Article:
Akhtar, M orcid.org/0000-0002-8135-362X and Ding, R orcid.org/0000-0002-6263-1524 (2017) Covalently cross-linked proteins & polysaccharides: Formation, characterisation and potential applications. Current Opinion in Colloid & Interface Science, 28. pp. 31-36. ISSN 1359-0294

https://doi.org/10.1016/j.cocis.2017.01.002

Covalently cross-linked proteins & polysaccharides: Formation, characterisation and potential applications

Mahmood Akhtar, Rui Ding

PII: S1359-0294(17)30004-3
DOI: doi:10.1016/j.cocis.2017.01.002
Reference: COCIS 1093

To appear in: *Current Opinion in Colloid & Interface Science*

Received date: 30 October 2016
Revised date: 5 January 2017
Accepted date: 20 January 2017

Please cite this article as: Akhtar Mahmood, Ding Rui, Covalently cross-linked proteins & polysaccharides: Formation, characterisation and potential applications, *Current Opinion in Colloid & Interface Science* (2017), doi:10.1016/j.cocis.2017.01.002

Covalently cross-linked proteins & polysaccharides: formation, characterisation and potential applications

Mahmood Akhtar* and Rui Ding

Food Colloids Group, School of Food Science & Nutrition, University of Leeds, West Yorkshire, LS2 9JT, UK

Abstract

This review presents recent research conducted on the development of various protein-polysaccharide conjugates, their functional properties and industrial applications. These conjugates are formed by the glycosylation of food proteins with carbohydrates via the Maillard reaction and are capable of improving the functional properties of proteins. The Maillard reaction facilitates covalent bonding between a reducing group of a carbohydrate and an amino group of a protein under controlled conditions of temperature, time, pH, and relative humidity. There is a great deal of interest in modifying the functional properties of proteins and in the use of novel conjugates for various industrial applications. This review discusses various methods and their implications for preparing and characterising these conjugates. Furthermore, the physicochemical properties of conjugates such as solubility, thermal stability, emulsifying activity, emulsion stabilising properties, gelling and foaming properties are also analysed. A novel processing technology, spinning disc reactor, could be an alternative process for the production of protein-polysaccharide conjugates, with desirable functionality in different food systems.

KEYWORDS: Protein-polysaccharide conjugates, Maillard reaction, Glycation, Functional properties

1. Introduction

Proteins are important food ingredients due to their provision of the essential amino acids that are not synthesized in the human body, despite these being necessary for cellular metabolism. Proteins are amphiphilic molecules, which are able to adsorb strongly at the oil-water interface as highly effective emulsifiers. They are commonly used in the food industry in the stabilisation of oil-in-water emulsions [1-3]. However, the functional

properties of proteins may be lost under acidic conditions, high ionic strength, high temperature, and the presence of organic solvents, which limits their industrial applications. Thus, if proteins could be converted into more stable forms they could be more versatile in the food industry and elsewhere [4••, 5].

Polysaccharides are high-molecule-weight, highly stable, hydrophilic, and biodegradable natural polymers which are usually used as thickeners to modify the viscosity of the aqueous phase in order to stabilise emulsions. Generally, they have little oil-water interfacial activity compared to proteins due to the lack of hydrophobic segments. However, some natural polysaccharides, such as gum arabic (*Acacia Senegal*), have emulsifying properties. Gum arabic is a highly branched carbohydrate polymer comprising ~2 wt% covalently-bonded protein and it is this that is responsible for the surface activity of the gum [6•].

Protein-polysaccharide conjugates are produced via the Maillard reaction, first reported by Maillard in 1912 [7]. It is a series of non-enzymatic browning reactions, which occur naturally between the reducing end of a sugar and an amino acid. We can combine the functional properties of proteins and polysaccharides by covalently linking proteins to polysaccharides via the Maillard reaction to prepare novel protein-polysaccharide conjugates [5, 8•-10•]. The covalent bonding between proteins and polysaccharides may be expected to lead to an enhancement of protein functionality both as emulsifiers and stabilisers. The basic mechanism of the coupling is illustrated in Figure 1 [11••].

High molecular weight glyco-conjugates possess the properties of the protein, strongly adsorbing at the surface of oil droplets and also possess the hydrophilic properties of the polysaccharide, in being highly solvated by the aqueous medium [12]. The conjugation between proteins and polysaccharides provides much more improved steric stabilisation of the emulsion droplets, as illustrated in Figure 2.

The functional characteristics of proteins can be enhanced by means of physical, chemical or enzymatic treatments to obtain food ingredients for different applications [11••, 13]. However, the use of modified proteins as food ingredients, when achieved by chemical methods, is still limited due to some of the chemicals being, or perceived as being, harmful to human health [14-16]. One example is the use of cyanogen bromide to form the

ovalbumin-dextran compound that exhibit improved emulsifying properties compared to matrices containing pure ovalbumin [10•]. Thus, the search for naturally occurring chemical reactions remains necessary.

The main advantage of linking proteins to polysaccharides via the Maillard reaction is enhanced solubility and functional properties of proteins over a wide range of environmental conditions, such as low pH and high ionic strength [6•]. For example, gum Arabic, a natural glycoprotein, is generally used in the emulsification of citrus flavoured oil for soft drink applications. It has been suggested that Gum Arabic could be replaced by whey protein-maltodextrin conjugates as the emulsifier and stabiliser instead [9•]. Well-prepared protein-polysaccharide conjugates can show substantial improvement in emulsifying and stabilising properties compared to native proteins, under both low and neutral pH conditions and with colouring agents. Furthermore, the conjugates exhibited effective stabilisation of emulsions over several weeks even after extensive emulsion dilution [9•]. Recently, a comprehensive critical review has been published on food protein-polysaccharide conjugates obtained via Maillard reaction [17]. The review discusses various stages of the Maillard reaction and the products formed at each stage.

