



## Protein-polysaccharide complexes and conjugates: Structural modifications and interactions under diverse treatments

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### ABSTRACT

Proteins and polysaccharides are two essential biopolymers, each with unique functional properties. Proteins are known for their foaming, emulsifying, and gelling abilities but often face stability and solubility challenges, making them sensitive to environmental factors like light, temperature, and pH. In contrast, polysaccharides are known for their solubility and strong mechanical properties. When combined, these biopolymers demonstrate enhanced functional characteristics, a phenomenon known as synergy, which opens up various opportunities in the nutraceutical, pharmaceutical, and food industries. This review explores the interactions between proteins and polysaccharides through both covalent and non-covalent bonds. It examines how these biopolymers respond to external conditions such as temperature, pH, humidity, pressure, and salt concentration. Additionally, it briefly addresses how variations in concentration, ratios, and types within the biopolymers influence protein-polysaccharide interactions. Moreover, protein-polysaccharide complexes are extensively utilized in various applications that involve both sophisticated and conventional treatments including gamma irradiation, high-pressure processing, microwave heating, UV radiation, electron beam irradiation, pulsed electric fields, fermentation, and ultrasonication. Therefore, studying their behaviour and changes in interactions under different treatments is crucial. This review offers a brief overview of how these interactions are affected by various treatment processes.

### 1. Introduction

Protein and polysaccharide are essential components of food that belong to the category of Biopolymers. The interactions between these two essential components have a tendency to influence and modify the structural and functional characteristics of food (Doost et al., 2019). The interactions mentioned have a significant impact on the rheological characteristics and shelf life of food [1,2]. Proteins are essential constituents of human nutrition, composed of amino acids that are connected by peptide bonds (Dolganyuk et al., 2023). Proteins are categorized into five groups based on their origins. These include animal sources such as milk, eggs, fish, meat, microalgae, insects, and micro-organisms (fungi, yeast, bacteria), as well as plant sources including grains, legumes, and oilseeds (Dolganyuk et al., 2023; [3]). The presence

of allergens and anti-nutritional elements in plant-based proteins can impede nutrient absorption, potentially having a detrimental effect on health. Additionally, the existence of undesirable off-flavors, such as beany or bitter aftertastes, poses sensory challenges in plant-based protein consumption (Wang et al., 2022; [4]).

Animal proteins are more costly than other sources and their production has a detrimental effect on the environment, including increased greenhouse gas emissions and the use of water, land, and other resources for processing. Additionally, excessive consumption of animal-based proteins can lead to health issues such as type 2 diabetes, obesity, and cardiovascular diseases due to the high cholesterol content in meat. Proteins are large molecules that have a significant impact on the functional and nutritional aspects of food. They possess important functional characteristics such as high solubility, emulsifying, foaming,

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gelling, water holding, binding, retention, and adhesion capacities. These characteristics make proteins widely used as additives in the preparation of meat analogs and packaging materials like edible thin film packaging and encapsulation [4,5]. However, proteins have a drawback in that they struggle to maintain stability under certain conditions, such as high temperatures, pH variations, and salt levels. This instability often leads to denaturation, limiting the applications of proteins [5,6]. To address this issue, the incorporation of a polymer with high stability would be beneficial. One such natural polymer is polysaccharides, which consist of monosaccharides linked together by glycosidic bonds to form a complex carbohydrate [7].

Polysaccharides are known for their biodegradability, non-toxicity, economic viability, easy accessibility, and most importantly, their excellent stability and solubility [8]. The synergistic interaction between protein and polysaccharide in food systems can have remarkable effects on the overall structure, techno-functional properties, and stability in dispersed phases. This mutual association enhances these aspects in a way that cannot be achieved by protein or polysaccharides individually [9]. Colloidal coalescence of proteins and polysaccharides in water can result in phase separation, either into segregative or associative forms. This outcome is mostly determined by the electrical charges present on the biopolymers. The modulation of these electrostatic interactions is highly dependent on several parameters, including pH, the size and structure of the biopolymer, and the charge density of the ionic strength [10]. The production of a composite in an aqueous solution involves three distinct processes. In the early stage, there is an immediate and random process in which electrostatic contact acts as a strong coulomb force between polysaccharide-protein complexes. The subsequent phase is characterized by the systematic formation of intricate compounds through the reorganization of the internal structure via the

establishment of chemical bonds and the adoption of specific shapes. The ultimate stage of the polysaccharide-protein complex is referred to as the aggregation phase, in which the reconfigured complexes primarily come together as a result of hydrophobic interaction [7]. Based on this the objective of the review is to examine the mechanism of protein-polysaccharide interactions, the factors that influence these interactions, the role of protein-polysaccharide interactions in the food system, and the effects of processing treatments on protein-polysaccharide interactions. This study would provide practical insights for developing innovative products and solutions with various applications in food processing.

## 2. Mechanism of protein and polysaccharide interactions

Interactions of protein and polysaccharide occur in two mechanisms either by covalent or by non-covalent interaction [3]. In the covalent bond type, the protein-polysaccharide conjugates with enhanced thermal stability are formed by means of a reaction called the Maillard reaction. Due to the Maillard reaction and in a non-covalent bond like Hydrogen bonding, van der Waals, hydrophobic and electrostatic interaction are responsible for altering the techno-functional properties of food [2]. Non-covalent forces such as steric exclusion, hydrogen bonding, hydrophobicity, and electrostatic interactions allow proteins and polysaccharides to form protein-polysaccharide complexes (PPCs), which can be either transient or permanent. Due to their non-covalent structure, these complexes provide better techno-functionalities like improved solubility, surface activity, gelling, emulsifying, and foaming capabilities. Conversely, protein-polysaccharide conjugates (PPCOs) are formed when amino and carbonyl groups from polysaccharide chains are grafted onto protein molecules using covalent bonds. Glycosylation

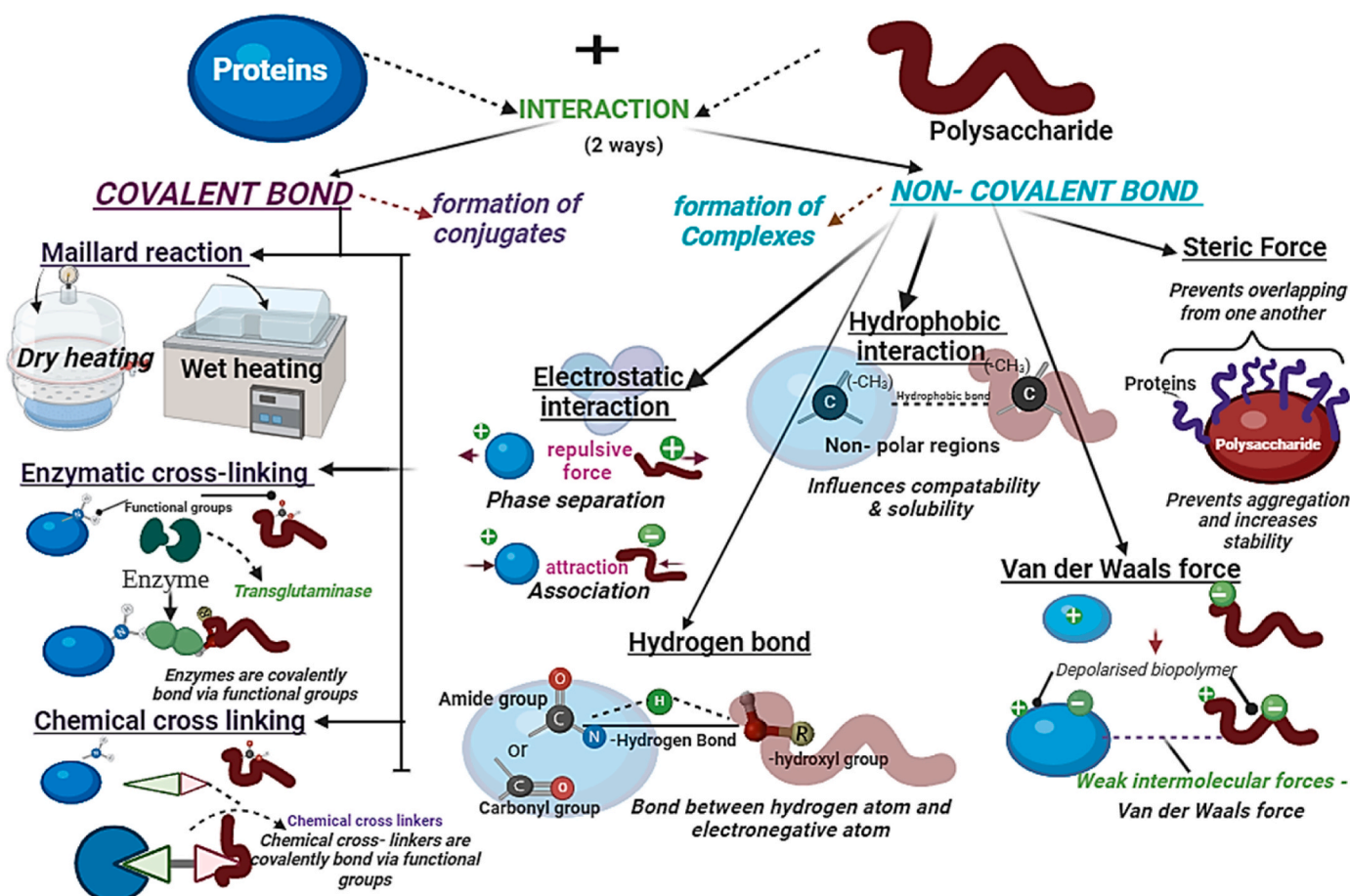


Fig. 1. Interaction of protein polysaccharide through covalent and non-covalent interaction.

causes conjugates to alter the secondary and tertiary structures, which results in improved techno-functionalities and modified micromorphological characteristics [7]. The interaction of protein polysaccharides through covalent and non-covalent interaction is presented in Figure (1).

### 2.1. Covalent bond between protein polysaccharide

Protein-polysaccharide conjugation involves enzymatic and chemical cross-linking conjugation. Protein and polysaccharide bonding are achieved through various enzymes such as transglutaminase, laccase, and peroxidase by enzymatic cross-linking which resulted in higher solubility and improved functional attributes such as stronger emulsifying ability and better gel properties [11,12]. Chemical cross-linking occurs when polymer chains are covalently linked and exposed to radiation, sulphur vulcanization, or a particular chemical reaction resulting in the formation of tri-dimensional networks which results in improving mechanical, barrier, and water-resistant characteristics [13]. Chemical cross-linking involves the use of short or long chain linkers or by use of cross-linking agents natural and synthetic are two types of agents. Chemical cross-link agents have high cross-linking efficiency and are easily accessible and affordable compared to natural cross linkers but certain chemical cross linkers are toxic/hazardous to tissues or cells. Glutaraldehyde is the most commonly used crosslinker for proteins or polysaccharides and on the other hand, natural cross-linkers are biocompatible, biodegradable, and less toxicity and some are challenging to handle leading to unpredictable and varying results such as cinnamaldehyde [13,14].

Maillard reaction is a non-enzymatic reaction also known as glycosylation, which is primarily centered around carbonyl interactions or by a covalent bond that occurs between carbonyl reaction between the polysaccharide terminal reducing carbonyl group and the free amino group of the protein and this chemical reaction occurs spontaneously by alteration and optimization of physical variables like pH, time, moisture control and temperature the interaction happens effortlessly without any involvement of additional additives [15,16]. Maillard reactions are broken down into three phases namely early, intermediate, and final these phases are interdependent and can take place concurrently, influenced by the conditions under which the reaction takes place [16]. During the early stages of the Maillard reaction, there is a formation of covalent bonds between protein and polysaccharide. Polysaccharides contribute to the unfolding of protein structure revealing the internal groups the structure shifts from being compact to more relaxed and illustrating in higher density of random coils like structure which is a secondary structure of protein [5]. Glycosylation occurs to generate N-glycosylamine which undergoes an irreversible rearrangement resulting in amadori product this phase has no discernible colour shift and because of the existence of reduced amino acids the nutritional value may drop. In the following phase, processes such as oxidation, fragmentation, enolization, dehydration, acid hydrolysis, and free radical reaction are done to modify amadori and external factors such as pH, time and temperature can contribute to the breakdown or degradation of amadori product. Furfural or hydroxymethylfurfural (HMF) depending on pH is formed when the amadori product enolizes which results in the production of fission products and reductones. In the advanced or intermediate stage carbonyl group condenses with the free amino group incorporating nitrogen into the reaction products Strecker degradation occurs where amino acids and dicarbonyl compounds react to form aminoketones and aldehydes additional processes that occur later at this phase include condensation, isomerization, rearrangement, cyclization, dehydration, and retro aldolization and the final stage results in the production of co-polymers and nitrogenous polymers called melanoidins which imparts in brown colour [16]. Reductone intermediates, heterocyclic compounds, and melanoidins are among the products of the Maillard reaction that have distinct biological capabilities such as antioxidant, antimicrobial, and antibacterial activities, and certain end

products such as acrylamide and heterocyclic amines because they might elevate the cytotoxicity and can lead to long term illness [5].

Dry heating and wet heating are the two conventional approaches to creating protein-polysaccharide conjugates via the Maillard process. A mixture of protein and polysaccharides are incubated and conditions such as humidity and temperature are regulated this process demands for extended reaction time which lasts up to several days or weeks hence this is not ideal for large-scale production additionally it poses challenges in controlling the reaction levels potentially resulting in the production of mixture of intermediate or advanced Maillard reaction which leads to yellow or brown colour and can also decline the protein quality [15,17]. The mix of protein and polysaccharide is dispersed in the water-based mixture and heated particular temperature and this process drastically reduces the reaction time to a few hours making it more suitable for large scale production in this process the Maillard reaction is deliberately regulated, focusing on the initial to intermediate stages which reduce the production of melanoidins which results in prevention of dark colouration and improved stability [15,17,18].

