

The application and mechanism of polysaccharides, proteins and their complexes on enhancing yogurt gel stability: a review

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Abstract: Yogurt is highly sought after by consumers because of its unique flavor, rich nutritional value and health care function. However, during its sale and storage, some undesirable phenomena such as unstable gel structure and whey precipitation are prone to occur, which has affected the further development of yogurt industry to some extent. Polysaccharides and proteins act as typical thickeners and stabilizers in food, enhancing the structural stability of yogurt gel through different mechanisms. Anionic polysaccharides interact with casein electrostatically to form complexes, while neutral polysaccharides rely on their hydration capacity and act as fillers to stabilise the three-dimensional structure of yogurt. Milk proteins and vegetable proteins play a stabilising role mainly through hydrophobic interactions and disulphide bonds. Gelatin, on the other hand, relies on its good hydration capacity and gelation properties. Polysaccharide and protein complexes mainly rely on non-covalent interactions to stabilise the yogurt gel structure. This paper reviewed the mechanisms of yogurt gel formation and the factors that affect yogurt texture. It also summarized the research progress on enhancing the structural stability of yogurt gel using polysaccharides and proteins as well as their complexes as additives, with a view to providing theoretical bases for improvement of the textural properties of yogurt.

Keywords: yogurt gel; stability; polysaccharide; protein; polysaccharide-protein complexes

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1 Introduction

Yogurt is one of the most widely consumed fermented dairy products in the world, which is prepared by lactic acid fermentation using microorganisms with fresh cow's milk as the main ingredient^[1-3]. Yogurt not only provides nutrients such as proteins and vitamins, but also has many health functions such as alleviating lactose intolerance, improving diarrhea/constipation, enhancing immunity, and lowering cholesterol and blood glucose levels^[4-8]. The microstructure of yogurt is a three-dimensional network structure, consisting mainly of a network skeleton of protein matrices, with interstices filled with substances such as free water and fat globules^[9]. A fundamental sensory characteristic of yogurt is texture, and textural defects such as unstable gel structure, whey precipitation, and protein layering can reduce consumer desire to buy^[10]. Various countermeasures have been taken to improve the texture including the addition of different thickening stabilizers such as proteins, polysaccharides, and their roles and mechanisms for stabilising the gel structure of yogurt will be elaborated in Sections 3 and 4 of this paper^[5,11]. In addition, different processing conditions can also have an effect on the texture of yogurt^[12]. The processing steps of yogurt are usually divided into milk standardization, homogenization, heat treatment and fermentation^[12-13]. During milk acidification, intermolecular interactions and macromolecular arrangements determine the final properties of the gel network. These interactions are influenced by processing conditions such as time, temperature, and shear strength^[14-16]. Research has shown that homogenization under pressures of 8–16 MPa can reduce the size of fat globules, promote the formation of fat clusters, improve the microstructure and

rheological properties of buffalo yogurt, and create a tighter three-dimensional network structure^[17]. Heat treatment can almost completely denature whey proteins, enhance yogurt viscosity and hardness, and improve yogurt consistency^[18]. Heat-treated milk treated with microfluidisation at 150 MPa improves the rheological and organoleptic properties of yogurt, especially low-fat yogurt^[19]. Fermentation parameters also significantly affect gel strength, and the fermentation temperature is usually 42–45 °C^[5,20]. Higher temperatures can lead to increased synchronization during storage, resulting in textural defects in yogurt^[5,21]. In addition, the leavening agent also has an effect on the texture of yogurt. Research has shown that adding *Lactiplantibacillus plantarum* ZFM55 can enhance the water-holding capacity (WHC), viscosity, and firmness of yogurt, improving its texture and taste^[22]. *Lactobacillus helveticus* MB2-1 can produce a large amount of extracellular polysaccharides (EPS), reducing the fermentation time of yogurt gel, and improving the structure and storage stability of yogurt^[23].

This paper reviewed the formation mechanism of yogurt gel and the main factors affecting the texture of yogurt, and summarized the methods of polysaccharides and proteins to improve the stability of the texture of yogurt gel, aiming to provide theoretical guidance and technical support for the enhancement of yogurt gel characteristics and yogurt quality.