As shown in Figure 2, the conjugate-stabilised emulsion droplets have a thicker stabilising layer than protein-stabilised emulsion droplets, when the molecular weight of polysaccharide moiety is sufficiently large. In other words, the molecular weight of the polysaccharide determines the thickness of the steric stabilising layer.

2. Preparation of protein-polysaccharide conjugates

Proteins used as functional ingredients in foods are derived mainly from animal products, e.g., meat, eggs and milk, but plant proteins are increasingly used, e.g., from soy protein and peas. Similarly, most research in preparing protein-polysaccharide conjugates has been done on animal-derived proteins (caseins, whey, egg white) [18-20], though there have been some investigations on conjugates derived from plant-based proteins [21-23].

In the case of polysaccharides, dextran and maltodextrin have been most commonly used for preparing these conjugates [18, 24, 25] while other saccharides have also been investigated such as glucose, lactose and chitosan [21, 26, 27].

Over the last 25 years, both dry and wet-heating methods have been used for developing protein-polysaccharide conjugates. In 1990, ovalbumin-dextran conjugates were prepared under controlled dry-heating method by a group of Japanese researchers [20]. Two years later, a similar method was adopted to synthesize hybrids between three different proteins (11S globulin *icia faba*, bovine serum albumin, β-casein) and dextran in the Procter Department of Food Science, University of Leeds [28•]. The researchers carefully selected the incubation conditions of temperature, relative humidity (RH) 65% and reaction time 3 weeks. In 2003, the key parameters for the dry-heating, such as the incubation temperature (80˚C) and relative humidity (79%), were carefully selected and the reaction time was significantly reduced (2 hours) for preparation of conjugates [8].

The coupling of proteins and polysaccharides in aqueous solution via the Maillard reaction was not considered until 2008 [29]. A few years later, more research was carried out using a similar wet-heating methods to produce protein-polysaccharide conjugates [30-32]. The main benefit of wet-heating was to maximise the contact between protein and polysaccharide and eliminate the freeze-drying step prior to incubation, since freeze-drying is energy and time consuming [30, 31]. However, the dry-heating method is more desirable than wet-heating from an industrial point of view, due to ease of handling and long-term storage [4••].

In order to eliminate freeze-drying, new drying processing technologies have also been be considered, such as spray drying and roller drying [4••]. More recently, a novel approach, utilising a spinning disc reactor (SDR), has been tested. The SDR offers a lean process with significant benefits including removal of freeze drying and other process aids, significant reductions in processing time, plus decreased energy use [33, 34].

In the SDR, protein + polysaccharide solution is fed into the centre of a rotating disc, and is forced towards the edge and onto the surrounding walls by centrifugal force. A schematic diagram showing various pathways for the flow is presented in Figure 3. The disc can be heated (or cooled) and the centrifugal force creates a very thin film (measurable in μm) [35], giving high heat transfer coefficients between the disc and solutions [36]. When the solution leaves the spinning disc, it can be re-circulated providing a continuous process. The residence time on the disc is short, of the order of 0.4 seconds, but it is possible to reduce significantly the reaction time compared to the classic dry-heating method. SDR technology

has also been used for other food applications including production of concentrated apple juice [37•].

3. Characterisation of protein-polysaccharide conjugates

According to the literature, there are many techniques that have been used to confirm the formation of conjugates including: spectrophotometry [19, 22, 38], gel electrophoresis [8•, 18, 39], and chromatography [20, 25, 27].

Spectrophotometry is a rapid and useful technique for monitoring the browning process of the Maillard reaction and its reaction products, in the wavelength range 270-500 nm [19, 22, 29, 30, 38, 40, 41]. Generally, Maillard browning process is monitored at 420nm, though Zhu et al., (2008) reported a maximum absorbance peak at 304 nm for the formation of the Schiff base. Spectrophotometry requires few pre-treatments before the measurement and is probably the easiest and cheapest way to characterise protein-polysaccharide conjugates but alone is not adequate to confirm the synthesis of conjugates.

During conjugate formation the concentration of free amino groups decreases as they react with the saccharide reducing group. Therefore, the analysis of free amino groups in the Maillard reaction products (MRPs) can provide more direct information on the extent or the degree of conjugation. A significant number of studies have utilised the 2,4,6-Trinitrobenzenesulfonic acid (TNBS) method to determine the free amino groups in the protein-polysaccharide mixture after the heat treatment [18, 19, 24, 40, 42-44]. The reaction is illustrated in Figure 4. The TNBS reagent reacts with the free amino groups to form an orange coloured compound which can be analysed for by absorbance at 335 nm [45]. The method is widely accepted for the determination of free amino groups during protein hydrolysis. However, Nielsen et al., (2001) reported several disadvantages of this method: it utilizes a toxic, high risk chemical; it is time-consuming and requires a dark environment. They developed a new method using o-phthalaldehyde (OPA) which was then tested/utilised by many researchers and is now widely accepted as a more accurate method than the TNBS reaction for determining the free amino groups in protein-polysaccharide mixtures [16, 31, 38, 41, 46]. Figure 5 [48] illustrates the reaction scheme, which it is easier and quicker than the TNBS reaction, whilst the OPA reagent is less toxic and more stable.

(The OPA method is now used to monitor the proteolysis of milk proteins in dairy science [47]).