### 2.2. Non-covalent bond between protein polysaccharide

The major non-covalent bond between protein and polysaccharide interaction involves hydrogen bonding, steric exclusion, electrostatic, and hydrophobic interactions. The interaction in a given system depends on the type, nature (molecular weight, pH charge, density, hydrophobicity), and properties of the biopolymer molecules' external factors such as temperature, pressure, pH, ionic strength of the solution and its composition [19]. The interaction between protein and polysaccharide varies based on the charges of biopolymers, particularly polysaccharides. The uncharged polysaccharides engage in hydrophobic interactions with proteins and with ionic polysaccharides electrostatic interactions play a major role in the complex building process [3]. In comparison with covalent bond interaction non-covalent bonds are weaker [9]. Through its ability to facilitate contacts between dextran molecules and rice protein for vegan yogurt, hydrogen bonding holds an essential role in the creation of a network structure in yogurt. These connections strengthen the network's ability to bind water and promote cohesiveness. Yogurt viscosity is increased and phase separation is prevented by means of physical crosslinking connections between dextran molecules and proteins or other constituents, which further stabilize the network. Dextran's strong ability to bind water plays a vital role in preserving the texture and moisture content of yogurt. Additionally, the steric hindrance effects of the dextran molecules increase viscosity and enhance texture by impeding the movement of other constituents. The overall effect of these interactions is to improve the viscosity, stability, and quality of rice protein yogurt by forming a stable network with enhanced water retention ability [20].

Complex formation and stabilization, especially in aqueous environments like food systems and emulsions, are greatly aided by hydrophobic interactions occurring between proteins and polysaccharides. Stable complexes are formed as the outcome of these interactions facilitating the binding of polysaccharides and nonpolar regions of proteins. Hydrophobic interactions at the interface between oil and water in emulsion systems affect the adsorption behaviour and molecular arrangement, ultimately enhancing stability. These interactions create a protective layer around oil droplets, reinforcing the emulsion stability. By exposing hydrophobic groups on proteins and changing the shape of polysaccharides, temperature changes can have an effect on these interactions and the stability of complexes and emulsions. Protein-polysaccharide complexes are generally much more stable due to hydrophobic interactions, which also enhances the complexes susceptibility to destabilizing factors such as phase separation or coalescence [21].

Hydrogen bonds play a significant role in influencing the features of the tri-component system. By rupturing intramolecular hydrogen bonds and causing conformational changes in the protein molecule by adding

sodium alginate (SA) to the hydroxyapatite/silk fibroin (HAP/SF) composite improves intermolecular interactions. Numerous hydrogen bond types, including those formed between two hydroxyl groups, hydroxyl group with nitrogen atom, oxygen atom, and  $\pi$  bond are revealed through FTIR analysis and have an effect on overall intermolecular interactions. According to the study, composite interfacial interactions are enhanced by enhanced intermolecular hydrogen bond strength at optimal SA concentration. Beyond this concentration, however, hydrogen bond strength decreases, indicating a complex link between hydrogen bond interactions and protein-polysaccharide ratios. Therefore, the tri-component system's characteristics are shaped by the composite's protein-polysaccharide ratios, which have a substantial impact on hydrogen bond strength [22]. The strong dipole-dipole attraction between a hydrogen atom shared by two electronegative atoms causes hydrogen bonding, which stabilizes the system and leads to a favourable drop in enthalpy ( $\Delta H$ ).

On the flip side, van der Waals forces are weak molecular attractions carried on by transient fluctuations in the distribution of electrons, which result in induced dipoles. van der Waals interactions, although weak, also exhibit a negative enthalpy change ( $\Delta H$ ), implying an effective interaction that maintains system stability (Yang et al., 2020). (Yang et al., 2020), lactoferrin (LF) and oat  $\beta$ -glucan (OG) complexes were examined at pH 5.0 and 25 °C using various OG concentrations including 0.9 %, 0.5 %, and 0.2%w/v). van der Waals forces are important in the interactions between proteins and polysaccharides because they are the result of attractive forces between molecules caused by transitory dipoles created by electron fluctuations. van der Waals forces with other forces such as electrostatic and hydrogen interactions specifically contribute to the self-assembling behaviour of LF-OG complexes, stabilizing the complexes and affecting their structural properties such as particle size, and zeta potential.

In protein-polysaccharide interactions, steric forces act as a physical barrier that prevents large polysaccharide molecules from coming close to one another, which inhibits clumping by preventing particle aggregation and hindering movement and helps maintain the stability of the system. Additionally, unlike the electrostatic repulsion seen in protein layers, the overlapping of polysaccharide layers creates a repulsive contact, primarily due to steric factors. Bigger molecules exert a repelling effect on one another, preventing them from aggregating and further improving stability. These steric forces are greatly influenced by the size and configuration of biopolymer species at the interface, which encourage repulsion between surfaces and stabilize the colloidal system via steric hindrance and repulsion mechanisms [23].

### 3. Factors affecting the protein-polysaccharide interaction

The formation and stability of protein-polysaccharide complexes involve certain factors which are classified as extrinsic and intrinsic factors. These factors are pivotal in facilitating both initial formation and subsequent maintenance and stability of protein-polysaccharide complexes. Extrinsic factors include a variety of environmental conditions such as pH, pressure, temperature, salt concentration, moisture or humidity as well as the involvement of chemicals and enzymes and intrinsic factors imply the type, structure, nature (ionic strength, charge density, conformation) of biopolymer ratio and concentration of biopolymer [24].

#### 3.1. Extrinsic factors

##### 3.1.1. Impact of temperature on protein-polysaccharide interaction

Temperature is a critical factor in the formation and stabilization of protein polysaccharide complexes particularly in the Maillard reaction, the thermal denaturation of proteins leads to the loosening of protein structure and exposure to all functional groups. when it is heated beyond its denaturation temperature, it induces changes in its molecular conformation altering into a secondary or tertiary structure and changes

in colour [5,24]. Thermodynamic incompatibility is mainly due to entropy and complex coacervation is responsible due to both entropy and enthalpy [25]. A study was conducted on soybean protein isolate-chitosan coacervation at different temperatures 50 °C, 70 °C and 90 °C on the Maillard reaction with 1.5 % (w/v) maltose in the preparation of microcapsule to initiate the Maillard reaction. At 50 °C the microencapsulation efficiency at 74.74 % and yield is increased by 36 % which is 76.59 % as well as enhanced emulsifying activity and bigger size of microcapsule i.e. 1.19  $\mu$ m compared to the size of the control 0.42  $\mu$ m. At 70 °C, further improvement in yield of 88.43 % and efficiency of 72.49 % with increased solubility and even bigger size microcapsules of 3.30  $\mu$ m were observed. The Maillard reaction improved stability against dissolution, while the microencapsulation performance slightly reduced at 90 °C. Comparatively, the optimal results were obtained at 70 °C, demonstrating enhanced solubility, stability, and performance. As a result, distinct temperatures can lead to variations in size, solubility, and conformational changes. At high temperatures, hydrophobic interaction becomes influential either by strengthening or weakening the interactions which have an effect on coacervation. furthermore, temperature changes have an impact on electrostatic interaction influencing the stability and functional properties (emulsion, foaming ability) of the resulting microcapsule (Hang et al., 2020). Heat can initiate the gelation process in the protein-polysaccharide complex leading to an increase in flexibility. At the same time, cooling the complex can lead to a firmer gel structure, which will influence the combination's overall elasticity [26]. In double emulsion of xanthan gum (XG) and pea protein isolate (PPI), temperature influences stability. PPI emulsions at higher temperatures are less stable. PPI-1% XG emulsion, on the other hand, exhibits greater stability at lower temperatures (5 °C–25 °C). However, increasing the temperature to 45 °C or 65 °C produced bigger droplets which may indicate the degradation of the protein polysaccharide network and droplet clumping [27]. Solubility in rice protein and dextrin is increased with increasing the temperature from 70 °C to 85 °C by 19.97 % and eventually, the solubility drops above 85 °C. Temperature plays a substantial role in triggering the glycation reaction with elevated temperature being associated with increased reaction time and also results in browning [28]. Exposure to temperatures beyond their denaturation threshold often leads to denaturation, and aggregation and ultimately forms a gel network in globular proteins [29]. The behaviour of protein polysaccharides during exposure to low and high temperatures is presented in Figure (2).

##### 3.1.2. Impact of pH on protein-polysaccharide interaction

The pH level greatly affects the stability of complexes because it affects how the polymer interacts with each other and with the surrounding solvent. Variations in pH could result in the separation of dispersed biopolymers from the solution [30]. The influence of pH on the charge density of the polysaccharides would lead to network formation [31]. The net charge of proteins and polysaccharides is broadly influenced by pH. The isoelectric point (pI) or the point at which a protein's charge changes from positive to negative or vice versa is an essential property. Changes in pH can alter the electrostatic interactions that cause proteins and polysaccharides to segregate or associate due to incompatibility with the solvents proteins and polysaccharides are least soluble near the isoelectric point (pI), which may cause proteins to precipitate [24,30]. Under high pH conditions (above the isoelectric point) stable dispersion can occur due to electrostatic repulsion between the negatively charged polysaccharides and protein. On the other hand, at low pH conditions, protein-polysaccharide complexes can form through electrostatic attraction between positively charged proteins and negatively charged polysaccharides [3].

pH fluctuations greatly impact biopolymers affecting the physical properties and weight of biomolecules. Proteins may denature at low pH which would increase weight and decrease solubility and higher elasticity in protein-polysaccharide mixtures, while at neutral pH, stability would enhance the stability and solubility by reducing the weight [26,

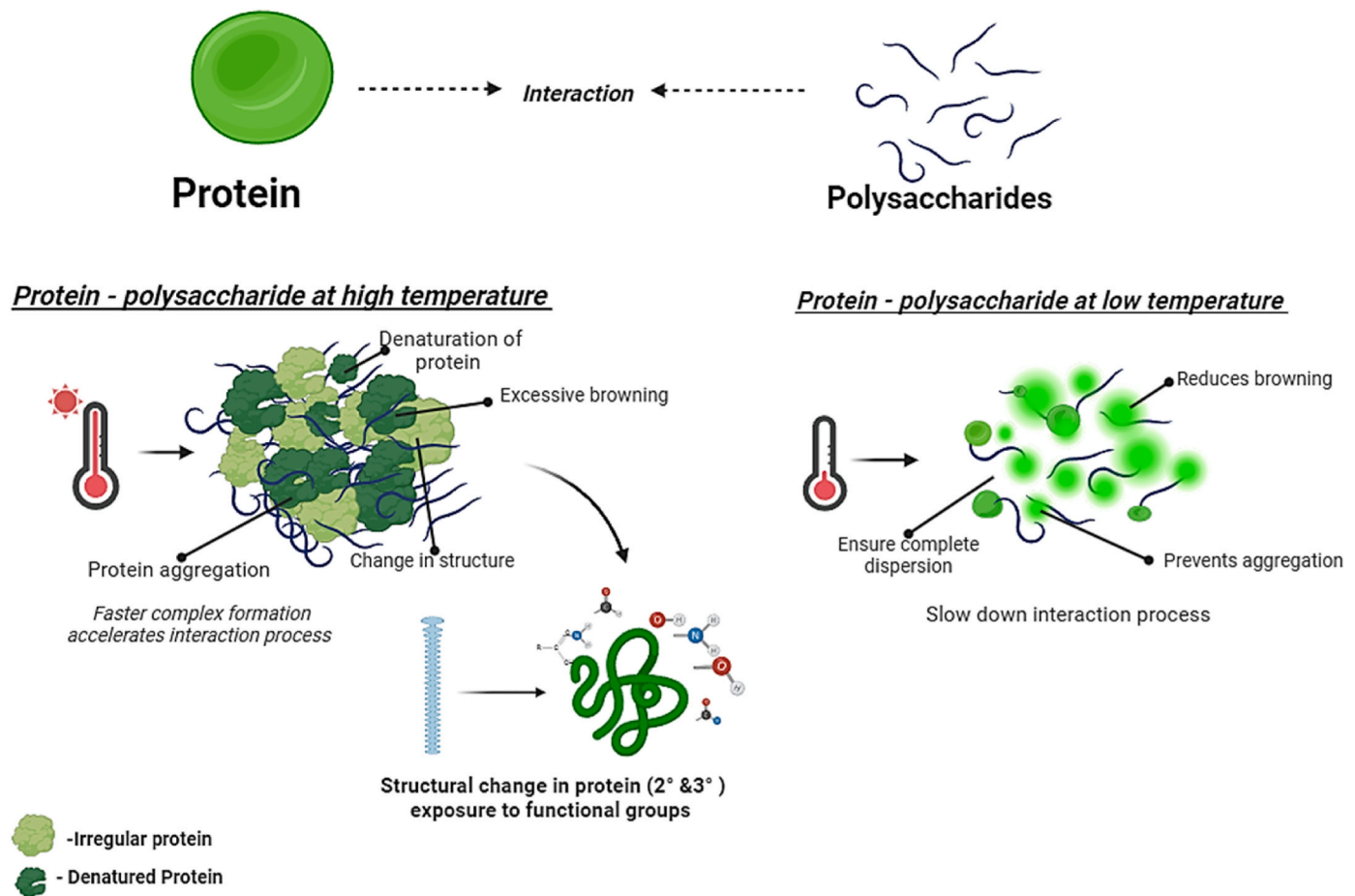


Fig. 2. Behaviour of protein polysaccharide during exposure to low and high temperature.

30]. Study of the interpolymeric complexing between xanthan gum and egg pH values ranging from 2.0 to 8.0. Variations in key pH values ( $pH_c$ ,  $pH_{41}$ , and  $pH_{max}$ ) and the protein-polysaccharide ratio significantly influenced the behaviour of these complexes. At particular pH levels insoluble complexes formed, indicating a transition from soluble to insoluble states driven by ratios and electrostatic interactions. Electrostatic forces played a crucial role in regulating complex behaviour, affecting protein binding availability. Variations in pH and salt concentration also altered the turbidity and kinetics of protein-polysaccharide complexes [10].

pH variations in the solution play a critical role in controlling how proteins like SC II interact with anionic polysaccharides. SC II exhibits a high positive charge at lower pH levels, which diminishes as pH rises until it reaches its isoelectric point around pH 5.2. While anionic polysaccharides remain negatively charged. This charge interplay is key for complex coacervation with pH also affecting SC II's secondary structure and its binding affinity with polysaccharides. The significance of pH control for protein-polysaccharide interactions is thus highlighted by the necessity of maintaining ideal pH settings for the development of stable complexes with desirable characteristics [32]. In the casein-carboxymethylcellulose (CA: CMC) system, pH significantly influences the production and behaviour of nano/microparticles. At lower pH (2.5–4), CMC chains are less negatively charged and interact more with larger, partially positively charged casein chains. As pH increases to moderate levels pH (5–7), CMC chains become more stretched and negatively charged, altering electrostatic interactions. At higher pH (8–10), both CMC and casein chains become more negatively charged, increasing electrostatic repulsion. These pH-driven changes regulate the characteristics of the complexes and nano/microparticles, influencing

their charge, size, interactions and rheological properties, with higher CMC concentrations leading to increased viscosity and viscoelasticity, especially at pH 4, which effects the system's stability and flow behaviour [33].

