillard reaction are promising emulsifiers as they possess higher solubility, emulsifying properties and thermal stability. When a conjugate is used as an emulsifier, its protein component rapidly adsorbs at the oil–water interface to stabilise oil and the polysaccharide component provides strong steric repulsion and prevents the coalescence of oil droplets effectively.[135] Moreover, the antioxidant activity of the conjugate provides additional protection to the susceptible oils. The presence of a carbohydrate as a component of the wall matrix

also lessens the drying-related stress to the protein and improves the powder properties.[136]

The efficiency and effectiveness of various protein–polysaccharide conjugates to stabilise susceptible oils are a well-research theme.[137–139] Zhang *et al.*[140] produced a hydrolysed soy protein isolate–maltodextrin conjugate and used it to stabilise fish oil *via* emulsion evaporation technology. The authors observed that the microcapsules stabilised with a soy protein isolate–maltodextrin conjugate had lower surface oil and higher encapsulation efficiency than the microcapsules produced using their mixture. This improvement in the microencapsulation efficiency was attributed to the better emulsifying properties of the conjugate. The conjugates effectively decreased the O/W interfacial tension and help produce droplets with smaller size.[140] The conjugate stabilised microcapsule also exhibited improved oxidative stability compared to the one with the mixture as the wall material, which was attributed to the improved antioxidant acidity and emulsifying properties of the conjugate. A similar trend was observed by Wang *et al.*[141] when hemp seed oil microcapsules were prepared with a soy protein isolate–maltodextrin conjugate. The encapsulation efficiency of the microcapsule was shown to increase with the increase of the glycation degree.

The microcapsules stabilised by protein–polysaccharide conjugates have shown improved ability to protect the encapsulated compounds from premature release in a harsh gastric environment and deliver them to the intestinal stage of digestion. Yang *et al.*[139] investigated the digestibility of a citral microcapsule in a simulated gastrointestinal environment with a soy protein-soy polysaccharide conjugate as the wall material. Compared with the microcapsule stabilised by a protein–polysaccharide mixture, the one stabilised by the conjugate exhibited increased encapsulation efficiency and enhanced resistance against gastric digestion.[142,143] Covalent bonding between proteins and polysaccharides partially masked the pepsin binding sites of proteins and slowed down their access.[139]

6.2 Microencapsulation of susceptible oils using protein–polysaccharide complex coacervates

Protein–polysaccharide complex coacervates are effective encapsulants of susceptible oils as they have a stronger protective effect compared to individual proteins or polysaccharides.[144] In general, the microencapsulation process through complex coacervation commences with the formation of an oil-in-water (O/W) emulsion using a protein or protein–polysaccharide mixture as the emulsifier, depending on whether the *in situ* or the *ex situ* complex coacervation method is used (Section 4.2.4). Subsequently, the polysaccharide is added to the emulsion (*in situ* method), followed by pH adjustment to induce complex coacervation and facilitate the migration of coacervates on the surface of oil droplets.[31]

The microcapsules produced using complex coacervates as wall materials have desirable features including a compact structure, high oil loading and high encapsulation efficiency improved controlled release properties (Table 4).[122–125] For example, Timilsena *et al.*[121] encapsulated chia seed oil using chia seed protein-chia seed gum complex coacervates as the wall material. Compared with the microcapsule produced using chia protein, the one produced using the coacervate as the wall material showed much higher encapsulation efficiency (93.9%) and smaller particle size. In Lan *et al.*'s[123] study, hemp seed oil was encapsulated in pea protein isolate-sugar beet pectin complex coacervates. The authors achieved higher oil loading as well as higher encapsulation efficiency. RiosMera *et al.*[126] crosslinked soy protein isolate–inulin complex coacervates with transglutaminase to stabilise fish oil. Crosslinking was implemented to make the complex coacervate compact and robust. Compared with the non-crosslinked microcapsules, the transglutaminase crosslinked ones showed improved oil retention even at 100 °C. It was because the crosslinking significantly enhanced the integrity of the complex coacervate wall material surrounding the oil.[131]

Compared with unmodified proteins as the wall material at the oil/water interface, those made with complex coacervates are usually more rigid and less porous. Thus, complex coacervates are more effective in protecting the encapsulated oil against environmental stresses.[145] Kaushik *et al.*[124] produced flaxseed oil microcapsules stabilised in flaxseed protein-flaxseed gum complex coacervates. The authors reported an improved oxidative stability of the encapsulated oil in terms of reduced peroxidative values during a 30 day storage. Similarly, mung bean protein isolate-apricot peel pectin complex coacervates were used to encapsulate rose essential oil.[128] These microcapsules exhibited remarkable stability during *in vitro* oral and gastric digestion. The steric hindrance provided by the polysaccharide component in the coacervates inhibited pepsin's access to the protein component of the wall matrix and helped preserve the integrity, especially in the gastric stage. The encapsulated rose essential oil was successfully delivered to the targeted (gastric) stage of digestion and achieved the desired targeted delivery.