Electrophoresis is another commonly used method to confirm the formation of protein-polysaccharide conjugates, especially sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) [8•, 9•, 38, 42, 49, 50]. The intensity of the bands due to pure (un reacted) protein declines relative to that of the new bands due to the conjugates, in positions of higher molecular weight. Chromatographic techniques are usually chosen to assess the molecular weight (M_w) distribution of MRPs [20, 25, 30, 51, 52]. Size exclusion high performance liquid chromatography (SE-HPLC) is the most popular approach to investigating the M_w profile of MRPs. MRPs typically dissolved in a sodium phosphate buffer are injected into a specific column at a controlled flow rate and the elution monitored by a light scattered photometer at a selected wavelength, e.g., 214nm [24, 25]. The M_w of the conjugates can be estimated based on the elution profiles against the standard protein references [25]. Other approaches that have been applied to characterise protein-polysaccharide conjugates include fluorescence measurement [26, 32, 40], circular dichroism for their amino acid profiles [32, 38, 41], and matrix-assisted laser desorption ionization-mass spectrometry (MALDI-MS) for structure characterisation of protein-polysaccharide conjugates [26, 53•].

4. Functional properties of protein-polysaccharide conjugates

4.1 Solubility and thermal stability

To be effective as proteinaceous emulsifiers, sufficient solubility is a critical requirement [54•]. However, high solubility is usually difficult to achieve when environmental conditions are 'harsh'. For instance, when the pH of the aqueous phase is not far from the isoelectric pH (pI) the solubility is markedly lower for most proteins. The covalent coupling of proteins and polysaccharides can improve the solubility of native proteins under unfavourable conditions, due to the attached hydrophilic polysaccharide moieties. A number of researchers have demonstrated improvement in the solubility of proteins after glycosylation [9•, 22, 31]. Akhtar and Dickinson (2007) reported that the solubility of whey proteins was significantly enhanced after coupling with polysaccharides

around the isoelectric point (pI) [9•]. Similarly, a considerable improvement in solubility of soy proteins coupled with acacia gum was observed compared to the mixture of protein-acacia gum without the Maillard reaction [31]. However, contradictory solubility results were reported by Al-Hakkak and Al-Hakkak (2010) for egg white protein-pectin conjugates [43].

Another important test for protein stability is resistance to denaturation. Thermal treatment is inevitable in food processing, such as pasteurisation, which can lead to the denaturation of proteins. Previously, many investigations suggested the improvement of protein thermal stability when grafted to polysaccharides [19, 42]. Their results suggested that a more stable structure was formed during the Maillard reaction, most likely due to protein-protein interactions between denatured protein molecules being more hindered by the attached polysaccharide molecules [11••].

4.2 Emulsifying properties

Generally, it is expected that protein-polysaccharide conjugates possess better emulsifying properties than native proteins, particularly at a pH near the pI. A large number of studies have been conducted to investigate the emulsifying properties of protein-polysaccharide conjugates [8•, 20, 28•, 41, 42, 55-58•]. The critical parameters that significantly influence the emulsifying properties of the conjugates are the molecular weight of polysaccharide and the ratio of proteins and polysaccharide, though this has been studied to a lesser extent.

4.2.1 Influence of molecular weight of polysaccharide

Shu et al. (1996) reported that the emulsifying properties of lysozyme-galactomannan conjugates were enhanced significantly by increasing the molecular weight of the polysaccharide [42]. An optimum molecular weight of the polysaccharide was recognized to be 6 kDa. Other researchers have argued that the emulsifying properties may not be improved if the molecular weight of the attached polysaccharides is less than 6 kDa [11]. Furthermore, Akhtar and Dickinson, (2007) also confirmed that whey protein isolate-maltodextrin (DE 19) (M_w =8.7 kDa) conjugates, exhibited better emulsifying properties than the conjugates with maltodextrin (DE 2 and 47) [9•]. Theoretically, larger sugar moieties should provide increased steric stabilisation. However, if the polysaccharide is too large

compared to the proteins, it will reduce the adsorption of the conjugates onto the oil-water interface detrimentally. Thus, an optimum M_w should exist for a particular type of polysaccharide chain.

4.2.2 Influence of protein-polysaccharide ratio

Dickinson and Semenova (1992) investigated the effect of molar ratio of protein-dextran hybrids [28•]. Four years later, Shu et al. (1996) reported studies on lysozyme attached with 1 and 2 mol galactomannan, which showed that conjugates synthesised with a higher proportion of polysaccharides may have better emulsifying properties than that with a relatively low proportion of polysaccharides [42]. Akhtar and Dickinson (2007) also found a similar improvement of emulsifying properties of whey protein isolate: maltodextrin conjugates by increasing the proportion of polysaccharide from 1:0.5 to 1:3 (w/w) [9•]. However, recently Lam and Nickerson (2013) warned that if the content of polysaccharides is above a certain level, the non-linked polysaccharides may destabilise emulsions due to the depletion effect [59]. Therefore, it is critical to control the molar or the weight ratio of proteins and polysaccharides for synthesising effective conjugates.

4.3 Gelling and foaming properties

Protein-polysaccharide conjugates have also been explored for their gelling and foaming properties compared to native proteins [16, 60, 61]. Matsudomi et al. [60] compared the gelling properties of egg white-galactomannan conjugates with egg white protein alone and showed an increase of gel strength and water retention capacity. More recently, Spotti et al. [16] reported that WPI-DX conjugates significantly enhanced the mechanical properties of the gels, which could be subjected to as high as 80% deformation in a uniaxial compression test without fracture, far higher than the gel prepared by non/unconjugated WPI-DX mixture [16]. Although majority of studies have shown that protein-polysaccharide conjugates can have improved the foaming properties, β-casein-dextran conjugates showed a negative effect [61••].

5. Site-specific conjugates

Practically, the attachment of polysaccharide molecules to specific sites on protein molecules is extremely challenging, especially in the context of food. However, computer simulation has been used to estimate the theoretical behaviours of such conjugates. The interactions between adsorbed layers of α_{s1}-casein- polysaccharide conjugates have been investigated using the lattice-based self-consistent-field (SCF) theory, suggesting that short polysaccharide chains covalently bonded to proteins may have detrimental effects on colloidal stabilisation, especially when the attachment position is in the middle of the protein [62••].