Black soldier fly larve albumin (BSFLA) undergoes structural changes when the pH is adjusted, especially at pH 12, which unfolds the structure and reveals interior hydrophobic groups. Interactions with pectin are facilitated by this change. Through a variety of interactions, including hydrophobic interactions, electrostatic attraction, and hydrogen bonding, the unfolded BSFLA structure bonds to pectin more successfully. These interactions lead to the formation of complexes with increased surface charge and smaller particle sizes, reducing protein aggregation. As a result, emulsifying capabilities are improved, leading to more stable emulsions, better adsorption at interfaces and overall enhanced functional properties of the system [34]. As the pH became closer to the protein isoelectric point (pI) it was observed that complexes between lentil proteins and pectin formed through electrostatic interactions. The pH-dependent interactions between protein and polysaccharides can influence the stability and strength of the complex and can also alter the type and nature of interaction affecting the overall structure and properties of the protein-polysaccharide complex [35]. The effect of pH and salt on protein-polysaccharide interaction is presented in Figure (3).

### 3.1.3. Impact of salt concentration on protein-polysaccharide interaction

A comparison of the gel-like complex between two egg proteins i.e. lysozyme and ovalbumin with xanthan gum and the inclusion of salts affects in several ways. In the ovalbumin/xanthan gum complex NaCl gradually reduced the turbidity curves and reached a minimum of 37 %

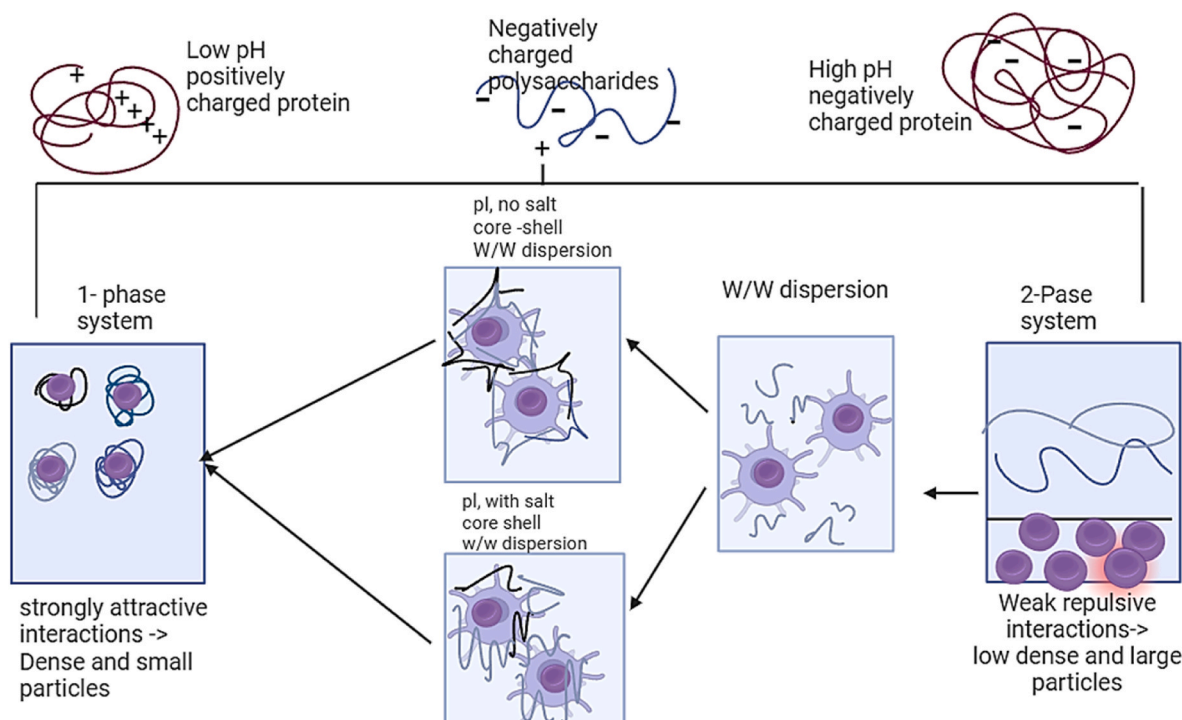


Fig. 3. PH and salt influencing the protein polysaccharide interaction.

with 0.4 mol/L NaCl and turbidity is mainly due to a decrease in protein solubility at high acid pH (2.0–1.0). On the contrary Lysozyme-xanthan gum complex, the turbidity has decreased from 0.1 mol/L to 0.4 mol/L NaCl, and the interaction between the polymers is suppressed by the screening effect. Increased concentration of NaCl in both complexes enabled  $\text{Na}^+$  to compete with the protein positive charge binding site and bind with polysaccharide. Meanwhile  $\text{Cl}^-$  competed with the negatively charged xanthan gum site which binds to protein eventually lowering binding affinity. High concentrations of salt ions have caused a reorganization around the protein molecules by forming a strong between water molecules and salt ions leading to weaker intermolecular electrostatic interaction between protein polysaccharides. Hence salt influences protein-polysaccharide interaction by screening effects, weakening electrostatic interactions, competing with binding sites, and affecting solubility resulting in the weaker gel-like coacervate and higher water content [10]. A greater level of salt ions can affect the stability of the protein [36]. Mesona blumes polysaccharide-soy protein isolates mixed gel is greatly influenced by the addition of salt ions, particularly sodium and calcium ions. Lower ion concentration (0.005–0.01M) induces the gel by enhancing the viscosity, and elasticity of the gel. It also shows improved thermal stability and water-holding ability meanwhile at higher concentration of ions (0.015–0.02M) leads to reduced strength of gel and calcium ions have a more noticeable influence than sodium ions. Salt ions can modify the crystalline structure of the gel system, enhancing crystals at low concentrations and new crystalline peaks are observed at higher ion concentrations [31].

Soy protein-polysaccharide coacervate undergoes changes upon the addition of salt. The viscosity significantly reduces giving the mixture a smoother appearance. Elastic and viscous parameters both decrease, modifying the rheological characteristics. The primary factor maintaining coacervate structure is weakened by the electrostatic shielding effect of salt ions. Moreover, there is a noticeable decline in zeta potential with the addition of NaCl [37]. Salt regulates the ionic strength of solutions, with salt such as potassium chloride (KCl) and sodium chloride (NaCl). This impacts the electrostatic interactions between proteins and polysaccharides during complex coacervation. Proteins separate from the solution as a result of the salting out process, which is triggered

by high salt levels. In the meantime, charge neutralization and competition for water molecules influence the formation of complexes. The stability and properties of these complexes are further influenced by the concentration of salt, with some salts helping to stabilize the connections between proteins and polysaccharides [32].

#### 3.1.4. Impact of pressure on protein-polysaccharide interaction

The restricted compatibility of protein and polysaccharides in water leads to incompatible at high pressure as a result they split into distinct phases. Decreasing the ability to come together and encouraging molecules to come together are the outcomes of excluded volume effects which involve modifications in the amount of space occupied by these large molecules in response to pressure. In phase separated system, pressure can cause viscosity to increase impacting the flow characteristics and properties of the mixture [38]. Protein-polysaccharide gel combinations at high pressure result in the formation of a stronger gel structure. This phenomenon is due to changes in the molecular interactions and organization of the biopolymers created by elevated pressure which leads to the formation of a long-lasting and better-organized gel network [38]. A mixture of peanut protein isolate (PPI) and different polysaccharides such as chitosan, guar gum, and xanthan gum are subjected to high-pressure homogenization leading to a reduction in gel strength. This is attributed to the rupture and breakdown of both PPI and polysaccharide chains, along with the involvement of electrostatic interactions. While the homogenization process has increased the gel firmness in pure PPI mixture [39]. Hydrostatic pressure partially desaturates proteins, revealing reactive groups that are within the molecules and facilitating the formation of complexes and emulsions. On the other side, high dynamic pressures use shear action, cavitation, and turbulent forces to reduce the size of complexes [21].

#### 3.1.5. Impact of humidity and moisture on protein-polysaccharide interaction

Humidity doesn't play a major role but it influences the Maillard reaction. In protein-polysaccharide combinations, humidity has a significant impact on phase behaviour and structure formation [24]. Both wet and dry heating techniques greatly influence the development of

protein-polysaccharide complexes depending on relative humidity. The efficiency and kinetics of the glycosylation reaction are influenced by the relative humidity in the dry-heating technique, which involves heating biopolymers in a dry condition. Relative humidity throughout the process affects the solubility and mixing of proteins and polysaccharides, which can change the reaction time and efficiency. On the contrary, in the wet-heating technique, where polysaccharides cause a molecular crowding effect, the relative humidity influences the direct interaction between proteins and polysaccharides, which in turn influences the kinetics and efficiency of glycosylation. Better interactions between proteins and polysaccharides in both techniques may be encouraged by higher relative humidity, which would ultimately affect the structure, stability, and functionality of the resulting complex [7]. Lower humidity in thin films leads to the production of topographies smaller than 200 nm, whereas higher humidity induces Ostwald ripening which forms a bigger structure up to 10  $\mu\text{m}$ . removing water from a protein-polysaccharide mixture can cause structural changes such as sol-gel transition that results in material that mimics paste having plastic characteristics. The type of biopolymer concentration, evaporation, temperature, mixing ratio, and extrinsic factors like pH and temperature impact the outcomes of the process [24]. A cohesive gel structure is formed more easily when there is moisture present because it hydrates the proteins and polysaccharides and promotes their connections. A dry and brittle gel network might result from inadequate moisture, which will lower the composite gel's overall texture and quality. The interactions between polysaccharides and myofibrillar proteins in a system can be affected by the amount of moisture present. These interactions are advantageous, improving the texture and characteristics of gels at precisely the right moisture levels. On the other hand, excessive moisture may cause unfavourable interactions or component separation, which could change the gel's overall structure and characteristics [40].

The hydration state has a major impact on how proteins and polysaccharides interact in food systems, which affects important parameters including viscosity and water-holding capacity (WHC). Increased WHC generally follows higher hydration, suggesting improved water binding capacity within the protein-dextrin matrix. This increased ability to bind water results in better texture and stability in products that incorporate in-situ-produced dextran, such as rice protein yogurt. By altering the interactions between proteins, dextrin and water molecules within the network, the degree of hydration has a direct impact on the viscosity of this type of yogurt. Viscosity is frequently increased at higher hydration levels because these components interact more strongly. Sustaining the appropriate texture and consistency of yogurt requires optimal moisture levels, which facilitate efficient protein-dextrin interactions. To attain the intended characteristics, these interactions can be modulated by regulating moisture levels during formulation [20].

### 3.2. Intrinsic factors

#### 3.2.1. Impact of type and nature of biopolymer on protein-polysaccharide interaction

The type and nature of biopolymers are important factors to consider when studying the interaction and formation of protein-polysaccharide interaction. The size and form of molecules can affect how proteins and polysaccharides interact with one another. The development of stable complexes between proteins and polysaccharides and the accessibility of binding sites are influenced by factors such as complementarity and steric hindrance [41]. When soluble undenatured type II collagen (SC II) interacts with various anionic polysaccharides, it alters the secondary structure of SC II. These modifications differ based on the polysaccharide involved. Changes in SC II's structure are mostly dependent on the molecular properties of the anionic polysaccharides, including their weight, size, and flexibility. Anionic polysaccharides with smaller molecular weights and shorter chain lengths, like chondroitin sulfate (CS) and pectin (PE), form smaller and denser complexes with SC II,

which ultimately affects the protein's secondary structure [32]. Polysaccharides that have highly flexible main chains tend to create coacervates with proteins, while those with rigid structures tend to form interpolymeric complexes. This indicates that the flexibility of polysaccharide chains is indeed a factor in how they interact with proteins. Rigid polysaccharides may be limited in their ability to bind because of their constricted shapes, whereas flexible polysaccharides may have a distinct advantage in adapting to the protein's surface and creating strong binding associations [32]. The size, compactness, and characteristics of the complexes that anionic polysaccharides, such as chondroitin sulfate (CS) and pectin (PE), form with soluble undenatured type II collagen (SC II), are significantly influenced by differences in their molecular weight and chain length relative to xanthan gum (XG) and carboxymethyl cellulose (CG). The secondary structure of SC II within the complexes and the infrared spectrum is influenced by the molecular conformation of these polysaccharides [41].

#### 3.2.2. Impact of chemical structure on protein-polysaccharide interaction

A diverse chemical structure is exhibited by polysaccharides, which are derived from monosaccharide units such as glucose, fructose, or galactose. Polysaccharide conformation is influenced by glycosidic linkages that diverge in orientation and position between monosaccharide units. Branching ranging intensity affects overall structural and functional characteristics this can range from highly branched to linear. Based on the composition of various monosaccharides, polysaccharides are classified into homopolysaccharides composed of single monosaccharide units, and heteropolysaccharides composed of various monosaccharides [42]. Through negative charges, heteropolysaccharides such as hyaluronic acid improve the interaction between myofibrillar protein which results in reduced particle size and increased emulsion stability. Increased negative charges strengthen the electrostatic interaction which improves protein adsorption and forms spherical myofibrillar protein-hyaluronic acid polymers. In comparison to heteropolysaccharides, homopolysaccharides such as cellulose produce weaker interactions which lead to decreased viscosity as well as less efficient emulsion stabilization [43]. On the other hand, biopolymer interactions are greatly influenced by the molecular structure of proteins are polypeptide structures consisting of one or more long chains of amino acids with various functional groups such as hydroxyl, carboxyl, and sulfhydryl groups. Water binding properties features of proteins can influence the interaction of protein-polysaccharide interaction with hydrogen bonding and electrostatic interaction [30,44,45]. Interaction between pea protein with four different polysaccharides which have distinct chemical compositions and structures, that impart functional and unique characteristics resulted in varying effects. Corn fibre gum (CFG) with protein interaction produced well-defined, uniformly distributed spherical particles and increased viscosity. High-methoxy pectin (HMP) stabilized through steric hindrance resulting in particles with irregular shapes. Carboxymethyl cellulose (CMC) achieved stability and exhibited spherical particles with smooth surfaces by electrostatic repulsion, steric hindrance, and high viscosity by maintaining negative charge mass at acidified pH. Konjac glucomannan (KGM) stabilized by elevated viscosity with monodispersed particles are individually separated and scattered randomly. Overall, these interactions highlighted the unique mechanism employed by each polysaccharide in stabilizing pea protein dispersion [46].