6.3 Microencapsulation of susceptible oils by using conjugate-based coacervates

A small number of publications recently applied covalent conjugation followed by complex coacervation to encapsulate oxygen-sensitive and unstable oils. In this approach, a protein–polysaccharide (*i.e.*, polysaccharide 1) conjugate is produced first. This conjugate is further coacervated with different polysaccharides (*i.e.*, polysaccharide 2). Here, the protein is modified twice, first by conjugation with polysaccharide 1 and then again using polysaccharide (2). The Maillard reaction is used to achieve conjugation. In some cases, complex coacervation is carried out between a protein and a polysaccharide and then heat treated to induce conjugation. In Huang *et al.*'s[133] study, vitamin E was encapsulated in soy protein isolate–chitosan complex coacervates followed by thermal treatment of microcapsules over 50 °C for 12 hours to induce the Maillard reaction between the protein and polysaccharide. Compared to the microcapsule produced using complex coacervates as the wall material, the ones produced using conjugated complex coacervates showed

significantly higher storage stability. Muhoza *et al.*[131] prepared gelatin-low methoxyl pectin conjugates *via* the Maillard reaction and used these conjugates to stabilise cinnamaldehyde. Then, the authors adjusted the pH to induce complex coacervation between the protein and polysaccharide components in the conjugate. Compared with microcapsules produced using conjugates as the wall material, the ones stabilised by conjugate-based complex coacervates showed higher encapsulation efficiency and stability against oxidation. The study conducted by Ifeduba and Akoh[129] also showed a similar trend in which stearidonic acid soybean oil was encapsulated in gelatin-gum Arabic conjugate-based complex coacervates. The authors reported that the formation of a secondary layer on the droplet surface through polysaccharide adsorption significantly enhanced the steric repulsion and improved the oxidative stability of the microcapsule.

Zhang *et al.*[99] developed a (protein–carbohydrate (1) conjugate)-polysaccharide (2) complex coacervate as a novel wall material for microencapsulation purposes. The schematic diagram illustrating the preparation of the microcapsule is presented in Fig. 5. Briefly, *Spirulina* protein and maltodextrin were conjugated *via* a controlled Maillard reaction in the initial reaction stage. The resulting conjugate was used as an emulsifier to stabilise canola oil in an O/W emulsion. Carrageenan gum was then added to the aqueous phase and the pH of the mixture was adjusted to induce complex coacervation. Finally, microcapsule powders were obtained through spray-drying. The authors reported that the microcapsules produced using the (protein–maltodextrin conjugate)-carrageenan complex coacervate exhibited enhanced encapsulation efficiency and oxidative stability in comparison to the ones produced using the (protein–maltodextrin mixture)-carrageenan complex