2 Methods

This review indexed through Web of Science. A total of 162 papers published between 2014 and 2024 used both the keywords “Yogurt” and “Gel stability”. The keyword co-occurrence network in Figure 1

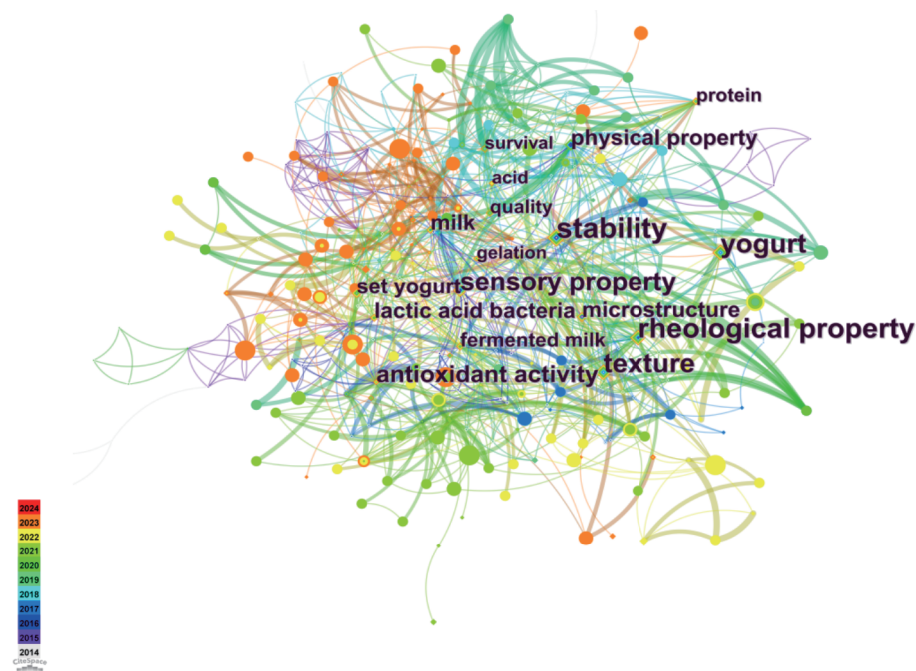


Figure 1 Keyword co-occurrence network visualization of published articles on yogurt and its gel stability (2014–2024).

indicates that yogurt stability, rheological properties, and structure are hot topics in the field because they are related to yogurt texture, which affects the development of the yogurt industry.

3 Mechanism and influencing factors of yogurt gel

3.1 Formation mechanism of gel network structure of yogurt

Milk proteins contain two types of colloidal particles, casein and whey proteins, with casein accounting for the largest proportion^[24–25]. Casein micelles consist of four caseins (α_{s1} -, α_{s2} -, β -, and κ -casein) and colloidal calcium phosphate, bound together by hydrophobic interactions and hydrogen bonding. κ -Casein is located on the surface of casein micelles and stabilizes the

aggregation of casein micelles by spatial and electronegative repulsive forces to prevent flocculation^[26–28].

The gelation process of milk is defined as the transformation from low viscosity Newtonian fluid to semi-solid or solid^[29]. During the acidification process, lactic acid bacteria convert lactose from milk to lactic acid and the pH decreases. As shown in Figure 2, the extension of κ -casein collapsed at pH 6.0, when the pH is reduced to 5.3, the micelle particles decompose and colloidal calcium phosphate is released from the interior of the micelles, whereas when the pH drops to the casein isoelectric point (pI) 4.6, casein micelles begin to aggregate the casein micelles started to aggregate and form a three-dimensional network gel structure together with whey proteins. Finally, the microstructure of yogurt gel is a three-dimensional network of aggregated casein with embedded fat

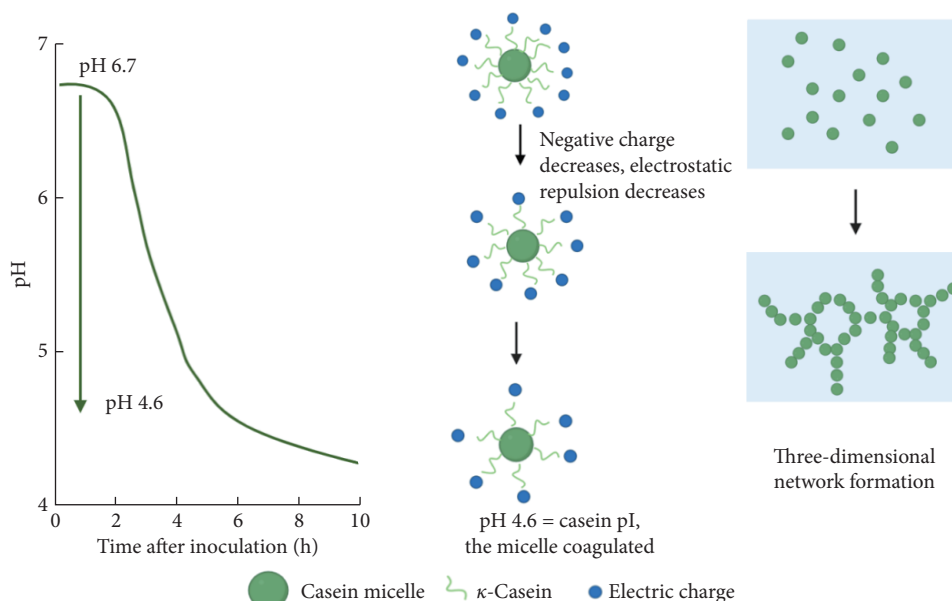


Figure 2 Schematic diagram of the formation mechanism of yogurt gel network structure.

globules and voids, and the center is filled with substances such as proteins and lactose^[27–28,30–32].