6. Conclusions

In the search for better and more environmentally friendly emulsifiers and stabilizers, the glycosylation of proteins via the Maillard reaction can enhance the functional properties of native proteins and the conjugates produced have many potential uses in foods and other, non-food applications, such as drug delivery systems for controlled release of bioactive agents. New processing technologies, such as spinning disc reactor, are being developed that could provide alternative methods for their production. There is a need for further exploration of the fundamentals of Maillard reaction between proteins and saccharides in order to achieve a better understanding of the structure and functional properties of the conjugates formed.

Acknowledgments

It has been a great pleasure to work with Professor Eric Dickinson in the School of Food Science & Nutrition on various research projects. MA would like to thank Prof. Dickinson for introducing him to the area of protein-polysaccharide conjugates – it has led to our understanding of the formation and functional properties of the conjugates, for which we are very grateful.

References:

1. Dickinson, E., *Hydrocolloids as emulsifiers and emulsion stabilizers.* Food Hydrocolloids, 2009. **23**(6): p. 1473-1482.
2. Dickinson, E., *Hydrocolloids and emulsion stability*, in *Handbook of hydrocolloids*, G.W. Phillips, PA, Editor. 2009, Woodhead Publishing Ltd.: Cambridge. p. 23-47.
3. McClements, D., *Food emulsions*. Vol. 24. 1999, Boca Raton: CRC Press.
4•• Oliver, C.M., L.D. Melton, and R.A. Stanley, *Creating proteins with novel functionality via the Maillard reaction: A review.* Critical Reviews in Food Science and Nutrition, 2006. **46**(4): p. 337-350.
   This review shows a deeper understanding of modified properties of proteins especially from the processing conditions perspectives in the Maillard reaction. Moreover, it also suggests critical factors for designing functional conjugates via the Maillard reaction.
5. Liu, J., Q. Ru, and Y. Ding, *Glycation a promising method for food protein modification: Physicochemical properties and structure, a review.* Food Research International, 2012. **49** (1): p. 170-183.
6• Dickinson, E., *Interaction of polysaccharides with adsorbed proteins*, in *An Introduction to Food Colloids*, E. Dickinson, Editor. 1992, Oxford University Press: United States. p. 170.
   It establishes the foundation of covalently bonded protein-polysaccharide complex via the Maillard reaction by mimicing the natural mateiral gum arabic.
7. Maillard, L.C., *The action of amino acids on sugar; The formation of melanoidin by a methodic route.* Comptes Rendus Hebdomadaires Des Seances De L Academie Des Sciences, 1912. **154**: p. 66-68.
8• Akhtar, M. and E. Dickinson, *Emulsifying properties of whey protein-dextran conjugates at low pH and different salt concentrations.* Colloids and Surfaces B-Biointerfaces, 2003. **31**(1-4): p. 125-132.
   This research paper investigates the emulsifying properties of MRPs (Maillard reaction products) under unfavourable environmental conditions, and shows that the functional properties of modified proteins can be enhaced.
9• Akhtar, M. and E. Dickinson, *Whey protein-maltodextrin conjugates as emulsifying agents: An alternative to gum arabic.* Food Hydrocolloids, 2007. **21**(4): p. 607-616.
   This paper is the practice of the fundenmental theory (Reference No. 6) and suggests that the conjugates have better emulsifying properties than natural gum arabic. This provides a strong evidence to support the theory and lays down a solid foundation for further research.
10• Kato, A., K. Murata, and K. Kobayashi, *Preparation and characterization of ovalbumin-dextran conjugate having excellent emulsifying properties.* Journal of Agricultural and Food Chemistry, 1988. **36**(3): p. 421-425.
   This paper was published at the very beginning of the research area on modifying proteins via dry-heat treatment. It also proposed the basic structure of conjugates preparation and characterisation techniques for further researches.
11•• Kato, A., *Industrial applications of Maillard-type protein-polysaccharide conjugates.* Food Science and Technology Research, 2002. **8**(3): p. 193-199.

This review provides a summary of previous research on the topic and proposes the general mechanism of conjugates formation. Importantly, it also suggests great potential of conjugates for industrial application as a novel emulsifier and stabiliser.