#### 3.2.3. Impact of polyelectrolyte on protein-polysaccharide interaction

A polyelectrolyte is a polymer when dissolved in a polar solvent can generate electrical charges due to the presence of certain groups that are prone to ionization. Polyelectrolytes are classified as either anionic which is negatively charged or cationic which is positively charged [47]. Polyelectrolytes such as whey protein isolate (WPI) and chitosan (CS) play a critical role in the interaction and development of polyelectrolyte nanoparticles (PENs) through electrostatic interactions. The negatively charged WPI interacts with positively charged CS, resulting in stable

nanoparticles. The presence of tripolyphosphate (TPP) further enhances stability by cross-linking the CS backbone and reducing free amino groups. This leads to the formation of compact structures with irregular edges as evidenced by scanning electron microscopy (SEM) images [48]. The interaction between protein polysaccharides is influenced by the charges present in the biopolymers. In protein-polysaccharide interaction, strong electrostatic interactions occur when oppositely charged biopolymers come together facilitating the formation of stable and soluble complexes [9].

Interaction between polysaccharides and proteins which possess different charges namely anionic and cationic can happen in different ways. The protein-polysaccharide complex binds and remains stable if cationic polysaccharides form strong bonds with negatively charged proteins on the other hand positively charged proteins interact with anionic polysaccharides influences how well the complex binds and functions [49]. When a single polysaccharide interacts with protein, ionic polysaccharides bind to charged protein groups through electrostatic forces. When protein and polysaccharides have opposite charges, they are drawn towards each other through electrostatic interaction at the uneven distribution of charges resulting in soluble complex and insoluble form when the charges are balanced. On the other hand, when the charges are identical repulsive forces develop, which can cause phase separation at high concentrations or electrostatic repulsion at low concentrations leading to co-solubility. Unlike charged polysaccharides, neutral polysaccharides primarily interact with proteins through less potent interactions like hydrogen bonding and hydrophobic interaction [50,51]. The interaction between soy protein isolate (SPI) with various polysaccharides of different charges such as guar gum (GUG), gellan gum (GEG), and chitosan (CS), was investigated at varying mass ratios and pH values between 8.0 and 2.0. When combined with cationic polysaccharide chitosan (CS) at a mass ratio of 10:1, SPI/CS complexes showed lower foamability compared to SPI alone, although their foam stability was significantly enhanced. However, their foam stability was noticeably improved. In comparison to SPI/GUG (neutral polysaccharide guar gum), the SPI/GEG (anionic polysaccharide gellan gum) complexes, with a mass ratio of 15:1, showed a less dense and less aggregated microstructure. This implies that SPI self-aggregation was effectively prevented by GEG. In addition, SPI/GEG complexes showed a stronger binding affinity than SPI/GUG at a mass ratio of 30:1, which led to a higher level of microstructural aggregation. Particularly, SPI/GEG complexes exhibited exceptionally excellent foam stability, which improved with increasing GEG concentrations. As a result, the behaviour and foamability properties of the SPI/polysaccharide complexes varied depending on the charges on the polysaccharides [51].

#### 3.2.4. Impact of molecular weight on protein-polysaccharide interaction

The Interaction with protein particularly bovine serum albumin (BSA), human serum albumin (HSA), and polysaccharide chitosan with varying molecular weights 200 kDa, 100 kDa, and 15 kDa. This exhibited that increasing chitosan molecular weight up to 200 kDa improved BSA-chitosan complex binding through hydrophobic interactions (affinity sequence as 200 kDa > 100 kDa > 15 kDa). On the other hand, HAS-chitosan complexes with chitosan- 100 kDa exhibited greater stability by electrostatic interactions (stability order as 100 kDa > 200 kDa > 15 kDa). Larger chitosan nanoparticles (chitosan-200kDa) induced greater alterations in protein secondary structure. These results highlight the crucial role of chitosan molecular weight in influencing protein binding affinity and conformational charges in the presence of chitosan nanoparticles [52]. The molar mass of proteins and polysaccharides affects their solubility, viscosity, and gelation properties. Higher molar mass polymers can form complex networks in solution, which results in increased viscosity and greater gelation [45].

#### 3.2.5. Impact of biopolymer concentration on protein-polysaccharide interaction

The concentration level of protein and polysaccharides has a

significant impact on how they behave in their interactions. These biopolymers tend to exhibit co-solubility at low concentrations, sustaining solubility while engaging in significant associations. On the flip side, biopolymers exhibit thermodynamic incompatibility or endure complex coacervation at larger concentrations. In such instances, they undergo phase separation, forming aggregates or coacervates [53]. The study found that the properties of the interaction between these two biopolymers were affected by varying the concentration of basil seed gum (BSG) in a blend with whey protein isolate (WPI). In particular, the study showed that when the concentration of BSG in the blends reduced, the BSG's intrinsic viscosity decreased as well going from 147.25 mL/g to 88.24 mL/g. This shows that rheological properties and interaction strength of the BSG-WPI blend are impacted by the concentration of BSG in the research conducted by Jones and colleagues, the study effectively showcased the impact of protein concentration on the formation of biopolymer particles through heating of protein-polysaccharide complexes. Higher initial protein concentration in protein-polysaccharide complexes leads to the formation of larger and more concentrated biopolymer particles during heating and also turbidity in heat-treated mixtures rose with higher concentration indicating enhanced light scattering [54]. At high concentrations, proteins and polysaccharides form insoluble complexes, resulting in phase separation. Soluble complexes are formed at low concentrations and dispersed uniformly throughout the system hence levels of concentration of biopolymer affect the solubility. Furthermore, as larger complexes or networks form at increasing concentrations, the solution viscosity increases, impacting the texture of the product [41].

#### 3.2.6. Impact of biopolymer mixing ratio on protein-polysaccharide interaction

The study explored the interactions between high methoxylated pectin and lysozyme at various ratios 1:1, 2:1, 3:1, 5:1, and 10:1 highlighting notable differences in behaviour. At lower ratios 1:1 and 2:1, there was a slight increase in complex size, along with an expansion of the pH range favourable for complex formation. A notable increase in complex size occurred at a 3:1 ratio due to the presence of more lysozyme-neutralizing pectin. However, at higher ratios 5:1 and 10:1 prevented the development of soluble complexes which caused a sharp rise in turbidity at pH above 11.50. This study demonstrates at varying biopolymer ratios, the particle size is influenced and along with changes in pH, these factors play a crucial role in shaping interaction dynamics [55]. The impact of varying ratios of basil seed gum (BSG) to whey protein isolate (WPI) on their interaction behaviour ratios of 1:4, 1:6.6, and 1:9 was examined. A ratio of 1:4 exhibited the strongest interaction due to biopolymer complex formation via electrostatic interaction and hydrogen bonding. Poor miscibility due to repulsion was evident in the 1:6.6 ratio. At a 1:9 ratio interactions varied with pH demonstrating poor combability at pH 6 and 7 but high turbidity is observed at pH 5. These findings provide insight into the complex behaviour of protein and polysaccharide interactions highlight the significance of pH and biopolymer ratio and show how these variables influence the protein-polysaccharide interaction and the structure [53]. When modifications in the system arise from variations in pH and ratio the interactions between components are predominately upheld through electrostatic forces or hydrogen bonds [30].

The formation of soluble complexes between proteins such as Sodium Caseinate (SC), Whey Protein Isolate (WPI), Pea Protein Isolate (PPI) and High Methoxyl Pectin (HMP) are influenced by mixing ratios and pH. In 1:1 and 2:1 ratios, SC-HMP mixes exhibited soluble complex formation at pH 4–5, but WPI-HMP mixtures revealed limited soluble complex formation, implying a combined influence of pH and ratio. PPI-HMP mixes show soluble complexes in a wider pH range at different ratios. While WPI-HMP and PPI-HMP mixtures show insoluble complex formation at pH 3 for certain ratios, SC-HMP forms insoluble complexes at pH 3 for all ratios and at pH 4 and 5 for specified ratios. Insoluble complex formation occurs at specific pH values and ratios. Ultimately,

the ratio of protein to pectin is critical in determining the formation of soluble and insoluble complexes, influenced by specific proteins, their interactions with HMP, and pH conditions [41].

#### 4. Processing treatments affecting the protein-polysaccharide interaction

##### 4.1. Heating treatment (conventional heating)

The various novel conventional and non-conventional techniques that alter the protein-polysaccharide complex are presented in Table (1). The research was conducted on the effects of heat treatment on whey protein-soluble soybean complexes, comparing conditions with and without heat treatment on whey protein-soluble soybean complexes, comparing conditions with and without heat treatment across different pH values 3.0, 3.5, and 4.0. In the absence of heat regardless of pH levels and biopolymer ratios, the complexes exhibited limited exposure of hydrophobic regions due to their retained consistent surface hydrophobicity. However, the complexes grew more compact and stable at lower pH levels specifically pH 3 as shown by a reduction in Z-average which indicated better compactness and stability, and at pH 4 whey protein isolate (WPI) became unstable signifying that WPI control is pH dependent. Heat treatment at 90 °C for 20 min, on the other hand, caused notable changes in that the complexes grew larger and demonstrated increased surface hydrophobicity revealing hydrophobic regions. Change in protein secondary structure and reorganization of protein-polysaccharide-water interactions which lead to changes in physical stability. The complexes' Z-average size increases as a result of the treatment, which could point to alterations in their interactions or structures. Additionally, it reveals ionic and hydrophobic residues on the complex surface, which affects the  $\zeta$ -potential and surface hydrophobicity. Physical stability is maintained for at least 56 days, during which the complex size remains stable and nanosized. To achieve desired properties including reduced size, higher hydrophobicity, enhanced stability, and a  $\zeta$ -potential near to zero, the ideal conditions to develop WPI-SSPS complexes include pH 3.5, a WPI: SSPS mass ratio of 1:0.5 with heat treatment [56]. Whey protein isolate (WPI) and soluble soybean polysaccharides (SSPS) to interact and form nanoparticles, and the dispersions were heated to  $90.0 \pm 1.0$  °C for 15 min. The structural and interfacial behaviour of the nanoparticles exhibited substantial changes upon heating at this temperature such as modifications to the molecular structure, denaturation of proteins, and interactions between WPI and SSPS molecules. The heat treatment resulted in a further reduction in non-sedimentable protein content for the  $WS_d$  complex, indicating its impact on protein-polysaccharide interactions and the formation of stable complexes. The complexes formed after heating, namely  $WS_d$  and  $Wd_s$ , exhibited larger particle sizes compared to those assembled from unheated proteins ( $WS_n$  and  $Wn_s$ ), highlighting the influence of heat treatment on the particle size of the complexes (Cabezas et al., 2019).

##### 4.2. Microwave heating

Microwave is a non-conventional heat method, which utilizes electromagnetic waves (frequency ranging from 300 MHz to 300 GHz) to directly excite the molecules with in the material resulting in the generation of heat. This method also incorporates volumetric heating, where the electric field component of the microwave induces the rotation of dipoles in foods leading to uniform heating throughout the food material compared to the conventional heating method [42,57,58]. Surmi is a paste made from processed fish meat which is the common base for various seafood products. Surmi paste is prepared by partially thawing the frozen surmi and chopping it into small pieces and further processed into a paste using specialized equipment. Polysaccharides like konjac glucomannan (KGM) are added to the surmi paste which binds with the protein in the surmi paste, forming a cohesive network structure and the gelation occurs when surmi paste is heated. By two means

the mixture is heated at 90 °C microwave set to 300W with varying duration such as 1,2, 5, and 10 min, and in water bath mixture is kept for 30 min. The microwave and water bath heating methods produced distinct effects on the gel. Gels heated in a microwave had a denser and more homogeneous network structure than gels heated in the water bath the cause for this was due to polysaccharide chains in gels heated by microwave exhibited a more expanded network whereas in gels heated by water bath observed as an aggregate embedded into proteins. Additionally, microwave-heated gels showed noticeably greater gel strength as well as stronger interactions between their molecular chains. Texture and water holding ability of gels are improved by microwave heating conversely water bath heating resulted in polysaccharide aggregation and had no discernible effect on protein structure across treatment and this study showed that microwave heating resulted in more noticeable modifications to the gels [59]. Rice protein (RP) and dextran in 1:3 ratio w/w were dispersed in deionized water and pH adjusted to 11 with NaOH and then subjected to microwave and water bath heating to investigate the influence of the Maillard reaction. The solubility of rice protein improved significantly to 64.25 % during 5 min of heating compared to the traditional approach which only achieved 24.22 %. This emphasizes microwave treatment facilitates the Maillard reaction between rice protein and dextran. Microwave heating can lower the energy barrier for the reaction resulting in a faster reaction rate as indicated by its requirement of only 46.70 kJ/mol compared to conventional heating which requires 75.09 kJ/mol and microwave treatment also enhances the functional properties such as solubility, emulsifying, foaming abilities [28].