3.2 Texture defects of yogurt and influencing factors

Yogurt is a healthy and tasty dairy product, and its texture is an important indicator of consumer acceptance. However, quality defects such as dehydration shrinkage or whey separation can occur during processing and storage, reducing consumer acceptance^[5]. Whey separation is a key defect of yogurt, which will precipitate liquid on the surface of yogurt gel, thus producing undesirable sensory characteristics. In addition, due to the loss of water-soluble nutrients, the nutritional value of yogurt may also decrease. In the process of yogurt formation, the interaction between macromolecules and colloids in yogurt forms a special network structure, and the gel structure is very important in determining the texture stability of yogurt^[5,33–34]. The rearrangement of three-dimensional network in the gel process leads to gel shrinkage, which may result in the expulsion of whey from the gel matrix, thus bringing quality defects to yogurt^[21,35]. The structural defect of yogurt is also a common quality defect, which mainly includes granular texture and undesirable precipitation. Factors such as mechanical vibration during fermentation, excessive shear rate, high fermentation temperature, reduce in protein and milk fat content affect the structural stability of yogurt gel, resulting in rough texture and poor taste of yogurt^[14–15,21,36–37].

Heat treatment not only kills some microorganisms unwanted for fermentation and promotes the growth of lactic acid bacteria, but also leads to the denaturation of whey proteins and promotes their binding with casein micelles to form a strong gel network structure, which enhances the WHC and improves the stability of yogurt^[38–40]. Generally, heat treatment at 95 °C for 5 min or 80 °C for 30 min can achieve complete denaturation of whey proteins and kill most of the useless microorganisms, which can enhance the gel strength of yogurt^[18]. High pressure homogenization (HPH) combined with heat treatment can improve the rheological properties and stability of yogurt. Homogenized milk before heat treatment has higher hardness and apparent viscosity compared to homogenized milk prepared yogurt after heat treatment, which is beneficial for protein interactions, gel formation, and these indicators increase with the increase of homogenization temperature^[41–42]. Homogenization (15–20 MPa) or HPH can improve the WHC of yogurt, enhance the structural stability of gel network and reduce whey precipitation by influencing protein interactions^[38]. In recent years, some scholars have improved the texture and efficacy of yogurt by improving fermentation agents. Some yogurt starters can release EPS, and yogurt prepared by fermenting them has a better texture compared to strains that do not produce EPS. For example, a novel synbiotic yogurt prepared with *Lactobacillus paracasei* was effective in increasing the total solids content, enhancing the WHC and improving the texture of yogurt^[43–44].

The influence of the milk base on the quality of the curd is mainly related to the protein and fat content^[45–46]. The ratio of protein to total solids and the ratio of whey to casein proteins affect the gel characteristics^[5]. Exogenously added proteins can act as fillers in the yogurt gel network and strengthen the three-dimensional network stability^[47–49]. Appropriately increasing the ratio of whey to casein proteins can improve the strength and elasticity of yogurt gels, form a denser network structure, improve WHC, and reduce whey separation^[50]. In addition, protein

functional properties can be improved by enzymatic reactions. For example, glutamine transferase induces intramolecular and intermolecular cross-linking of proteins through acyl transfer reactions, promoting the formation of a denser and more homogeneous network structure in yogurt^[16,48,51–52]. Milk fat content also has an important effect on the stability of the gel structure, decreasing the fat content reduces the strength and viscosity of the yogurt gel, leading to textural defects such as whey separation and granular texture^[29,46,53–54]. Enhancement of milk fat content is accompanied by enhancement of milk fat globule content, and the smaller size of fat globules after homogenization may contribute to their interaction with proteins and enhance gel network binding stability. Thus full fat yogurt generally has a better texture than low fat yogurt^[1,29].

Improving the stability of yogurt is key to promoting the sustainable and stable development of the yogurt industry. In addition to improving its stability by adding polysaccharides and whey protein through physical methods, yogurt gel can also induce whey protein aggregation through chemical methods such as heat treatment, acid induction, enzyme-induced cross-linking, and other strategies to promote gelation and influence the stability of yogurt^[16,55–57]. This review mainly summarized the effects and mechanisms of polysaccharides, proteins, and their complexes on the stability of yogurt gel.

4 The application and mechanism of polysaccharides, proteins and their complexes in improving the stability of yogurt gel

Polysaccharides and proteins are hydrophilic, and are often used as thickeners and stabilizers to improve food texture. Different kinds of polysaccharides and proteins can improve the WHC of yogurt through different mechanisms, and improve the texture, stability, taste and appearance of yogurt^[57].