12. Dickinson, E. and Y.B. Galazka, *Emulsion stabilization by ionic and covalent complexes of beta-lactoglobulin with polysaccharides.* Food Hydrocolloids, 1991. **5** (3): p. 281-296.
13. Du, Y., et al., *Physicochemical properties and emulsion stabilization of rice dreg glutelin conjugated with kappa-carrageenan through Maillard reaction.* Journal of the Science of Food and Agriculture, 2013. **93** (1): p. 125-133.
14. Hattori, M., et al., *Functional improvement of alginic acid by conjugating with beta-lactoglobulin.* Journal of Food Science, 1996. **61**(6): p. 1171-1176.
15. Nagasawa, K., et al., *Role of the polysaccharide content and net charge on the emulsifying properties of beta-lactoglobulin-carboxymethyldextran conjugates.* Journal of Agricultural and Food Chemistry, 1996. **44**(9): p. 2538-2543.
16. Spotti, M.J., et al., *Gel mechanical properties of milk whey protein–dextran conjugates obtained by Maillard reaction.* Food Hydrocolloids, 2013. **31**(1): p. 26-32.
17. de Oliveira, F.C., et al., *Food Protein-polysaccharide Conjugates Obtained via the Maillard Reaction: A Review.* Critical Reviews in Food Science and Nutrition, 2016. **56**(7): p. 1108-1125.
18. Kato, A., et al., *Functional casein-polysaccharide conjugates prepared by controlled dry heating.* Bioscience Biotechnology and Biochemistry, 1992. **56**(4): p. 567-571.
19. Jiménez-Castaño, L., M. Villamiel, and R. López-Fandiño, *Glycosylation of individual whey proteins by Maillard reaction using dextran of different molecular mass.* Food Hydrocolloids, 2007. **21**(3): p. 433-443.
20. Kato, A., et al., *Funtional protein polysaccharide conjugate prepared by controlled dry-heating of ovalbumin dextran mixtures.* Agricultural and Biological Chemistry, 1990. **54**(1): p. 107-112.
21. Usui, M., et al., *Enhanced bactericidal action and masking of allergen structure of soy protein by attachment of chitosan through Maillard-type protein-polysaccharide conjugation.* Nahrung-Food, 2004. **48**(1): p. 69-72.
22. Qi, J.-R., X.-Q. Yang, and J.-S. Liao, *Improvement of functional properties of acid-precipitated soy protein by the attachment of dextran through Maillard reaction.* International Journal of Food Science and Technology, 2009. **44**(11): p. 2296-2302.
23. Li, C., et al., *Physicochemical Properties of Dry-Heated Peanut Protein Isolate Conjugated with Dextran or Gum Arabic.* Journal of the American Oil Chemists Society, 2013. **90**(12): p. 1801-1807.
24. Nakamura, S., A. Kato, and K. Kobayashi, *New antimicrobial characteristics of lysozyme dextran conjugate.* Journal of Agricultural and Food Chemistry, 1991. **39**(4): p. 647-650.
25. Wong, B.T., et al., *The Effect of Maillard Conjugation of Deamidated Wheat Proteins with Low Molecular Weight Carbohydrates on the Secondary Structure of the Protein.* Food Biophysics, 2009. **4**(1): p. 1-12.
26. Medrano, A., et al., *The effect of glycation on foam and structural properties of beta-lactoglobulin.* Food Chemistry, 2009. **113**(1): p. 127-133.
27. Gu, F.-L., et al., *Structure and antioxidant activity of high molecular weight Maillard reaction products from casein-glucose.* Food Chemistry, 2010. **120**(2): p. 505-511.
28•. Dickinson, E. and M.G. Semenova, *Emulsifying properties of covalent protein dextran hybrids.* Colloids and Surfaces, 1992. **64**(3-4): p. 299-310.
This paper proposed the reasons of improved emulsifying properties of modified proteins and most importantly showed that the protein-protein interactions on the adsorbed interface were not significantly affected after the conjugation.

29. Zhu, D., S. Damodaran, and J.A. Lucey, *Formation of whey protein isolate (WPI)-dextran conjugates in aqueous solutions.* Journal of Agricultural and Food Chemistry, 2008. **56**(16): p. 7113-7118.
30. Zhu, D., S. Damodaran, and J.A. Lucey, *Physicochemical and Emulsifying Properties of Whey Protein Isolate (WPI)-Dextran Conjugates Produced in Aqueous Solution.* Journal of Agricultural and Food Chemistry, 2010. **58**(5): p. 2988-2994.
31. Mu, L., et al., *Physicochemical Properties of Soy Protein Isolates-Acacia Gum Conjugates.* Czech Journal of Food Sciences, 2011. **29**(2): p. 129-136.
32. Niu, L.-Y., et al., *Characteristics and functional properties of wheat germ protein glycated with saccharides through Maillard reaction.* International Journal of Food Science and Technology, 2011. **46**(10): p. 2197-2203.
33. Burns, J.R. and R.J.J. Jachuck, *Recent advances on spinning disc reactor technology: Better Processes for Bigger Profits*, in *5th International Conference on Process Intensification*. 2003: Maastricht, The Netherlands.
34. Jachuck, R.J., et al., *Process Intensification for Energy Saving.* Applied Thermal Engineering, 1997. **17**: p. 861-867.
35. Burns, J.R., C. Ramshaw, and R.J. Jachuck, *Measurement of liquid film thickness and the determination of spin-up radius on a rotating disc using an electrical resistance technique.* Chemical Engineering Science, 2003. **58**(2245-2253).
36. Auoune, A. and C. Ramshaw, *Process intensification: heat and mass transfer characteristics of liquid films on rotating discs.* International Journal of Heat Mass Transfer, 1999. **42**: p. 2543-2556.
37•. Akhtar M, C.P., Safriani N, Murray B, Clayton G, *Concentration of Apple Juice Using Spinning Disc Reactor Technology.* Journal of Food Processing & Technology, 2011. **2**(108).
This research paper is not directly related to protein-polysaccharide conjugates. However, the use of the SDR (spinning disc reactor) provides a a novel continous and wet-heating process which has a great potential to optimise the preparation of protein-polysaccharide conjugates for industrial applications (see Reference No. 11).
38. Xu, C.-h., et al., *Emulsifying properties and structural characteristics of beta-conglycinin and dextran conjugates synthesised in a pressurised liquid system.* International Journal of Food Science and Technology, 2010. **45**(5): p. 995-1001.
39. Li, Z., Y. Luo, and L. Feng, *Effects of Maillard reaction conditions on the antigenicity of alpha-lactalbumin and beta-lactoglobulin in whey protein conjugated with maltose.* European Food Research and Technology, 2011. **233**(3): p. 387-394.
40. Li, Y., et al., *Functional properties of Maillard reaction products of rice protein hydrolysates with mono-, oligo- and polysaccharides.* Food Hydrocolloids, 2013. **30**(1): p. 53-60.
41. Sun, W.-W., et al., *Study on the rheological properties of heat-induced whey protein isolate-dextran conjugate gel.* Food Research International, 2011. **44**(10): p. 3259-3263.
42. Shu, Y.W., et al., *Effects of the length of polysaccharide chains on the functional properties of the Maillard-type lysozyme-polysaccharide conjugate.* Journal of Agricultural and Food Chemistry, 1996. **44**(9): p. 2544-2548.
43. Al-Hakkak, J. and F. Al-Hakkak, *Functional egg white-pectin conjugates prepared by controlled Maillard reaction.* Journal of Food Engineering, 2010. **100**(1): p. 152-159.
44. Álvarez, C., et al., *Functional properties of isolated porcine blood proteins modified by Maillard's reaction.* Food Hydrocolloids, 2012. **28**(2): p. 267-274.
45. Adlernissen, J., *Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid.* Journal of Agricultural and Food Chemistry, 1979. **27**(6): p. 1256-1262.
46. Markman, G. and Y.D. Livney, *Maillard-conjugate based core-shell co-assemblies for nanoencapsulation of hydrophobic nutraceuticals in clear beverages.* Food & Function, 2012. **3**(3): p. 262-270.