##### 4.3. Ohmic heating

Ohmic heating (OH) is a highly efficient method that involves treating food passing an AC electrical current generates heat within the material itself resulting in rapid and uniform heat generation and this process is also known as direct joule heating [60]. The impact of moderate electric field (MEF) on thermal gelation and network structures of Patatin-enriched potato protein (PPI) gel. 9 % protein maintained at pH 7 and 25 mM NaCl were heated from 25 to 65 °C using OH (3–24V/cm) and by conventional heating with various come up and holding times. Self-standing gels were formed by both techniques, but OH resulted in less protein denaturation. Analysis revealed that OH- treated gels retained more native patatin, exhibited gap-like structures and frayed patches, and had poorer water holding ability compared to COV- treated gels. Molecular analyses revealed that MEF causes modification to beta sheet structures and less hydrophobic interactions with in the protein network additionally OH- treated gels demonstrated lower rigidity and higher flexibility impacting their gelling functionality [29]. The solubility of soya milk protein decreases as both voltage and temperature increase during ohmic heating. For instance, at 70 °C for 160V, the solubility is  $18.11 \pm 0.63$  % while at 90 °C for 200V, it decreases to  $5.08 \pm 0.017$  %. This illustrates the inverse relation between solubility and voltage temperature conditions during ohmic heating [61]. OH has displayed its ability to develop novel protein-based structures like films or gels with desirable techno-functional characteristics by altering the kinetics at which proteins unfold, denature, and aggregate [62]. The structural alterations seen in whey protein isolate solutions are greatly influenced by the type of electrode material used during ohmic heating treatments. Because of their high electro-activity, stainless steel electrodes have the ability to interact with whey proteins on the metal surface, which may result in metal release and subsequent changes to the structure of the protein. While stainless steel electrodes are vulnerable to acid erosion, especially at pH 3, indicating the importance of solution conditions in metal release, titanium electrodes also release metals into the solution, which influences protein aggregation. On the other hand, graphite electrodes are favoured for minimizing metal contamination during ohmic heating [62]. Ohmic heating has diverse impacts on the structure of lentil protein based on temperature and

**Table 1**  
Novel conventional and non-conventional techniques which alter the protein polysaccharide complex.

| Type of protein               | Type of polysaccharide                | Ratio                               | Type of interaction                           | Type of treatment         | Temperature       | pH              | Process parameters                                                                                                                                                              | Observations                                                                                                                                                                                                                                                                                         | Reference            |
|-------------------------------|---------------------------------------|-------------------------------------|-----------------------------------------------|---------------------------|-------------------|-----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|
| Rice protein                  | Dextran                               | 1:3                                 | Maillard reaction (Dry heating)               | Microwave heating         | Heating for 5 min | pH 11.0         | Microwave heating for 5 min                                                                                                                                                     | Improved solubility by 64.25 %, can lower the energy barrier for the reaction resulting in faster reaction rate by 46.70 kJ/mol and enhanced emulsifying and foaming ability                                                                                                                         | [28]                 |
| Soya protein                  | Soyabean- soluble polysaccharide      | 1:3                                 | Non-covalent interaction (Nano gel formation) | UV radiation              | 90 °C for 1 h     | pH 4.0          | UV radiometer with a peak wavelength of 365 nm and power of 500 W. Samples irradiated for 1 h at a distance of 30 cm from the UV source                                         | UV radiation leads to photodegradation this is used to assess the protection efficiency of folic acid before and after the radiation – fluorescence analysis                                                                                                                                         | [66]                 |
| Fish gelatin (G)              | Chitosan (CS)                         | 50 % of G: 50 % CS                  | (Non- covalent bond Blended films)            | Electron beam irradiation |                   | pH 5.6          | A linear electron accelerator with an energy of 2.2 MeV and a dosage rate of 0.3 kGy/s was used to deliver the radiation treatment. Dosages of 20, 40, and 60 kGy               | Irradiation films exhibits smoother, denser, and arranged more compactly In 60 kGy irradiation dosage - the blend films YM increased by 28 %, indicating a change in the stiffness of the film, improved moisture barrier efficiency and oxygen permeability and surface hydrophobicity also reduced | [68]                 |
| Whey protein isolate (WPI)    | Soluble soybean polysaccharide (SSPS) | 1:0.50 to 1:0.17                    | Electrostatic interaction                     | Heat treatment            | 90 °C for 20 min  | 3.0,3.5 and 4.0 |                                                                                                                                                                                 | Complexes grew larger and demonstrated increased surface hydrophobicity revealing hydrophobic regions Increased Z- average (<200 nm), uniform size distribution (PDI value low <0.2), zeta potential near to zero.                                                                                   | [56]                 |
| Whey protein isolate (WPI)    | Soluble soybean polysaccharide (SSPS) | Mass ratio 4.6                      | Electrostatic and hydrophobic interaction     | Heat treatment            | 90 °C for 15 min  | 3.0             |                                                                                                                                                                                 | Reduction in non- sedimentable protein content indicating change in particle size, complexes formed after heating, namely Wsd and WdS, exhibited larger particle sizes compared to those assembled from unheated proteins.                                                                           | Cabezas et al., 2019 |
| Surimi protein (Fish protein) | Konjac glucomannan (KGM)              |                                     | Steric effects                                | Microwave heating         | 90 °C             |                 | Microwave at 300 W                                                                                                                                                              | Microwave heating for 5 or 10 min improved gel strength and enhanced water holding capacity of mixed gels and microwave heating resulted in stronger interactions between the biopolymer                                                                                                             | [59]                 |
| Pectin                        | α – amylase                           | 32:1,4:1and 1:1 (α-amylase: pectin) | Electrostatic interaction                     | Pulsed electric field     |                   | pH 5.0          | A bidirectional square-wave pulse with a frequency of 1 kHz and a duration of 40 μs cycle times (1–6), flow rate (m 25–250 mL/min), and electric field intensity (E 0-20 kV/cm) | Change in molecular conformation – intrinsic fluorescence of α-amylase quenched and change in structure of α-amylase. 80 % of enzyme activity reduced after PEF treatment. Particle size increased and complex tend to exhibit branched ring but zeta potential of complexes did not change.         | [72]                 |
| Patatin potato protein (PPI)  |                                       | 9 % wt                              | Maillard reaction                             | Ohmic heating             | 25–65 °C          | pH 7.0          | OH for (3–24V/cm) with 25 mM NaCl                                                                                                                                               | Lower denaturation during thermal gelation and lower water holding capacity is observed and also less hydrophobic interaction is exhibited                                                                                                                                                           | [29]                 |
| Lentil protein                | Pectin                                |                                     | Electrostatic interaction                     | Ohmic heating             | 40–80 °C          | pH 3.0          | Electric field 5–75 V/cm for 10 s                                                                                                                                               | Modification in lentil protein structure, increase in hydrophobic surface and sulfhydryl group also decreases extrinsic fluorescence                                                                                                                                                                 | [35]                 |

(continued on next page)

Table 1 (continued)

| Type of protein                                   | Type of polysaccharide                              | Ratio                                                                                                                            | Type of interaction                                                     | Type of treatment        | Temperature                                                                           | pH      | Process parameters                                                                                                                                                | Observations                                                                                                                                                                                                                                                                                                                                                     | Reference |
|---------------------------------------------------|-----------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------|--------------------------|---------------------------------------------------------------------------------------|---------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| Calcium caseinate (CC) Whey protein isolate (WPI) | Alginate, Potato starch (PS), Soluble potato starch | 1:0.1                                                                                                                            |                                                                         | Gamma irradiation        | For Gel formation heated protein polysaccharide in CaCl <sub>2</sub> for 4 h at 90 °C |         | Total dose –32kGy. Films were conditioned at 56 % humidity for 48 h                                                                                               | Irradiated films demonstrated strong and rigid structure and also enhanced barrier qualities and improved resistance water vapor transfer                                                                                                                                                                                                                        | [64]      |
| Soya protein                                      | Pectin                                              | 3:3.1                                                                                                                            | Hydrophobic interaction and hydrogen bonding                            | High pressure processing |                                                                                       |         | Pressure levels of 100,200,300 and 400 MPa for 15 b min                                                                                                           | soy protein-pectin-coumarin (S-P-C) complexes exhibited better physical and chemical stability. more compact structures with spherical shapes and uniform sizes were formed. (S-P-C) complexes with HPP reduced the ability to scavenge                                                                                                                          | [76]      |
| Black soldier fly larvae (BSFLA)                  | Pectin                                              | (BSFLA) solutions containing 10 mg/mL were added with pectin. Pectin concentration - 0.0 %, 0.5 %, 1.0 %, 1.5 %, and 2.0 % (w/v) | Hydrogen bonding, electrostatic interaction and hydrophobic interaction | Ultra sonication         |                                                                                       | pH 12.0 | ultrasonic bioreactor for 30 min at 25 °C power density of 50 W/L, ultrasonic frequency of 60 kHz, and on/off times of 10 and 3 s                                 | Ultrasonication disrupts protein aggregates, promoting dispersion and enabling better binding and complex formation with polysaccharides. Addition of pectin to BSFLA led to an impressive rise, with EAI and ESI reaching 174.41 % and 643.22 %, respectively, demonstrating the significant improvement in emulsification performance.                         | [34]      |
| Zein                                              | Chitosan                                            | –                                                                                                                                | Non – covalent bond interaction                                         | Pulsed electric field    | 22.5 ± 1.5, 25.2 ± 3.2, and 37.6 ± 2.1 (22–37 °C)                                     |         | Voltage Input (Vi) -10-45 % of the maximum equipment capacity<br>Electric Field Strength (EP)- 0.8 kV/cm, 1.6 kV/cm, and 3.4 kV/cm<br>Pulse Frequency (f) - 50 Hz | Change microstructure, impact on viscosity, thermal stability. composite films exhibited increased thermal stability when the dispersions were exposed to specific energy levels (QP) exceeding 160 kJ/kg. Changes in chemical bonding and interactions within the films were indicated by alterations in functional group peak locations seen by FTIR analysis. | [73]      |

electric field strength. Ohmic heating modifies the secondary structure of lentil protein at different temperatures, affecting the content of  $\alpha$ -helix and  $\beta$ -sheet. On the flip hand, the protein structure is measured by the intensity of intrinsic fluorescence, which remains unchanged. At 40 °C, 70 °C, and 80 °C, ohmic heating causes a reduction in extrinsic fluorescence. Ohmic heating in a modest electric field (5 V/cm) enhances the exposure of hydrophobic surfaces and increases the amount of sulfhydryl groups in lentil protein when evaluating electric field strength at 80 °C. The protein structure of lentils is affected by varying electric field strengths in comparison to conventional heating, OH-5 V/cm also significantly boosts intrinsic fluorescence intensity, revealing variations in protein unfolding and exposure to hydrophobic sites [35].

#### 4.4. Gamma irradiation

Gamma irradiation utilizes radioactive sources like cobalt-60 and cesium-137, which emit high-energy photons. These photons have strong penetrating abilities, and their energy levels typically fall within the range of 0.662–1.33 MeV [63]. The mechanical strength and barrier properties of calcium caseinate (CC) and whey protein isolate (WPI) films were enhanced by 32 kGy gamma irradiation. The characteristics of both irradiated and non-irradiated films were further improved by the addition of certain polysaccharides. In particular, films made from irradiated solutions were more rigid and strong compared to those made from non-irradiated solutions. The barrier qualities of irradiated films outperformed those of non-irradiated ones when particular polysaccharides were added such as sodium alginate and soluble potato starch. Irradiated films demonstrated reduced water vapor permeability (WVP) values, which show improved resistance to water vapor transfer [64].

#### 4.5. UV radiation

Ultraviolet (UV) radiation is a form of electromagnetic radiation that has a shorter wavelength than visible light ranging from 100 to 400 nm. According to wavelength, these are divided into four categories vacuum UV (100–200 nm), UVA (315–400 nm), UVB (280–315 nm), and UVC (200–280 nm). It is widely known for its germicidal properties (Delorme et al., 2020). Proteins can undergo structural changes when exposed to UV light, such as unfolding or a change in their original shape. When compared to proteins that have not been altered, these modifications may have an impact on the binding affinity, stability, and functional characteristics of proteins when they interact with polysaccharides. Polysaccharide structures can be altered by UV exposure, resulting in modifications such as depolymerization. These changes could affect the development of protein-polysaccharide complexes, which could have an impact on their potential uses by altering their binding affinity, stability, and functional characteristics [65]. Soy protein films underwent significant alterations in their chemical and physical characteristics due to UV exposure. The films' tensile strength substantially increased after being exposed to UV light, indicating the occurrence of cross-linking. The increase in strength was directly proportional to the UV dosage. Conversely, the elongation at break decreased as UV exposure increased, revealing a less flexible but denser and stronger protein matrix. Interestingly, UV irradiation did not significantly affect the water vapor permeability of the films, suggesting that extensive cross-linking may not have occurred. Additionally, electrophoretic patterns revealed further development of covalent cross-links in the treated films, indicating molecular alterations induced by UV treatment (Gennadios et al., 1998). The study utilized UV radiation to examine the degradation of folic acid and evaluate the protective capabilities of soy protein/soy polysaccharide complex nanogels against folic acid degradation under UV exposure. Degradation of folic acid was detected at 365 nm, which is the highest wavelength of the UV light that was used. The degree and length of UV exposure, the pH of the solution, and the existence of other

substances interacting with the folic acid molecule were found to have an impact on the pace of degradation [66].

#### 4.6. Electron beam irradiation

Electron beam irradiation is a non-thermal technology that utilizes high energy electrons produced from a linear accelerator and are directed towards food products resulting in disruption of molecular or atomic bonds and are ideally used for phytosanitary treatments ([67]; Pillai et al., 2017). Chitosan-fish gelatin mix films exhibited significant changes in their microstructure, mechanical characteristics, barrier qualities, and spectroscopic features upon exposure to electron beam irradiation at doses of 20 kGy, 40 kGy, and 60 kGy. The microstructures of the irradiation films were smoother, denser, and arranged more compactly. This is attributed to the formation of new linkages within or between polymer chains. After a 60 kGy irradiation dosage, the tensile strength of the blend and chitosan films stayed mostly unchanged, but the chitosan films' Young's modulus (YM) doubled and the blend films YM increased by 28 %, indicating a change in the stiffness of the film. Furthermore, for both chitosan and mix films, the elongation at break (E) dramatically dropped as irradiation doses increased, indicating decreased ductility and flexibility. Irradiated blended films showed slight enhancement in the moisture barrier efficiency particularly under conditions of 30–84 % relative humidity gradient. After being exposed to a dose of 60 kGy radiation, the chitosan and mix films' oxygen permeability and surface hydrophobicity also declined. Electron spin resonance analysis detected the presence of free radicals within the polymer matrix of the blended films when subjected to irradiation and these free radicals play a significant role in initiating intermolecular cross-linking within the polymer matrix forming covalent bonds between polymer chain and resulting in three-dimensional network structure [68]. As the high-energy electron irradiation doses increased there was a decrease in viscoelastic properties by 20 % compared to initial moduli of the agarose hydrogel [69].