4.1 Polysaccharides

Addition of polysaccharides improves yogurt's WHC and gel structure^[38]. One possible mechanism by which polysaccharides improve WHC is that polysaccharide molecules are hydrophilic and can retain water in the three-dimensional gel network, increasing the rigidity of the protein gel network^[2]. The improvement of yogurt texture by polysaccharides depends on their ionic state, and anionic and neutral polysaccharides can improve the stability of yogurt through different mechanisms, respectively^[5].

4.1.1 Anionic polysaccharides

After the addition of anionic polysaccharides to yogurt, the oppositely charged casein micelles and anionic polysaccharides (e.g., modified starch, pectin, and sodium alginate (SA)) form complexes through electrostatic interactions between the COO[−] of the anionic polysaccharides and the NH₃⁺ of the casein after passing through the pI of the proteins to improve the structural stability of the gel and to reduce the dehydration and condensation of yogurt^[2,35]. There are two main mechanisms by which neutral polysaccharides promote yogurt gelation: one is that neutral polysaccharides do not interact electrostatically with casein, but act as fillers by increasing the viscosity of the continuous phase in the gel network; the other is that neutral polysaccharides have a high hydration capacity of their own, and form a three-dimensional gel network in the yogurt system, which is helpful to lock water, increase the viscosity of yogurt, and reduce whey separation^[2,55].

Modified starch is favored by manufacturers because of its high production efficiency^[5,59]. It is divided into anionic polysaccharide and neutral polysaccharide. The interaction between starch and casein includes electrostatic adhesion, spatial stability, and hydrogen bonding. Phosphate starch, due to the introduction of phosphate groups, has a large number of anionic charges. When mixed with casein, it is more prone to electrostatic adsorption, resulting in a continuous and tight gel network structure, stabilizing the gel structure^[27]. Cross-linked acetylated starch can adsorb on the surface of casein to form a thicker adsorption layer, which can be overlapped and penetrated when micelle molecules collide with each other, but cannot be compressed. The concentration of polymers increases, leading to the occurrence of an osmotic effect in the yogurt system. The osmotic effect plays a dominant role in spatial stability in the yogurt system. Cross-linked acetylated starch maintains the stability of the yogurt gel structure through electrostatic interactions and spatial stability^[60]. Hydroxypropyl starch and octenyl succinate starch ester are neutral polysaccharides, and their main interaction with casein is spatial stability to make the gel structure more compact and improve its stability^[27]. Although starch and chemically refined thickening stabilizers are cost-effective, they are perceived by consumers as “unnatural” ingredients. With the growing consumer demand for “clean label” products, cellulose, SA, and EPS are emerging as new potential alternatives to improve yogurt gels^[61–62].

Pectin is a type of anionic polysaccharide that has been widely studied in recent years for its interaction mechanism with casein. Pectin-casein interactions are mainly influenced by the degree of pectin esterification, pH in yogurt, polysaccharide charge density and polysaccharide concentration^[55,63]. High-methoxy pectin protects casein micelles from aggregation at acidic pH and prevents yogurt gelation. Low-methoxy pectin can form gels by bridging with casein micelles and slow down syneresis^[64].

Adding fiber-rich fruit extract to yogurt can not only increase the flavor of yogurt, but also improve the nutritional value of yogurt. Soluble and insoluble dietary fiber in fruits can reduce the phase separation between casein micelle and whey through different mechanisms^[65]. The soluble fiber in apple pomace (AP), such as pectin, dominates the gel structure at low fiber concentrations in the casein network, and below pH 5.0, pectin carboxylate groups adsorb to the surface of the casein micelles by electrostatic interaction^[63–64]. At higher fiber concentrations (1.0%), pectin is embedded in the casein network to form casein-pectin complexes, which reduce the gaps between the protein clusters and make the protein network structure more compact, thus stabilizing the yogurt gel structure^[2,64,66]. When the AP concentration was 0.5%, there was no significant effect on the yogurt gel structure^[61]. A high concentration of anionic polysaccharides leads to larger gaps between protein clusters due to increased bridging of polysaccharides, which in turn reduces the stability of the gel structure. Organic acids in fruits can also disrupt the three-dimensional gel network structure of yogurt by inducing casein denaturation and aggregation^[2,64]. In addition, carboxyl groups between the chains of pectin molecules can interact with calcium ions released from casein micelles to promote gel network stabilization^[64].