47. Church, F.C., et al., *Spectrophotometric assay using ortho-phthaldialdehyde for determination of proteolysis in milk and isolated milk-proteins.* Journal of Dairy Science, 1983. **66**(6): p. 1219-1227.
48. Nielsen, P.M., D. Petersen, and C. Dambmann, *Improved method for determining food protein degree of hydrolysis.* Journal of Food Science, 2001. **66**(5): p. 642-646.
49. Liu, J.H., Q.M. Ru, and Y.T. Ding, *Glycation a promising method for food protein modification: Physicochemical properties and structure, a review.* Food Research International, 2012. **49**(1): p. 170-183.
50. Laemmli, U.K., *Cleavage of structural proteins during assembly of head of bacteriophage-T4.* Nature, 1970. **227**(5259): p. 680-&.
51. Kasran, M., S.W. Cui, and H.D. Goff, *Covalent attachment of fenugreek gum to soy whey protein isolate through natural Maillard reaction for improved emulsion stability.* Food Hydrocolloids, 2013. **30**(2): p. 552-558.
52. Bund, T., et al., *Chromatographic purification and characterization of whey protein–dextran glycation products.* Journal Chromatography, 2012. **A**(1244): p. 98-105.
53•. Corzo-Martinez, M., et al., *Interfacial and foaming properties of bovine beta-lactoglobulin: Galactose Maillard conjugates.* Food Hydrocolloids, 2012. **27**(2): p. 438-447.
This paper clearly shows the enhancement of foaming properties of modified globular proteins especially in acidic environment close to the pI of the protein.
54•. Halling, P.J., *Protein-stabilized foams and emulsions.* Crc Critical Reviews in Food Science and Nutrition, 1981. **15**(2): p. 155-203.
This reviews presents the critical importance of protein solubility in food colloidal systems such as emulsions and foams.
55. Tamnak, S., et al., *Physicochemical properties, rheological behavior and morphology of pectin-pea protein isolate mixtures and conjugates in aqueous system and oil in water emulsion.* Food Hydrocolloids, 2016. **56**: p. 405-416.
56. Pirestani, S., et al., *Effect of glycosylation with gum Arabic by Maillard reaction in a liquid system on the emulsifying properties of canola protein isolate.* Carbohydrate Polymers, 2017. **157:** p. 1620-1627.
57•. Chen, L., et al., *Improved Low pH Emulsification Properties of Glycated Peanut Protein Isolate by Ultrasound Maillard Reaction.* J. Agric. Food Chem., 2016. **64**: p. 5531-5538.
This recent paper provides a brand new method to prepare conjugates via ultrasound Maillard reaction instead of tradational heat treatments.
58. Bi, B., et al., *Characterization and emulsifying properties of β-lactoglobulin-gum Acacia Seyal conjugates prepared via the Maillard reaction.* Food Chemistry, 2017. **214**(1): p. 614-621.
59. Lam, R.S.H. and M.T. Nickerson, *Food proteins: A review on their emulsifying properties using a structure-function approach.* Food Chemistry, 2013. **141**(2): p. 975-984.
60. Matsudomi, N., et al., *Improvement of gel properties of dried egg white by modification with galactomannan through the Maillard reaction.* Journal of Agricultural and Food Chemistry, 2002. **50**(14): p. 4113-4118.
61••. Dickinson, E. and E. Izgi, *Foam stabilization by protein-polysaccharide complexes.* Colloids and Surfaces a-Physicochemical and Engineering Aspects, 1996. **113**(1-2): p. 191-201.
This paper highlights important factors for obtaining improved foaming properties of proteins: process time of preparation. Moreover, the type of protein is also critical for preparing conjugates.
62••. Akinshina, A., et al., *Interactions between Adsorbed Layers of alpha(S1)-Casein with Covalently Bound Side Chains: A Self-Consistent Field Study.* Biomacromolecules, 2008. **9**(11): p. 3188-3200.
This paper established a new method to investigate proteins modified with polysaccharides by computational simulation. It provided interesting evidence to explain the behaviours of conjugated proteins from theoretical point of view.