Chitosan-fish gelatin films containing quercetin exhibited enhanced mechanical, oxygen barrier, and thermal characteristics upon exposure to electron beam irradiation. This included improved thermal stability, a 65 % reduction in oxygen permeability, and a 42 % increase in tensile strength when 5 wt percent of quercetin was added and exposed to 60 kGy of radiation. The retention and release of quercetin from the films were impacted by the cross-linking caused by radiation [70]. Electron Beam Irradiation (EBI) treatment changed the okara protein's tertiary structure by reducing the proportion of  $\alpha$ -helix and  $\beta$ -sheet structures. This reduction indicates that the regular, organized structure of the protein has been disrupted, resulting in a less compact tertiary shape. The protein's three-dimensional structure opens up and becomes more flexible in this less compressed arrangement., according to intrinsic fluorescence studies. Protein conformation alterations had a major impact on its functional qualities, including solubility, thermal stability, and emulsification. As the radiation dose rose, the solubility of okara protein significantly increased and peaked at 6 kGy. The observed rise in solubility was ascribed to modifications in protein conformation and unfolding of the protein structure upon exposure to EBI treatment. This resulted in amplified interactions with water molecules as a result of an amplification of hydrophobic, hydrophilic, and polar groups on the protein surface. However, solubility did not always rise with increasing radiation doses at 8 and 10 kGy, solubility dropped perhaps as a result of protein molecules cross-linking and forming insoluble aggregates. Additionally, emulsions' microstructures exhibited considerable changes as the amount of radiation increased. The emulsions that were subjected to a 6 kGy treatment displayed a more uniform appearance with smaller droplets. This was attributed to enhanced protein adsorption at the oil-water interface, culminating in a stable emulsion with reduced interfacial tension. On the other hand, emulsion droplet aggregation occurred at higher radiation dosages (8–10 kGy) because the okara protein was less soluble and had weaker emulsification [71].

#### 4.7. Pulsed electric field treatment

Pulsed Electric Field (PEF) is an innovative food processing technique that operates without the need for heat. Rather, it employs short bursts of high voltage that induce cellular stress, which results in structural alterations in food and the formation of specific compounds. PEF has demonstrated effectiveness in microbial inactivation, improving molecular transport in food products, and reducing energy consumption compared to traditional heat-based methods (Arshad et al., 2021).  $\alpha$ -Amylase/pectin complexes had significant structural and physico-chemical changes after being treated for five processing cycles at an electric field strength of 20 kV/cm and duration of 1.02 ms. These changes included the creation of branching, ring- or circle-like structures, decreased enzyme activity, and an increase in complex size. Dipole-dipole interactions and fast particle movement in the field affected the complexes' ultimate shape, whereas the external electric field caused the redistribution of surface charges within the complexes, influencing their overall distribution and orientation [72]. The rheological behaviour of chitosan-zein-poly (vinyl alcohol)-polyethylene glycol composite dispersions is considerably altered by pulsed electric field (PEF) treatment, resulting in modifications to their viscoelastic characteristics, particle size, and zeta potential. Additionally, this treatment affects the development of biodegradable films with improved tensile and mechanical barrier qualities. PEF improves the mechanical characteristics of biopolymers by causing stretched chain conformation, exposing reactive regions, and facilitating bioconjugation and intermolecular entanglements. Additionally, it fosters the development of regular, compact, and crystalline structures in the films, further improving their mechanical and barrier qualities. This raises the crystallinity of cast films, which improves their mechanical strength and barrier qualities. A more compact film matrix is formed when polymer-polymer interactions are improved by treatment at particular energy levels [73].

#### 4.8. High pressure processing (HPP)

An innovative method utilizing protein polysaccharide mixture to develop hydrogels is high pressure technology. This method can change the structure of proteins by rupturing the hydrophobic and electrostatic bonds leading to protein denaturation, unlike thermal induced technique which primarily disrupts the hydrogen bonds [39]. The optimal parameters for high-pressure processing (HPP) differ based on the target system and result. In order to prevent denaturation of proteins, general guidelines include pressure ranges of 100–1000 MPa, treatment periods ranging from seconds to minutes, and temperatures below 40 °C. The results of HPP may also be influenced by pH levels. In particular, protein-polysaccharide interactions may be strengthened in acidic environments. It can be essential to adjust HPP conditions for every system in order to get the intended outcomes. In mixed systems, high-pressure treatment causes protein gelation, which produces phase-separated gels with different microstructures and textures and affects the mixture's and the product's rheological characteristics. The specific system under investigation determines the effects on protein-polysaccharide interactions [74]. HPP can enhance the emulsifying properties of both proteins and polysaccharides by modifying their molecular structures. It increases the flexibility of proteins, resulting in peptides with a more balanced distribution of polar and hydrophobic amino acids, thus improving their ability to emulsify. HPP also boosts the emulsifying activity of proteins and stabilizes emulsions by altering protein molecular weight and surface hydrophobicity. These alterations contribute to enhanced stability and rheological properties in emulsion-based delivery systems [75]. Compared to soy protein-coumarin (S-C) complexes, the physical and chemical stability of soy protein-pectin-coumarin (S-P-C) complexes was enhanced by the addition of pectin and high-pressure processing (HPP). This combination modified the complexes' charge, porosity, and size, which had an effect

on their ability to donate hydrogen atoms. As a result, more compact structures with spherical shapes and uniform sizes were formed. At pressures higher than 200 MPa HPP strengthened the hydrophobic interaction and hydrogen bonding between soy protein and coumarin. Pectin also increased coumarin's resistance to heat and UV deterioration and increased the stability of the complex particles. However, the combined effect of HPP and pectin reduced the ability of S-P-C complexes to scavenge radicals [76].

#### 4.9. Ultra sonication treatment

Ultrasonication significantly influences the interaction between pectin and black soldier fly larvae albumin (BSFLA) by inducing structural changes in proteins, improving solubility, enhancing emulsification properties, increasing binding affinity, promoting stability, and modifying protein surfaces. This process exposes hydrophobic regions and functional groups, providing additional binding sites for interactions like hydrogen bonding and hydrophobic interactions. Additionally, ultrasonication disrupts protein aggregates, promoting dispersion and enabling better binding and complex formation with polysaccharides. Moreover, it modifies protein structure and surface properties, improving their emulsifying capabilities and leading to stable emulsion formation. Ultrasonication enhances the binding affinity between proteins and polysaccharides through various mechanisms, crucial for forming stable complexes with improved resistance to environmental factors. Overall, ultrasonication plays a vital role in enhancing the interaction between pectin and BSFLA by affecting multiple aspects of their complexation process [34].

#### 4.10. Fermentation

Fermentation is a dynamic biological process where starch and sugars undergo transformation into various compounds like lactic acid, volatile fatty acids, alcohol, methane, and carbon dioxide. Traditionally employed to enhance storage qualities and minimize health risks in food products, this process relies on microorganisms such as lactic acid bacteria, yeasts, and coliforms. These microbes play a vital role in converting carbohydrates into fermentation products. The predominant production of organic acids, particularly lactic acid, decreases pH levels, leading to the breakdown of InsP6, thereby enhancing the digestibility of minerals and nutrients in diets (Humer et al., 2016). The interaction between proteins and polysaccharides in dairy products is significantly influenced by fermentation. As fermentation progresses, the gradual increase in acidity changes the charges of molecules and releases calcium from casein micelles, affecting the ionic environment. These changes alter the way polysaccharides interact with casein micelles. Moreover, lactic acid bacteria produce exopolysaccharides (EPS) during fermentation, which act as natural stabilizers and thickeners, affecting texture and water retention. The presence of EPS can cause phase separation under neutral pH and interact with negatively charged polysaccharides under acidic conditions. Studying EPS during structure formation in fermented milk systems is complex due to changes in polymer concentration, casein micelle charge, and ionic balance [77]. Yogurt manufacturing is one example of how fermentation can affect the rheological properties of protein-polysaccharide complexes. Variations in the viscosity and texture of the final product might arise during fermentation due to interactions between proteins, polysaccharides, and other components in the system [74]. The stability and characteristics of colloidal systems, such as fermented milk products, can be influenced by the interactions between proteins and polysaccharides during fermentation. It is essential to comprehend the qualitative and quantitative examination of exopolysaccharides (EPS) and how they interact with the product's microstructure. In fermented milk, these interactions have an impact on properties like viscosity, syneresis, and gel hardness. Studies on the precise impacts of EPS on these characteristics have produced contradictory results, indicating the complexity of their action during

fermentation [78].

## 5. Protein-polysaccharide interaction in the food system

As the polysaccharides and proteins co-exist in a food system, their interaction with each other plays a major role in how the food behaves to different kinds of processing and applications [2]. These interactions also vary with the type of food systems that are being employed in the interactive system. For example, the outcome of these interactions could be different for solid food, semi-solid food, and liquid food (M. Zhang et al., 2018). Some of the major outcomes due to the protein-polysaccharide interactions that arise in different types of food systems are given below.

### 5.1. Dairy

Milk is called a complete food in terms of its constituents from a nutritional point of view. An ardent source of vitamins and minerals, milk is also a very significant source of carbohydrates in the form of lactose and proteins, such as casein, and whey respectively [79]. Various dairy products such as cheese, butter, cream, yogurt and ice cream are derived from milk, which contains significant amounts of proteins like casein and whey. However, milk proteins are not stable on their own [80]. Adding polysaccharides to these dairy products improve their stability as well as their texture, rheological and organoleptic properties [81]. The relationship between proteins and polysaccharides in milk is intricate with two primary types of interactions namely associative and segregative have a major impact on the functional properties of milk and dairy products [77]. In ice cream, interaction between skim milk protein and polysaccharides such as flexible types like locust bean gum and guar gum exhibited high viscosity even at lesser concentrations leading to a creamier, softer ice cream with higher overrun (47–58 %) and easier scoopability. In contrast rigid polysaccharides such as xanthan gum and iota carrageenan create a denser, harder texture with lower overrun and more grittiness [82]. Different types of polysaccharides interact uniquely with the same protein, leading to variations in their functional properties. The incorporation of 1 % jujube polysaccharide (JP) in goat milk cheese enhanced water-holding capacity, hardness and rheological properties by creating a more compact and stable casein network. JP strengthened intermolecular interactions, which fortified the protein structure and improved its ability to retain water. The addition of lyceum barbarum polysaccharide (LBP) produced a softer cheese with higher moisture content due to its induced effect on the structure (Wang et al., 2023). The concentration of polysaccharides in proteins can have both positive and negative effects. For instance, at 0.5 % mulberry pomace polysaccharide (MPP) significantly reduced syneresis, enhanced gel strength, viscosity, texture and enhanced protein cluster formation, resulting in a thicker, more stable yogurt structure. These improvements were due to stronger intermolecular interactions between yogurt proteins and MPP, leading to enhanced gel properties and stability. However, higher concentration of MPP had a detrimental impact on yogurt quality [83].

### 5.2. Meat

Just like milk, meat is also rich in different nutrients, especially fats and proteins. On the other hand, they are also rich in structural polysaccharides and proteins. So, the interaction between the various nutrients are inevitable, especially the protein-polysaccharides as they are abundant in nature [84]. The interaction of different polysaccharides like dietary fibre (IDF), modified starch (MS), and konjac glucomannan (KG) with the myofibrillar protein (MP) in the meat especially the gelation and rheological properties. MS enhances MP gel strength by physically swelling and absorbing moisture, though its effect is limited. IDF significantly improves gelation by stabilizing moisture and promoting hydrophobic interactions, resulting in the highest gel strength

and water-holding capacity. In contrast, KG weakens the gel structure by creating interpenetrating network that disrupts protein aggregation [40]. J-carrageenan (CG) forms an interpenetrating porous network enhancing hydrophobic interactions and disulfide bonds, which increases salt release and enhances saltiness. Locust bean gum fills the protein network physically, while xanthan gum and LBG results in a softer, stickier mouthfeel which reduces perception of saltiness. These modifications help to tailor the texture and flavour of sausages, making them suitable for lower fat formation while maintain sensory quality [85].

### 5.3. Fish

The interaction between fish gelatin and polysaccharides profoundly influences the properties and applications of these materials. These alterations involve the formation of stable complexes through various bonding mechanisms like electrostatic interactions, hydrogen bonding, and hydrophobic interactions, enhancing structural stability and resistance to mechanical stress. Improved mechanical properties such as increased tensile strength, elasticity, and toughness make them suitable for applications like food packaging and biomedical materials. Furthermore, these interactions alter barrier properties, functional characteristics, and bioactivity, expanding their potential in industries like food, cosmetics, and pharmaceuticals, ultimately leading to the development of bioactive materials with enhanced functionality [86]. Myofibrillar protein (MP) and sodium alginate (SA) from turbot fish interact to form complexes through a combination of hydrophobic interaction and electrostatic repulsion. Myofibrillar protein regions that are similarly charged are repelled by SA's negative charge, which inhibits aggregation and improves dispersion stability. Furthermore, SA's hydrophobic portions interact with the proteins' nonpolar regions to facilitate the formation of complexes. Additionally, by creating connections between SA and myofibrillar proteins, hydrogen bonding fortifies the complexes and enhances hydrophobic and electrostatic interactions (Zhao et al., 2020).

### 5.4. Poultry

The study explored into how different saccharides, such as glucose, maltodextrin, dextran T20, and dextran T40, interacted with egg white protein (EWP). These saccharides have been found to have an impact on the water mobility, gel formation, and texture of EWP. To be more precise, their inclusion produced denser gels than the original EWP, which provides more elastic gels. Saccharides also reduced gel hardness and made the EWP solution less fluid, especially when the saccharides had a larger molecular weight. The study also observed modifications to the  $\alpha$ -helix and  $\beta$ -sheet composition of the protein gel resulting from saccharide inclusion in the secondary structure of EWP. Saccharides also inhibited the movement of immobile water, which slowed the formation rate of EWP gels, according to low-field nuclear magnetic resonance research [87]. Ovalbumin (Ova) and lysozyme (Lyso), egg white proteins, interact with polysaccharides like xanthan gum (XG) to form insoluble complexes, particularly at specific pH values. Lyso-XG complexes are prepared at pH 7.0, while Ova-XG complexes are at pH 3.0. The egg protein-to-polysaccharide ratio affects critical pH values and complex characteristics, with higher protein content raising critical pH values and impacting the formation of denser complexes. These complexes exhibit a gel-like network structure, influenced by the protein/polysaccharide ratio and ionic strength. Lyso-XG complexes form stronger gels with smaller loss tangents compared to Ova-XG complexes. Salt ions also affect complex formation by suppressing electrostatic attraction ([10]).