The main component of insoluble fiber is cell wall particles (CWP), which have strong hydrophilicity and hold large amounts of water through a porous fiber structure^[65–66]. With the increase of CWP addition, the hardness and elasticity of yogurt gel increased

and whey separation phenomenon decreased, but the gel structure was unstable after the concentration exceeded 2%, and the same phenomenon was observed with the addition of different concentrations of insoluble soybean fiber^[65,67–68]. This may be because at low CWP concentrations, the yogurt gel structure is mainly composed of proteins, and the slight spatial hindrance and electrostatic interaction between proteins and a small amount of cellulose make the gel network more uniform, and the high WHC of CWP stabilizes the water content in the network voids. At high concentrations, the structure of yogurt is mainly dominated by proteins and insoluble fibers together, and CWP's ability to form a strong gel network structure adversely affects yogurt texture^[65,68–69].

It has been shown that under acidic conditions, anionic polysaccharides enhance the network structure formed by casein micelles in yogurt mainly through electrostatic interactions, and at the same time, increase the WHC of yogurt through hydrogen bonding with free water in the network interstitial space^[55,70–72]. Most polysaccharides improve the network structure of yogurt gels with increasing concentration, but then destabilize the yogurt gels when the concentration is too high. For example, at low SA concentrations ($\leq 0.2\%$), the thickening effect dominates and SA enhances the viscosity of yogurt and improves the yogurt gel structure stability. In contrast, at high concentrations ($\geq 0.3\%$), the incompatibility of casein micelles with SA predominates, and phase separation of SA impairs yogurt gel stability^[73].

EPS is a class of natural products secreted by microbial growth and metabolism during yogurt fermentation^[74–75]. The addition of small amounts of EPS to yogurt improves yogurt texture due to the repulsive forces between EPS and casein micelles, which are tightly bound together, resulting in an increase in the WHC of the three-dimensional lattice structure^[76]. The ability of EPS to modulate the texture of yogurt is not only related to their concentration, but also to their structural features, such as charge, molecular weight, monomer composition, and branching degree^[75]. *Streptococcus thermophilus* strains produce pod-like, negatively charged EPS (cEPS[−]). *Lactococcus lactis*-producing strains produce free, neutral EPS (fEPS⁰). Strains producing cEPS[−] induced a tighter network structure than strains producing fEPS⁰. cEPS[−] interacted with casein through hydrogen bonding to form an electrostatic complex, improving the stability of the gel structure. fEPS⁰ increased adsorptive linkages between caseins, filling the three-dimensional network structure of the casein clusters, and improving the viscosity within the reticular pores and improve the gel texture^[77–79].

4.1.2 Neutral polysaccharides

Neutral polysaccharides (such as konjac glucomannan, guar gum, β -glucan, inulin (IN)) have a high hydration capacity by themselves, which can increase the solution viscosity, and can form gel particles with a high WHC to fill in the voids of the casein micelles, stabilize the structure of the yogurt gels, and reduce the dehydration and condensation^[2,5,35,55,80]. Within a certain range, with the increase of neutral polysaccharide concentration, the WHC and viscosity of yogurt gel are enhanced.

IN is a linear fructose polymer widely found in plant tissues with antioxidant activity, regulation of blood sugar and lipids, and other physiological functions. Natural inulin has different degrees of polymerization (DP), ranging from 2 to 60 monomers. The physicochemical properties of IN vary with different DPs, and long-chain IN has stronger hydration ability and lower solubility

compared to short-chain IN, which can substitute for fat and improve yogurt texture and sensory properties more effectively^[5,73,81]. The addition of IN brought the final hardness of low-fat yogurt closer to that of full-fat yogurt, with a more stable gel network structure and tighter pores^[73]. This may be attributed to the hydroxyl groups of IN enveloping water, forming gel particles with high WHC. IN acts as a filler to form a secondary gel structure between the casein micelle aggregates, and as its concentration increases, the secondary gel structure increases and the yogurt gel structure becomes more stable^[5,81–82].

In recent years, there have been conflicting results on the effect of IN on the texture and rheological properties of low fat yogurt. Some scholars have found that the rheological properties and sensory scores of low-fat yogurt with 1% and 2% IN were closer to those of full-fat yogurt, whereas hardness and viscosity were decreased with a high concentration of 3% IN. It is possible that the high concentration of polysaccharides affects the solubility of the polymers by strong cross-linking with proteins, which leads to a decrease in the hydration capacity and viscosity, and destabilizes the yogurt gel structure^[73,83–84]. It has also been shown that the hardness of yogurt is directly proportional to fat and inulin concentration. The differences in results may be due to variations in total protein or total solids content in the samples^[85]. In addition to the above-mentioned common polysaccharides, some polysaccharides that have an improving effect on the stability of yogurt gel have been compiled (Table 1).

4.2 Protein

Adding protein to fortified milk before fermentation is one of the important means to improve the texture of yogurt. The increase in protein content in the milk base leads to the increase of hardness, elasticity and WHC of yogurt, forming a denser gel network structure. This is due to the increase in the number of protein participating in the three-dimensional gel network, while casein is cross-linked with whey protein^[86–88].