Figures

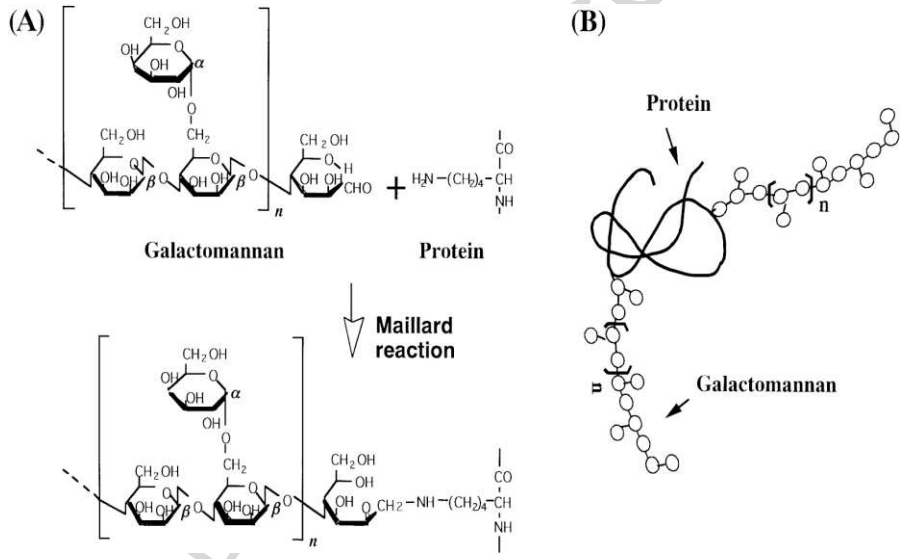
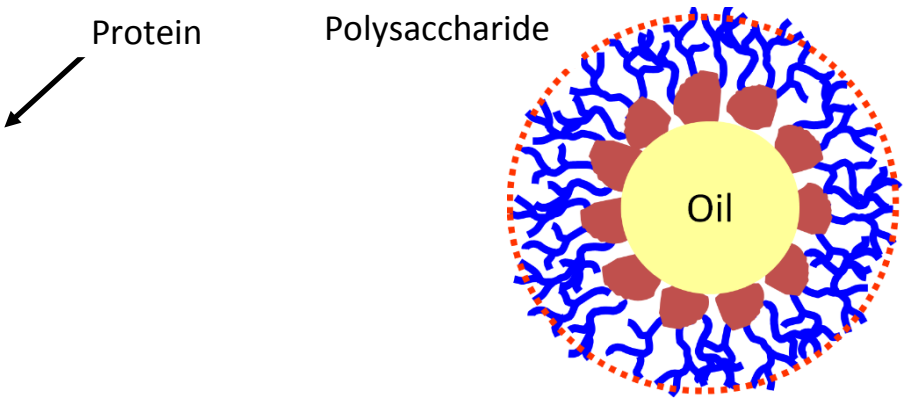


Figure 1. (A) The basic chemical reaction mechanism for the formation of protein-polysaccharide conjugates via the Maillard reaction (B) the overview structure of the conjugate [11].



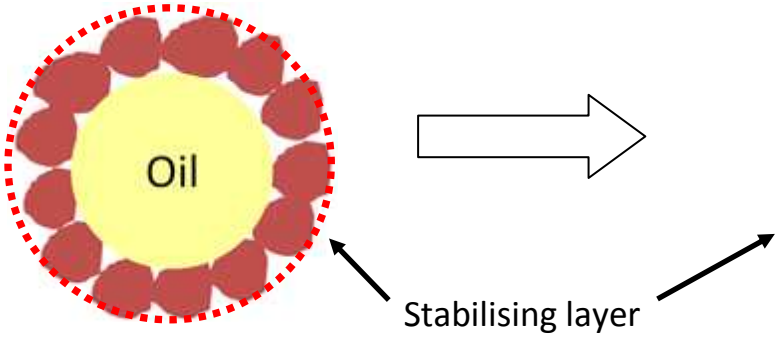


Figure 2. Comparison between protein- and conjugate-stabilised oil droplets in O/W system. The red blocks represent proteins; the blue branches represent polysaccharides.

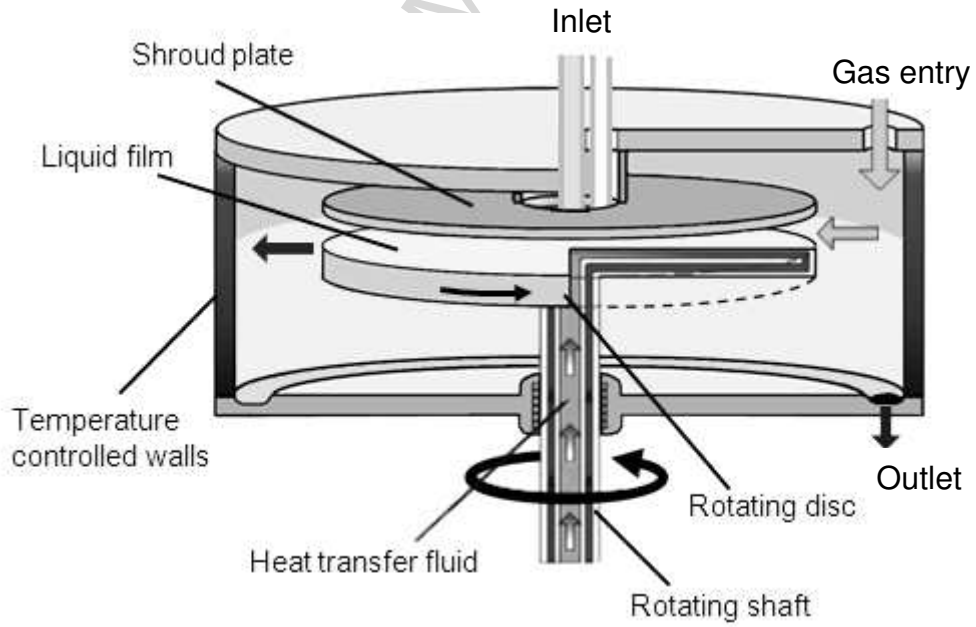


Figure 3. A schematic diagram of an SDR.