### 5.5. Encapsulant materials

Encapsulation is a type of encasing in which the substance of interest is packed inside a packaging material in the form of a capsule and is

placed inside the food. This method found a wide range of applications in various industries especially in Food and pharmaceuticals. In the food industry, they are widely used in active packaging technology where the active substances are encapsulated and is placed inside the packaging material along with the food [88]. A general study about the protein-polysaccharide interactions that could act as a tool for innovative delivery vehicles for bioactive like polyphenols, essential oils, and probiotics. These interactions could be achieved by various methods like physical mixing, enzymatic conjugation, chemical crosslinking and Maillard reaction etc but could depend upon various factors like pH, molecular interactions and protein-polysaccharide ratio. Some of the major delivery systems that could be developed from these kinds of interactions are emulsion-based delivery systems, capsule-based delivery systems, molecular complexes, nano gels, core-shell particles, composite nanoparticles, and micelles. They could be effectively used to deliver curcumin, resveratrol, EGCG, polyphenols, essential oils, carotenoids, vitamins, minerals phytosterols and probiotic bacteria (Wei & Huang et al., 2019). The microencapsulation technology, which leverages on blending soy protein isolates – low methoxy pectin interactions at 1:1 ratio resulted in formation of microcapsule to effectively encapsulate mulberry leaf extract's bioactive compounds and employed a solvent extraction technique using ethanol as a solvent for flavonoid extraction, subsequently evaluating their total phenolic content and antioxidant potential. The impact of both pH and coating material concentration on the microcapsules physical and chemical attributes. Various combinations of pH and coating material concentration were tested to study the effect of these two on the encapsulation process. It was identified that at pH 4.0 and a 7.5 % coating material concentration, yields microcapsules with superior encapsulation yield, efficiency, phenolic content, and antioxidant activity [89]. The potential of Maillard-conjugated sodium caseinate (NaCas)-lactose as a novel encapsulating material for oil-in-water (O/W) emulsions, with a particular focus on pH-mediated stability control. The capsule and encapsulation characteristics like encapsulation efficiency, insolubility, wettability, and microstructure of the resultant oil microcapsules are studied after the capsules are formed by spray drying technology. The study revealed that there will be a significant enhancement in NaCas-lactose interactions at pH 11, leading to superior oil droplet particle adsorption and consequently boosted emulsifying properties and long-term stability. Furthermore, adjusting the NaCas-lactose mixture pH to 11 yielded a remarkable improvement in oil entrapment ( $95.2 \pm 3.7$  %) compared to pH 7.5 ( $73.1 \pm 24.2$  %) [90]. Probiotic bacteria and canola oil were encapsulated through a combination of emulsification and ionotropic gelation. Whey proteins isolate WPI-dextran (DX) conjugate produced by Maillard reaction were served as the emulsifier in an emulsification/gelation process to encapsulate a potential probiotic bacterium, *Bifidobacterium animalis* subsp. *lactis*, and canola oil. By using controlled dry heating, WPIDX conjugates with dextran of 6 and 100 kDa (WPI-DX6 and WPI-DX100, respectively) were produced. Higher canola oil encapsulation efficiency in WPI-DX conjugates emulsion-gel gel beads was correlated with higher stability [91].

### 5.6. Formation of film

The interactions between soy protein isolate (SPI) and carboxymethylcellulose (CMC) on the physicochemical and morphological characteristics of SPI-based films. Zeta potentials were used to study the protein-polysaccharide interactions, and the results indicated that the concentration of polysaccharide, pH, and ionic strength were all related. Different CMC concentrations (0–0.5 %) were used to prepare SPI-based films at pH 11 and the pH of biopolymer coacervation (pHc). All of the films mechanical strengths increased with the addition of CMC, but the films made at pHc showed a decrease in the percentage of elongation at break and became more brittle than those made at pH 11. While the oxygen permeability of films made at pH 11 dropped with increasing CMC concentration, the water vapor permeability did not strictly

depend on either parameter [92]. The physicochemical, mechanical, and structural characteristics of edible composite films made of psyllium seed gum (PSG) and whey protein isolate (WPI). Compared to films formed entirely of WPI or PSG, these composite films showed reduced oxygen permeability, increased water contact angles, and water vapor permeability. They also showed lower light transmittance. The films showed less overall colour variation and more brightness and whiteness at higher PSG concentrations. The composite film with a 1:1 WPI/PSG ratio had the maximum tensile strength and elastic modulus. When compared to single films, these composite films demonstrated a greater ability to reduce surface fractures. The composite films had a semi-crystalline amorphous structure, according to XRD examination, with increased crystallinity and diffraction peak strength at greater PSG concentrations. Overall, the WPI/PSG composite film displayed superior properties compared to PSG or WPI films alone, particularly at a 1:1 ratio, showing the highest comprehensive physicochemical and mechanical performance [93].

## 6. Conclusion

Protein-polysaccharide complexes have gained significant interest due to their superior techno-functional properties compared to individual biopolymers, leading to widespread applications in nutraceuticals, pharmaceuticals, food, and cosmetics. These complexes are safer, more economical, and offer nutritional benefits while enabling the development of innovative biomaterials for specific biomedical applications. Their use in encapsulation is particularly prominent in nanotechnology and nanomedicine. These complexes facilitate the creation of biopolymer-based packaging materials with enhanced barrier properties and sustainability, providing alternatives to petroleum-based plastics and reducing plastic pollution. Additionally, incorporating them into functional foods improves nutritional value, stability, and sensory characteristics. Protein-polysaccharide complexes undergo notable modifications when subjected to both conventional and non-conventional treatments—such as gamma irradiation, high-pressure processing, microwave heating, UV radiation, electron beam irradiation, pulsed electric fields and ultrasonication along with external factors like temperature and pH. These treatments impact their functional properties, including solubility, viscosity, emulsifying and gelling abilities, as well as structural characteristics such as conformational changes and denaturation. Understanding these effects is crucial for optimizing the applications of protein-polysaccharide complexes in various industries moving forward.

### CRedit authorship contribution statement

**Ashley Babu:** Writing – review & editing, Writing – original draft, Visualization, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Rafeeya Shams:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kshirod Kumar Dash:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ayaz Mukarram Shaikh:** Visualization, Validation, Software, Resources. **Bela Kovács:** Visualization, Validation, Software, Funding acquisition, Resources, Supervision.

### Data availability

No data is generated as this is a review article.

## Human and animal rights and informed consent

This article does not include any experiments with human or animal subjects done by the authors.

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## References

- [1] E. Dickinson, Interfacial structure and stability of food emulsions as affected by protein–polysaccharide interactions, *Soft Matter* 4 (5) (2008) 932–942.
- [2] L. Gentile, Protein–polysaccharide interactions and aggregates in food formulations, *Curr. Opin. Colloid Interface Sci.* 48 (2020) 18–27.
- [3] D. Lin, W. Lu, A.L. Kelly, L. Zhang, B. Zheng, S. Miao, Interactions of vegetable proteins with other polymers: structure–function relationships and applications in the food industry, *Trends Food Sci. Technol.* 68 (2017) 130–144.
- [4] M. Tan, M.A. Nawaz, R. Buckow, Functional and food application of plant proteins—a review, *Food Rev. Int.* 39 (5) (2023) 2428–2456.
- [5] C. Ke, L. Li, Influence mechanism of polysaccharides induced Maillard reaction on plant proteins structure and functional properties: a review, *Carbohydr. Polym.* 302 (2023) 120430.
- [6] Q. Ru, Y. Wang, J. Lee, Y. Ding, Q. Huang, Turbidity and rheological properties of bovine serum albumin/pectin coacervates: effect of salt concentration and initial protein/polysaccharide ratio, *Carbohydr. Polym.* 88 (3) (2012) 838–846.
- [7] Q. Zhang, Y. Zhou, W. Yue, W. Qin, H. Dong, T. Vasanathan, Nanostructures of protein–polysaccharide complexes or conjugates for encapsulation of bioactive compounds, *Trends Food Sci. Technol.* 109 (2021) 169–196.
- [8] B.S. Paliya, V.K. Sharma, M. Sharma, D. Diwan, Q.D. Nguyen, T.M. Aminabhavi, V. K. Gupta, Protein–polysaccharide nanoconjugates: potential tools for delivery of plant-derived nutraceuticals, *Food Chem.* 428 (2023) 136709.
- [9] W. Wijaya, A.R. Patel, A.D. Setiowati, P. Van der Meeren, Functional colloids from proteins and polysaccharides for food applications, *Trends Food Sci. Technol.* 68 (2017) 56–69.
- [10] C.J. Souza, E.E. Garcia-Rojas, Interpolymeric complexing between egg white proteins and xanthan gum: effect of salt and protein/polysaccharide ratio, *Food Hydrocolloids* 66 (2017) 268–275.
- [11] T. Wu, C. Liu, X. Hu, Enzymatic synthesis, characterization and properties of the protein–polysaccharide conjugate: a review, *Food Chem.* 372 (2022) 131332.
- [12] Z. Wei, Q. Huang, Assembly of protein–polysaccharide complexes for delivery of bioactive ingredients: a perspective paper, *J. Agric. Food Chem.* 67 (5) (2019) 1344–1352.
- [13] H.M. Azeredo, K.W. Waldron, Crosslinking in polysaccharide and protein films and coatings for food contact—A review, *Trends Food Sci. Technol.* 52 (2016) 109–122.
- [14] A.C. Alavarse, E.C.G. Frachini, R.L.C.G. da Silva, V.H. Lima, A. Shavandi, D.F. S. Petri, Crosslinkers for polysaccharides and proteins: synthesis conditions, mechanisms, and crosslinking efficiency, a review, *Int. J. Biol. Macromol.* 202 (2022) 558–596.
- [15] T.D. Capar, H. Yalcin, Protein/polysaccharide conjugation via Maillard reactions in an aqueous media: impact of protein type, reaction time and temperature, *Lwt* 152 (2021) 112252.
- [16] F.C. de Oliveira, J.S.D.R. Coimbra, E.B. de Oliveira, A.D.G. Zuñiga, E.E.G. Rojas, Food protein–polysaccharide conjugates obtained via the Maillard reaction: a review, *Crit. Rev. Food Sci. Nutr.* 56 (7) (2016) 1108–1125.
- [17] C.W. Seo, B. Yoo, Preparation of milk protein isolate/k-carrageenan conjugates by maillard reaction in wet-heating system and their application to stabilization of oil-in-water emulsions, *Lwt* 139 (2021) 110542.
- [18] X. Zhang, Y. Zhao, T. Zhang, Y. Zhang, L. Jiang, X. Sui, Potential of hydrolyzed wheat protein in soy-based meat analogues: rheological, textural and functional properties, *Food Chem. X* 20 (2023) 100921.
- [19] D.J. McClements, Non-covalent interactions between proteins and polysaccharides, *Biotechnol. Adv.* 24 (6) (2006) 621–625.
- [20] Y. Lu, C. Jia, M. Niu, Y. Xu, S. Zhao, The in-situ dextran produced in rice protein yogurt: effect on viscosity and structural characteristics, *Carbohydr. Polym.* 311 (2023) 120767.
- [21] K.M. Albano, A.L.F. Cavallieri, V.R. Nicoletti, Electrostatic interaction between proteins and polysaccharides: physicochemical aspects and applications in emulsion stabilization, *Food Rev. Int.* 35 (1) (2019) 54–89.
- [22] J. Mobika, M. Rajkumar, S.L. Sibi, V.N. Priya, Investigation on hydrogen bonds and conformational changes in protein/polysaccharide/ceramic based tri-component system, *Spectrochim. Acta Mol. Biomol. Spectrosc.* 244 (2021) 118836.
- [23] R. Ettelaie, A. Akinshina, Colloidal interactions induced by overlap of mixed protein+ polysaccharide interfacial layers, *Food Hydrocolloids* 42 (2014) 106–117.
- [24] J. Weiss, H. Salminen, P. Moll, C. Schmitt, Use of molecular interactions and mesoscopic scale transitions to modulate protein–polysaccharide structures, *Adv. Colloid Interface Sci.* 271 (2019) 101987.
- [25] S.L. Turgeon, M. Beaulieu, C. Schmitt, C. Sanchez, Protein–polysaccharide interactions: phase-ordering kinetics, thermodynamic and structural aspects, *Curr. Opin. Colloid Interface Sci.* 8 (4–5) (2003) 401–414.
- [26] E.J. Bealer, S. Onissem-Karimu, A. Rivera-Galletti, M. Francis, J. Wilkowski, D. Salas-de la Cruz, X. Hu, Protein–polysaccharide composite materials: fabrication and applications, *Polymers* 12 (2) (2020) 464.
- [27] Aniya, Y. Cao, C. Liu, S. Lu, Y. Fujii, J. Jin, Q. Xia, Improved stabilization and in vitro digestibility of mulberry anthocyanins by double emulsion with pea protein isolate and xanthan gum, *Foods* 12 (1) (2022) 151.
- [28] Y.H. Cheng, D.C. Mu, Y. Jiao, Z. Xu, M.L. Chen, Microwave-assisted maillard reaction between rice protein and dextran induces structural changes and functional improvements, *J. Cereal. Sci.* 97 (2021) 103134.
- [29] E. Joeres, S. Drusch, S. Töpfl, A. Juadpur, U. Bindrich, T. Völker, N. Terjung, Ohmic vs. conventional heating: influence of moderate electric fields on properties of potato protein isolate gels, *Innovat. Food Sci. Emerg. Technol.* 85 (2023) 103333.
- [30] E.A. Cortés-Morales, G. Mendez-Montealvo, G. Velazquez, Interactions of the molecular assembly of polysaccharide–protein systems as encapsulation materials. A review, *Adv. Colloid Interface Sci.* 295 (2021) 102398.
- [31] C.S. Wang, N. Virgilio, P.M. Wood-Adams, M.C. Heuzey, A gelation mechanism for gelatin/polysaccharide aqueous mixtures, *Food Hydrocolloids* 79 (2018) 462–472.
- [32] R. Xu, L. Zheng, S. Zhang, M. Huang, M. Zhao, Complex coacervation of type II collagen with anionic polysaccharides: effects of solution pH and molecular conformation of polysaccharide, *Food Hydrocolloids* (2023) 108990.
- [33] D.S.D.S. de Souza, V.A.P. Tartare, B. da Silva Bega, G.C. Zambuzi, T.S. Ribeiro, C. Ribeiro, K.R. Francisco, The pH role in casein-carboxymethylcellulose nano/microparticles formation, *Colloids Surf. A Physicochem. Eng. Asp.* 682 (2024) 132953.
- [34] J. Pan, H. Xu, M. Dabbour, B.K. Mintah, L. Huang, C. Dai, H. Ma, Effect of pectin concentration on emulsifying properties of black soldier fly (*Hermetia illucens*) larvae albumin modified by pH-shifting and ultrasonication, *Int. J. Biol. Macromol.* 257 (2024) 128779.
- [35] C.G. Miranda, R.M. Rodrigues, R.N. Pereira, P. Speranza, L.E. Kurozawa, A. A. Vicente, A.C.K. Sato, Influence of ohmic heating on lentil protein structure and protein–pectin interactions, *Innovat. Food Sci. Emerg. Technol.* (2023) 103413.
- [36] M. Zhao, H. He, A. Ma, T. Hou, Sources, chemical synthesis, functional improvement and applications of food-derived protein/peptide-saccharide covalent conjugates: a review, *Crit. Rev. Food Sci. Nutr.* 63 (23) (2023) 5985–6004.
- [37] K. Liu, Y.Y. Chen, X.Q. Zha, Q.M. Li, L.H. Pan, J.P. Luo, Research progress on polysaccharide/protein hydrogels: preparation method, functional property and application as delivery systems for bioactive ingredients, *Food Res. Int.* 147 (2021) 110542.
- [38] M. Michel, K. Autio, Effects of high pressure on protein-and polysaccharide-based, Ultra High Pressure Treatment of Foods 189 (2012).
- [39] B. Jiao, A. Shi, H. Liu, X. Sheng, L. Liu, H. Hu, Q. Wang, Effect of electrostatically charged and neutral polysaccharides on the rheological characteristics of peanut protein isolate after high-pressure homogenization, *Food Hydrocolloids* 77 (2018) 329–335.
- [40] X. Zhuang, L. Wang, X. Jiang, Y. Chen, G. Zhou, The effects of three polysaccharides on the gelation properties of myofibrillar protein: phase behaviour and moisture stability, *Meat Sci.* 170 (2020) 108228.
- [41] M. Zioga, V. Evageliou, Formation and physicochemical properties of insoluble complexes resulted from high methoxyl pectin–protein interactions, *Food Hydrocolloids* 142 (2023) 108806.
- [42] M.Q. Guo, X. Hu, C. Wang, L. Ai, Polysaccharides: structure and solubility, *Solubility of polysaccharides* 2 (2017) 8–21.
- [43] K. Wang, Y. Li, J. Sun, Y. Zhang, The physicochemical properties and stability of myofibrillar protein oil-in-water emulsions as affected by the structure of sugar, *Food Chem. X* 18 (2023) 100677.
- [44] T. Sanvictores, F. Farci, Biochemistry, Primary Protein Structure, 2020.
- [45] K.K. Goh, A. Teo, A. Sarkar, H. Singh, Milk protein–polysaccharide interactions, in: *Milk Proteins*, Academic Press, 2020, pp. 499–535.
- [46] Y. Wei, Z. Cai, M. Wu, Y. Guo, R. Tao, R. Li, H. Zhang, Comparative studies on the stabilization of pea protein dispersions by using various polysaccharides, *Food Hydrocolloids* 98 (2020) 105233.
- [47] G. Chakraborty, A. Bhattarai, R. De, Polyelectrolyte–dye interactions: an overview, *Polymers* 14 (3) (2022) 598.
- [48] Z. Yadollahi, M. Motiei, N. Kazantseva, J. Cisar, P. Saha, Whey protein isolate-chitosan PolyElectrolyte nanoparticles as a drug delivery system, *Molecules* 28 (4) (2023) 1724.
- [49] F. Comert, F. Azarikia, P.L. Dubin, Polysaccharide zeta-potentials and protein-affinity, *Phys. Chem. Chem. Phys.* 19 (31) (2017) 21090–21094.
- [50] Y. Han, L. Zhu, E. Karrar, X. Qi, H. Zhang, G. Wu, Pickering foams stabilized by protein-based particles: a review of characterization, stabilization, and application, *Trends Food Sci. Technol.* 133 (2023) 148–159.
- [51] Y. Han, L. Zhu, H. Zhang, T. Liu, G. Wu, Characteristic of the interaction mechanism between soy protein isolate and functional polysaccharide with different charge characteristics and exploration of the foaming properties, *Food Hydrocolloids* 150 (2024) 109615.