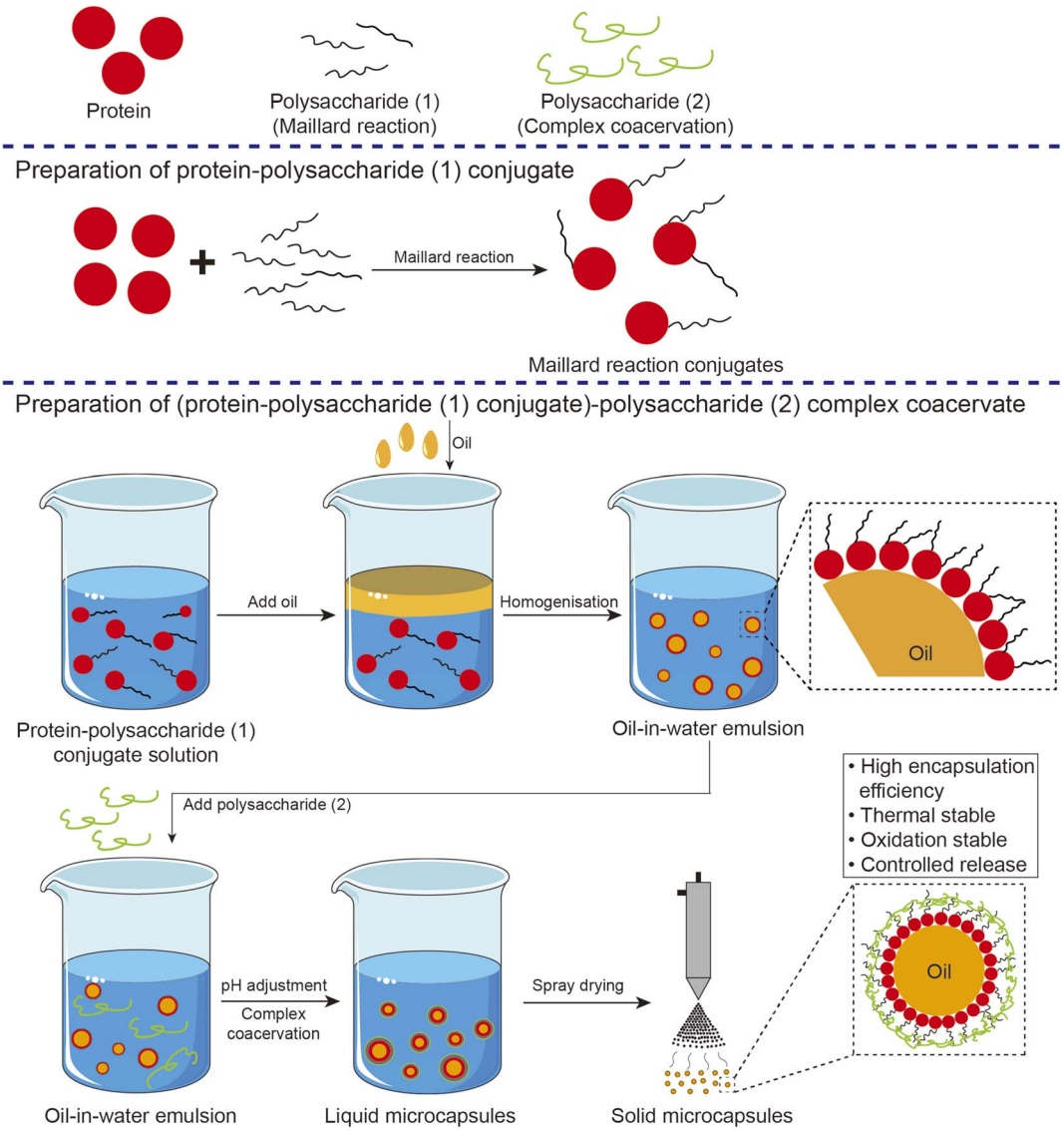

Fig. 5 Schematic diagram of microencapsulation using (protein–polysaccharide (1) conjugate)-polysaccharide (2) complex coacervates as wall materials. Here two different polysaccharides are involved. The polysaccharide (1) is used to form a conjugate with the protein and the polysaccharide and (2) is used to form a complex coacervate with the already conjugated protein.

coacervate as the wall material. The improved encapsulation efficiency was due to the improved emulsifying properties and antioxidant activity of the conjugate. The conjugate also helped form a rigid wall material, inhibiting oxygen access to the encapsulated oil.[99] These conjugate-based coacervate delivery system showed notable resistance against digestion in the *in vitro* oral and gastric environment, evidenced by limited protein hydrolysis and oil release. It was primarily attributed to the covalent bonding between the protein and carbohydrates in the conjugate and the formation of the robust microcapsule walls during the complex coacervation process.[134] Ultimately, the stabilised canola oil was released and hydrolysed in the simulated intestinal digestion stage achieving more efficient targeted delivery of the oil.

7. Concluding remarks and prospects

The modification of non-animal proteins, especially plant and algal ones, is gaining increasing research interest due to the need to improve their functional properties. Covalent bonding (*i.e.*, conjugation) and non-covalent complexation that can occur between proteins and polysaccharides can be used to alter the structure of proteins and enhance their techno-functional properties. The resulting conjugates and complex coacervates can be preferably used as emulsifiers and encapsulating shell materials of unstable and oxygen-sensitive oils. This review presents the underpinning science and associated technologies based on the Maillard reaction and complex coacervation whereby they are used to modify and improve the physicochemical properties. The protein–polysaccharide conjugates formed through the Maillard reaction are able to improve the solubility, thermal stability, antioxidant activity and emulsifying properties of these proteins. The protein–polysaccharide complex coacervates are also quite effective as emulsifiers and encapsulating shell materials. Several recent studies reported that the covalent conjugation followed by complex coacervation or complex coacervation followed by covalent conjugation is a much more effective approach to create protein–polysaccharide based emulsifiers and encapsulating shell materials. These conjugate-based complex coacervates are much more effective in encapsulating unstable and oxygen-sensitive oils. However, there is still a paucity of information, particularly on how to control and optimise conjugation and complex coacervation processes. The wet route of carrying out Maillard reaction-based conjugation is attractive; however, it is important to precisely control the temperature and time of the reaction to prevent it from progressing to the advanced stage. These parameters depend on the nature of the protein and polysaccharide used. The question of whether conjugation should precede complex coacervation or *vice versa*, *i.e.*, the optimal sequence, remains unresolved. The strength or tendency of the conjugated protein to electrostatically bind with a second polysaccharide remains unknown. The digestion or breakdown of conjugate-based complex coacervates under digestion conditions is still poorly understood. Most importantly, these approaches are not sufficiently applied to algal proteins.

Author contributions

Zijia Zhang has drafted this manuscript with input from and supervision of Benu Adhikari, Bo Wang, and Jie Chen. All authors provided critical feedback, wrote some parts, and contributed to the final version of this manuscript.

Conflicts of interest

The authors declare that there is no conflict of interests.

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