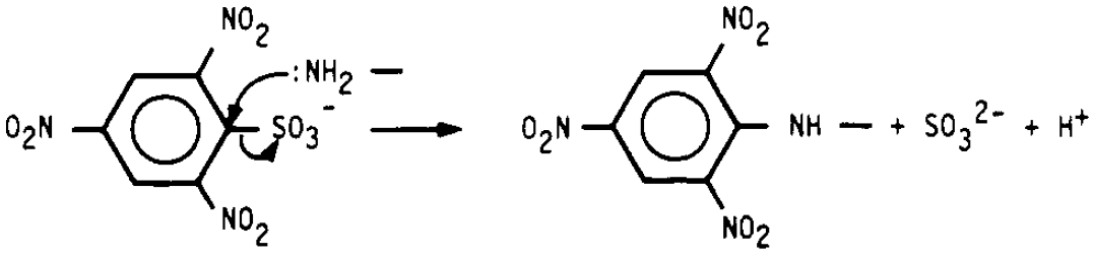


Figure 4. A schematic illustration of reaction between TNBS and amino groups.

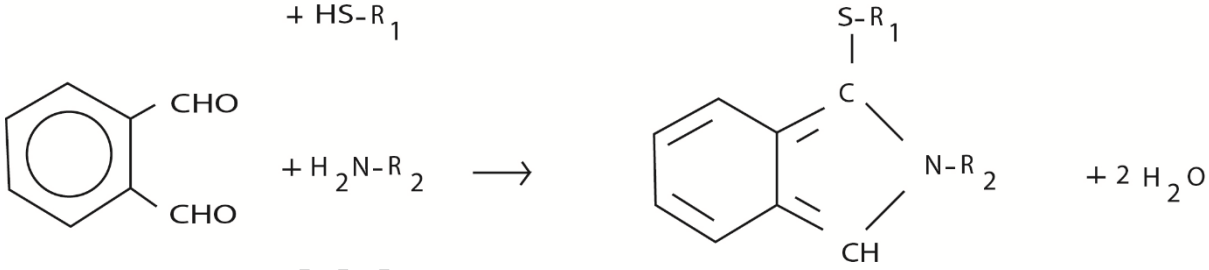
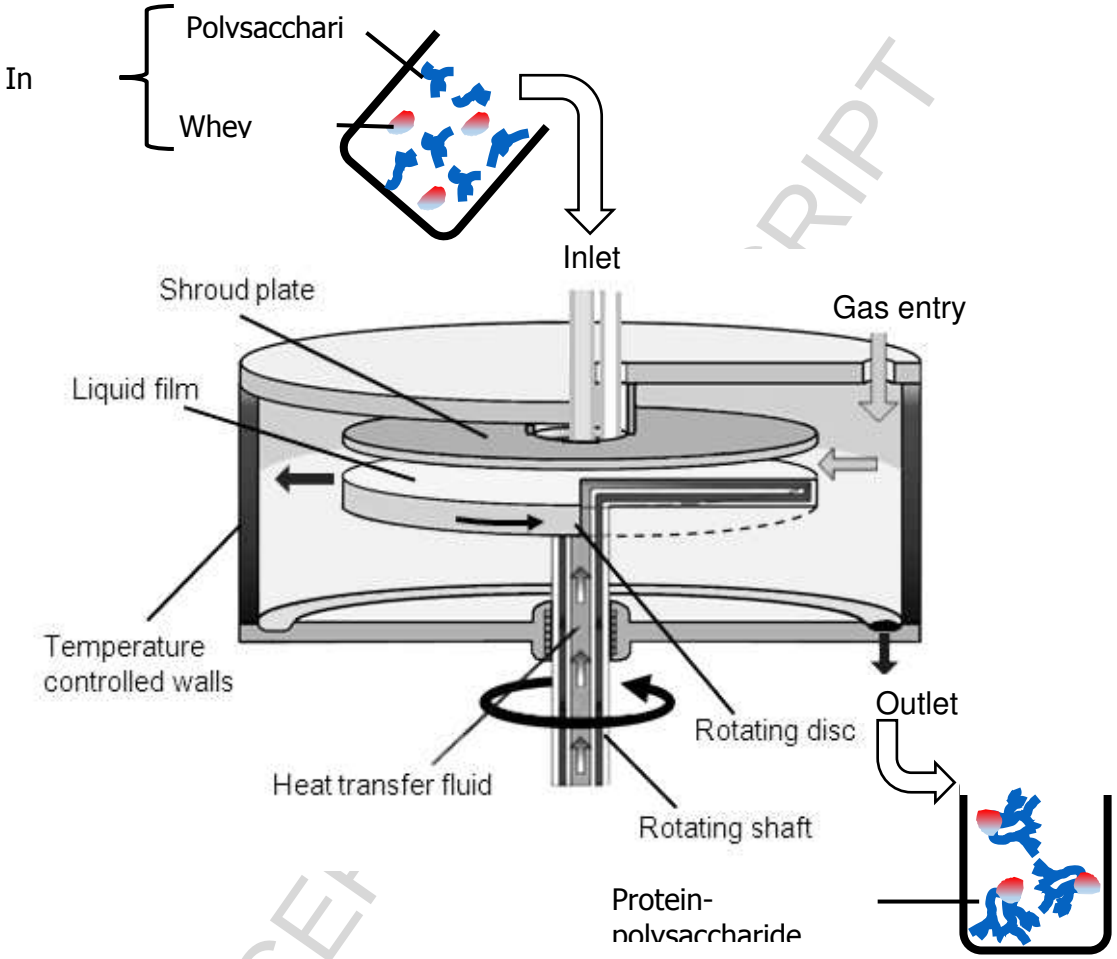


Figure 5. A chemical reaction of OPA with SH- and NH_2- groups to form detectable compound at 340nm.

Graphical Abstract



Highlights

- A novel process for preparing conjugates via Spinning Disc Reactor (SDR) has been presented.
- Solubility of proteins and their functional properties can be improved significantly by coupling with polysaccharides.
- Advances in protein-polysaccharide conjugates by dry and wet heating methods are compared.