- [52] L. Bekale, D. Agudelo, H.A. Tajmir-Riahi, Effect of polymer molecular weight on chitosan–protein interaction, *Colloids Surf. B Biointerfaces* 125 (2015) 309–317.
- [53] F. Behrouzain, S.M. Razavi, H. Joyner, Mechanisms of whey protein isolate interaction with basil seed gum: influence of pH and protein-polysaccharide ratio, *Carbohydr. Polym.* 232 (2020) 115775.
- [54] O.G. Jones, D.J. McClements, Biopolymer nanoparticles from heat-treated electrostatic protein–polysaccharide complexes: factors affecting particle characteristics, *J. Food Sci.* 75 (2) (2010) N36–N43.
- [55] C.J. Souza, A.R. da Costa, C.F. Souza, F.F.S. Tosin, E.E. Garcia-Rojas, Complex coacervation between lysozyme and pectin: effect of pH, salt, and biopolymer ratio, *Int. J. Biol. Macromol.* 107 (2018) 1253–1260.
- [56] D.E. Igartua, F.A. Platania, A. Balcone, G.G. Palazolo, D.M. Cabezas, Impact of heat treatment in whey proteins-soluble soybean polysaccharides electrostatic complexes in different pH and biopolymer mass ratio conditions, *Applied Food Research* 2 (2) (2022) 100184.
- [57] H. Jiang, Z. Liu, S. Wang, Microwave processing: effects and impacts on food components, *Crit. Rev. Food Sci. Nutr.* 58 (14) (2018) 2476–2489.
- [58] S. Chandrasekaran, S. Ramanathan, T. Basak, Microwave food processing—a review, *Food Res. Int.* 52 (1) (2013) 243–261.
- [59] L. Ji, Y. Xue, T. Zhang, Z. Li, C. Xue, The effects of microwave processing on the structure and various quality parameters of Alaska pollock surimi protein-polysaccharide gels, *Food Hydrocolloids* 63 (2017) 77–84.
- [60] V.L. Silva, L.M. Santos, A.M. Silva, Ohmic heating: an emerging concept in organic synthesis, *Chem.–Eur. J.* 23 (33) (2017) 7853–7865.
- [61] J.B. Shivaji, R.J. Mohan, A. Rawson, A. Pare, Effect of ohmic heating on protein characteristics and beany flavor of soymilk, *Int. J. Chem. Stud.* 9 (1) (2021) 248–252.
- [62] S. Ferreira, L. Machado, R.N. Pereira, A.A. Vicente, R.M. Rodrigues, Unraveling the nature of ohmic heating effects in structural aspects of whey proteins—The impact of electrical and electrochemical effects, *Innovat. Food Sci. Emerg. Technol.* 74 (2021) 102831.
- [63] S.D. Pillai, S. Shayanfar, Electron beam technology and other irradiation technology applications in the food industry, *Applications of radiation chemistry in the fields of industry, biotechnology and environment* (2017) 249–268.
- [64] K. Cieřla, S. Salmieri, M. Lacroix,  $\gamma$ -Irradiation influence on the structure and properties of calcium caseinate– whey protein isolate based films. Part 2. Influence of polysaccharide addition and radiation treatment on the structure and functional properties of the films, *J. Agric. Food Chem.* 54 (23) (2006) 8899–8908.
- [65] X. Chen, R. Zhang, Y. Li, X. Li, L. You, V. Kulikouskaya, K. Hileuskaya, Degradation of polysaccharides from Sargassum fusiforme using UV/H<sub>2</sub>O<sub>2</sub> and its effects on structural characteristics, *Carbohydr. Polym.* 230 (2020) 115647.
- [66] X. Ding, P. Yao, Soy protein/soy polysaccharide complex nanogels: folic acid loading, protection, and controlled delivery, *Langmuir* 29 (27) (2013) 8636–8644.
- [67] H.M. Lung, Y.C. Cheng, Y.H. Chang, H.W. Huang, B.B. Yang, C.Y. Wang, Microbial decontamination of food by electron beam irradiation, *Trends Food Sci. Technol.* 44 (1) (2015) 66–78.
- [68] N. BenBettaieb, T. Karbowiak, S. Bornaz, F. Debeaufort, Spectroscopic analyses of the influence of electron beam irradiation doses on mechanical, transport properties and microstructure of chitosan-fish gelatin blend films, *Food Hydrocolloids* 46 (2015) 37–51.
- [69] C. Krömmelbein, M. Mütze, R. Konieczny, N. Schönherr, J. Griebel, W. Gerdes, S. Riedel, Impact of high-energy electron irradiation on mechanical, structural and chemical properties of agarose hydrogels, *Carbohydr. Polym.* 263 (2021) 117970.
- [70] N. BenBettaieb, O. Chambin, T. Karbowiak, F. Debeaufort, Release behavior of quercetin from chitosan-fish gelatin edible films influenced by electron beam irradiation, *Food Control* 66 (2016) 315–319.
- [71] Xumei Feng, Hua Chen, Yaru Liang, Mengjie Geng, Mingyu He, Yuyang Huang, Yang Li, Fei Teng, Effects of electron beam irradiation treatment on the structural and functional properties of okara insoluble dietary fiber, *J. Sci. Food Agric.* 103 (2022), <https://doi.org/10.1002/jsfa.12131>.
- [72] W. Jin, Z. Wang, D. Peng, W. Shen, Z. Zhu, S. Cheng, Q. Huang, Effect of pulsed electric field on assembly structure of  $\alpha$ -amylase and pectin electrostatic complexes, *Food Hydrocolloids* 101 (2020) 105547.
- [73] S.G. Giteru, A. Ali, I. Oey, Understanding the relationship between rheological characteristics of pulsed electric fields treated chitosan-zein-poly (vinyl alcohol)-polyethylene glycol composite dispersions and the structure-function of their resulting thin-films, *Food Hydrocolloids* 113 (2021) 106452.
- [74] F. Van de Velde, E.H. de Hoog, A. Oosterveld, R.H. Tromp, Protein-polysaccharide interactions to alter texture, *Annu. Rev. Food Sci. Technol.* 6 (2015) 371–388.
- [75] S.M.T. Gharibzadeh, C. Hernández-Ortega, J. Welte-Chanes, P. Putnik, F.J. Barba, K. Mallikarjunan, S. Roohinejad, High pressure processing of food-grade emulsion systems: antimicrobial activity, and effect on the physicochemical properties, *Food Hydrocolloids* 87 (2019) 307–320.
- [76] B. Jin, X. Zhou, Y. Zhong, Q. Li, S. Zhang, H. Mo, J. Liang, The synergistic effect of high pressure processing and pectin on the physicochemical stability and antioxidant properties of biopolymer complexes composed of soy protein and coumarin, *Process Biochem.* 104 (2021) 46–54.
- [77] M. Corredig, N. Sharabafi, E. Kristo, Polysaccharide–protein interactions in dairy matrices, control and design of structures, *Food Hydrocolloids* 25 (8) (2011) 1833–1841.
- [78] S. Mende, H. Rohm, D. Jaros, Influence of exopolysaccharides on the structure, texture, stability and sensory properties of yoghurt and related products, *Int. Dairy J.* 52 (2016) 57–71.
- [79] D. Roy, A. Ye, P.J. Moughan, H. Singh, Composition, structure, and digestive dynamics of milk from different species—a review, *Front. Nutr.* 7 (2020) 577759.
- [80] D.A. Goulding, P.F. Fox, J.A. O'Mahony, Milk Proteins: an Overview. *Milk Proteins*, 1020, pp. 21–98.
- [81] I.T. Smykov, Protein-polysaccharide interactions in dairy production, *Food systems* 3 (4) (2021) 24–33.
- [82] X. Liu, G. Sala, E. Scholten, Role of polysaccharide structure in the rheological, physical and sensory properties of low-fat ice cream, *Curr. Res. Food Sci.* 7 (2023) 100531.
- [83] H. Du, X. Wang, H. Yang, F. Zhu, J. Cheng, X. Peng, X. Liu, Effects of mulberry pomace polysaccharide addition before fermentation on quality characteristics of yogurt, *Food Control* 153 (2023) 109900.
- [84] P.M.D.C.C. Pereira, A.F.D.R.B. Vicente, Meat nutritional composition and nutritive role in the human diet, *Meat Sci.* 93 (3) (2013) 586–592.
- [85] X. Wang, T. Feng, S. Xia, Saltiness perception related to salt release of surimi emulsified sausages: modulation in texture and microstructure by polysaccharides, *Int. J. Food Sci. Technol.* 56 (8) (2021) 3893–3902.
- [86] X.D. Shi, J.J. Huang, J.L. Wu, X.X. Cai, Y.Q. Tian, P.F. Rao, S.Y. Wang, Fabrication, interaction mechanism, functional properties, and applications of fish gelatin-polysaccharide composites: a review, *Food Hydrocolloids* 122 (2022) 107106.
- [87] Y. Mu, J. Sun, M. Obadi, Z. Chen, B. Xu, Effects of saccharides on the rheological and gelling properties and water mobility of egg white protein, *Food Hydrocolloids* 108 (2020) 106038.
- [88] S. Levi, V. Rac, V. Manojlović, V. Rakić, B. Bugarski, T. Flock, V. Nedović, Limonene encapsulation in alginate/poly (vinyl alcohol), *Procedia Food Science* 1 (2011) 1816–1820.
- [89] M. Peanparkdee, S. Iwamoto, C. Borompichaichartkul, K. Duangmal, R. Yamauchi, Microencapsulation of bioactive compounds from mulberry (*Morus alba* L.) leaf extracts by protein–polysaccharide interactions, *Int. J. Food Sci. Technol.* 51 (3) (2016) 649–655.
- [90] K. Li, M.W. Woo, H. Patel, C. Selomulya, Enhancing the stability of protein-polysaccharides emulsions via Maillard reaction for better oil encapsulation in spray-dried powders by pH adjustment, *Food Hydrocolloids* 69 (2017) 121–131.
- [91] P.A. Loyeau, M.J. Spotti, G. Vinderola, C.R. Carrara, Encapsulation of potential probiotic and canola oil through emulsification and ionotropic gelation, using protein/polysaccharides Maillard conjugates as emulsifiers, *Lwt* 150 (2021) 111980.
- [92] M.M.G. de Oliveira, K. de Souza Silva, M.A. Mauro, Evaluation of interactions between carboxymethylcellulose and soy protein isolate and their effects on the preparation and characterization of composite edible films, *Food Biophys.* 16 (2021) 214–228.
- [93] X. Zhang, Y. Zhao, Y. Li, L. Zhu, Z. Fang, Q. Shi, Physicochemical, mechanical and structural properties of composite edible films based on whey protein isolate/psyllium seed gum, *Int. J. Biol. Macromol.* 153 (2020) 892–901.