4.2.1 Milk protein

Milk-based protein can not only improve the gel stability of yogurt, but also enhance the nutrition of yogurt. Milk-based proteins that are often used to strengthen yogurt are casein derivatives, whey proteins and their processed products. It has been shown that yogurt prepared with sodium calcium caseinate (SCaCN) has a shorter fermentation time than yogurt with skim milk powder added, with smaller interstitial pores, higher adhesiveness and elasticity of yogurt, reduced dehydration and shrinkage, and improved texture of yogurt^[89]. In comparison to SCaCN, although yogurt supplemented with concentrated whey protein does not

shorten the fermentation time, its gel structure is finer and it has higher WHC^[90].

The addition of whey protein prior to fermentation usually enhances the WHC, hardness, and structural stability of the gel network of yogurt gels^[91–92]. This may be due to the denaturation of whey proteins by heat treatment prior to fermentation, and the creation of disulfide bonds and hydrophobic interactions on the surface of denatured whey proteins, leading to the formation of whey protein aggregates (WPA)^[93]. WPA interact with κ -casein to form micellar complexes, which lead to the formation of a more homogeneous and tightly packed porous structure of yogurt gels, with a highly linked gel network, which traps more free water in the pore space, thus improving yogurt texture^[56,94–97]. Covalent bonding has been found to have a greater effect on yogurt gel strength, with the strength of gels primarily stabilized by covalent bonds generally higher than those primarily stabilized by non-covalent bonds^[98]. For a given total protein content, reducing the proportion of casein improves the WHC of yogurt and produces a more stable protein network structure. This is due to the fact that casein is more likely to aggregate in the vicinity of its pI rather than bind to water^[87,99–100].

Processed whey protein improves yogurt texture and stability more effectively than natural whey protein. Microparticulated whey protein (MWP) can be used as a fat replacer, providing a similar texture to milk fat. Studies have shown that a higher proportion of natural whey protein in MWP may result in higher WPA formation during heating, which can form higher strength or number of bonds with casein micelles during the acidification stage, contributing to the improvement of yogurt texture^[101–103]. The loose and porous structure of whey protein concentrate (WPC) facilitates the bridging of casein and whey proteins, resulting in a more homogeneous and stable gel network structure, smaller pores, and higher yogurt viscosity and WHC^[96]. It has been shown that the ability of WPC to reduce osmotic effects is concentration dependent, with 0.7% WPC significantly inhibiting whey separation than 0.35% WPC^[99]. In addition, the negative charge of the phosphate group makes it easier for the whey protein isolate-sodium triphosphate (WPI-STPP) to unfold during heat treatment and to interact directly with casein, increasing the hardness, elasticity and viscosity of yogurt and stabilizing the structure of the yogurt gel network. The interaction between the WPI and the STPP also prevents delamination of the proteins precipitated due to the reduction of the porosity of the gel^[56].

4.2.2 Gelatin

Gelatin is an animal protein extracted from collagen^[104–105]. It is a popular stabilizer with good hydration, gelling properties and thermal reversibility of its own, which enhances the gel strength,

Table 1 Application and role of polysaccharides in yogurt.

Polysaccharides source	Application	Role	References
Butternut squash (<i>Cucurbita moschata</i>) seeds	Stabilizer	Improve the physical and rheological properties, sensory scores, and texture of yogurt	[131]
Okra polysaccharide	Stabilizer	Improve the WHC, viscosity, and elasticity of yogurt, reduce the gaps between the gel network, and form a dense network structure	[2]
Curdlan	Stabilizer	By embedding additional gel structures in the casein coagulum network, the WHC and textural stability of yogurt are improved	[35]
Salecan	Stabilizer	Enhance WHC and strengthen the yogurt gel network structure	[70]
Agave fructans	Stabilizer	Self-binding water and interacting with proteins to maintain the stability of the gel structure, and as the concentration increases, it deposits on the casein micelles to form a denser network structure	[82]

viscosity and WHC of yogurt^[105–106]. Gelation and melting of gelatin are associated with a reversible curl-helix transition at around 30 °C. Gelatin molecules gradually transform from an irregularly curled state to an ordered helical state at 30 °C, forming a gel. When the gelatin gel is heated above the melting temperature, the gel melts to form a solution, i.e., the gel is unspooled from the helical structural state to the irregularly curled state. Gelation is also concentration dependent. When the gelatin concentration is low (below 0.4%), the three regions of the helix may come from one chain, forming an intramolecular collagen-like structure, but there is no significant effect on the gel network structure. At higher gelatin concentrations (0.8% and 1.0%), the formation of helices may involve different chains, so that connecting regions inducing gelation can be formed. The spatial interference of gelatin may dominate at low concentrations, leading to a decrease in gelatin hardness, with no significant effect on the gel. The WHC, hardness, and stability of yogurt gels were significantly improved when gelatin was added at concentrations of 0.4%–1.0%. High concentration gelatin itself can interact with water molecules to form hydrogen bonds, leading to a reduction in free water, promoting the formation of a tighter gel network structure and thus inhibiting whey separation^[105,107].

Gelatin is mainly a product extracted from mammals such as pigs and cows, and consumers with certain religious beliefs do not accept such products. Fish gelatin is currently considered a potential alternative. Studies have shown that adding 0.4% tilapia skin gelatin can significantly improve gel WHC and inhibit whey separation in yogurt. The structural strength of the yogurt gel network formed with fish gelatin was weaker than that of mammalian gelatin. When the concentration reached 2.5%, the gel hardness was too high for yogurt production^[108].

4.2.3 Plant protein

In recent years, due to religious beliefs, vegetarianism and the shortage of animal protein resources, the use of plant protein instead of animal protein has gradually attracted the attention of scholars and businessmen. Plant proteins, like whey proteins, are spherical proteins with a clear secondary and tertiary structure. Under low pH conditions, peptide chains can be made more mobile by heating and enzymolysis, and exposed amino acids can combine with other proteins to promote gel formation^[108–109]. The main plant proteins that are currently being used to replace animal proteins in yogurt are legume proteins, cereal proteins and pseudo-cereal proteins^[110].

Beans are the main source of plant yogurt protein because they are rich in high-quality protein. Protein plays a major role in plant protein gels, with legumin playing a dominant role in legume

protein gels^[111]. The addition of soy protein was found to improve the WHC and gel strength of yogurt. Upon heat denaturation of soy protein, its hydrophobic groups are exposed and soluble aggregates are formed, inducing the formation of gel network structure^[112]. In addition, mung bean proteins showed higher hardness, elasticity, and water retention than plant-based yogurts prepared from pea proteins. Pea yogurt had higher adhesion, while WHC was higher than chickpea yogurt^[113–115]. Hydrophobic interaction dominates in the formation of gel structure of pea-based protein yogurt, followed by disulfide bonding, while ionic and hydrogen bonding play a lesser role in the gel structure^[111,114]. Differences in the texture of soy protein yogurts may be attributed to differences in the subunit composition and β -sheet content of the proteins, which affect the viscoelasticity and hardness of the gel network, respectively. The major globulin subunits in soybean and pea proteins are 7S and 11S, and the content of 7S and 11S in pea proteins is slightly higher than that of soybean proteins, whereas the major globulin subunit of mung bean proteins is 8S^[113–114,116–117]. The network gel structure formed by 11S has a higher hardness and strength, and the gel speed is faster, while 7S forms the gel structure mainly through hydrogen bonding, and 8S does not have a disulfide bond, which are mainly connected by hydrophobic interactions and hydrogen bonds. It has been found that strengthening 7S or 11S alone improves the gelation properties of soy protein, but too high a content of 11S (40%) leads to a more loose pore structure^[112,116–118]. Higher β -sheet content increases gel hardness and contributes to a denser gel structure^[111,119]. Although bean-based yogurt shows potential as a substitute for milk-based yogurt in terms of WHC, adhesiveness, and gel structure stability, further exploration and improvement are needed in terms of lactic acid bacteria inoculation dosage, pH, and acidity^[113].

Common cereal proteins include oat, corn, millet, and rice, all of which are rich in protein and fiber. Oat is rich in β -glucan, which has excellent gelation properties, allowing oat yogurt to have good WHC and a stable gel structure. Corn protein has hydrophilic ends and charged side chains that improve yogurt WHC. These cereals can provide methionine that legumes lack, but they are deficient in lysine and tryptophan, and allergy should be considered when cereals containing gluten are added. Quinoa and amaranth are nutritious pseudo-cereals. Quinoa is rich in globulin and albumin, which contribute to gel formation. Amaranth protein can absorb water and oil during gel formation and maintain emulsion stability by preventing aggregation and flocculation of oil droplets. Furthermore, as these pseudo-cereals are naturally gluten-free, they also address issues related to gluten allergens^[110,120–121]. In addition to the above-mentioned common plant proteins, some plant proteins that can improve the stability of yogurt gel structure have also been collected (Table 2).

Table 2 Application and role of plant proteins in yogurt.

Plant proteins	Application	Role	References
Millet protein	Stabilizer	Millet proteins interact with each other and also with lipids and carbohydrates as stabilizers for emulsions, and the use of modification techniques such as fermentation can improve the gelling ability of proteins. It can also act as a prebiotic to promote the role of probiotics in yogurt	[110,132]
Potato protein isolate (PPI)	Stabilizer	By using HPH pretreatment before emulsification, the emulsion droplets become smaller. With increasing pressure, the stability of PPI yogurt gel becomes stronger, and it is most stable at 200 MPa	[123]
Hemp protein	Stabilizer, flavoring	Soybean and hemp protein fermented yogurt fixing the other ingredients, as the hemp protein content increases, the WHC of the yogurt increases, the whey precipitation decreases, the network of pores formed is dense and homogeneous, and the texture improves. The texture and flavor are optimal when 10% hemp protein is added	[133]
Flaxseed	Stabilizer, thickener, gelling agent	Flaxseed mucilage can enhance the WHC of yogurt, reduce syneresis, and stabilize gel structure, and flaxseed without mucilage can not only reduce the dehydration contraction of yogurt, and improve texture, but also have no adverse effects on sensory properties	[134–135]

Plant protein yogurt has received much attention in recent years as a plant-based alternative to dairy products. Future research directions include the following aspects. First, formula optimization, by adjusting the formula of yogurt, including plant protein sources, stabilizers, etc., to improve its stability. For example, increasing the protein content in yogurt appropriately results in a tighter gel structure^[122]. Second, exploring innovative ingredients, such as root vegetables like potatoes and sweet potatoes, rich in starch to improve the yogurt's texture. Third, technical research, developing new processing techniques to improve the stability of yogurt gel. For instance, using techniques like ultra-high pressure processing, microwave-assisted processing, enzymatic hydrolysis, etc., can enhance the stability of the product. The choice of fermentation agents is also crucial, as different strains have varying compatibility with plant substrates, affecting the texture, taste, and functionality of the product^[110,123]. Fourth, gelation mechanism research, further studies the interaction between plant protein molecules, and the contributions of other plant-based yogurt components such as polysaccharides and lipids to gel formation^[120].

4.3 Polysaccharide-protein complexes

The complex of proteins and polysaccharides can effectively solve the adverse effects such as rougher yogurt gel structure and decreased WHC caused by excessive addition of a single protein or polysaccharide^[109,124]. The interaction between proteins and polysaccharides is mainly based on non-covalent interactions to enhance the stability of the gel structure, including electrostatic, hydrophobic, hydrogen interactions, and van der Waals forces, with electrostatic interactions being the most common^[63,104,124]. There are mainly two kinds of interactions between proteins and polysaccharides, one is attraction, the other is repulsion, which depends on their properties and concentration, as well as the pH value of the solution, ionic strength and other factors. When they carry opposite charges and the pH is lower than protein pI, they form a complex under electrostatic attraction. After the charge is neutralized to a certain extent, the complex is further aggregated to form a gel network structure. When they carry the same charge, no interaction occurs^[63,124].

Many studies have demonstrated that the fat content has an impact on the gel system of dairy products^[97,125–126]. The increase of fat globule content may lead to enhanced interactions with proteins, thus increasing gel hardness and stability, resulting in a better texture of full-fat yogurt than low-fat yogurt^[1,125]. Proteins and polysaccharides can act as fat substitutes in two ways. First, as macromolecules with different physical, chemical and sensory properties, they can be added to low-fat products to form a taste and texture similar to full-fat products. Second, polymers of both can form microparticles similar in size and shape to fat globules, mimicking the structure of fat morphology^[104]. The addition of WPI with pectin to low-fat yogurt promotes the balance of gravity and repulsion between polypeptide chains and maintains the stability of the three-dimensional network structure of the gel. Pretreated vegetable oil was then added as a filler uniformly distributed in the yogurt gel to form a denser network structure, which enhanced the WHC of the yogurt and improved the stability of the yogurt gel^[127]. Xanthan/locust bean gum can increase the consistency of yogurt like gelatin, which is favorable to the formation of a three-dimensional gel network, but does not significantly improve the WHC. The addition of WPI can improve the WHC without destroying the gel structure. However, the combination of WPI

with carrageenan negatively affected the gel instead. This is because carrageenan interacts with milk proteins and inhibits casein-whey protein interaction^[128].

5 Future development trends

Yogurt gel stability is an important indicator of yogurt product quality, affecting product texture and shelf life. In order to meet consumers' demand for high-quality yogurt, researchers need to conduct in-depth studies on raw material selection, stabilizer selection, strain improvement, process optimization, etc., promoting the stable and rapid development of the yogurt industry. With increasing attention to sustainability and healthy eating, plant proteins can be used as alternatives to animal proteins for preparing plant-based yogurts. It has been proven that various plant proteins can improve the stability of yogurt gel structure. Mixing grains, pseudocereals, and legume proteins can achieve the best product texture and nutritional quality, addressing the issue of a single plant protein lacking certain essential amino acids^[110,122]. With consumers' preference for natural products, the future trend is to gradually increase the use of natural stabilizers, such as pectin and SA^[62,64]. In the future, different strains of bacteria can be studied for their effects on the gelation of yogurt, for example, strains that produce EPS result in yogurt gels with better stability than those that do not produce EPS^[129]. More advanced production processes and equipment may emerge in the future, methods such as magnetic fields and ultrasonic processing can be employed to enhance the stability of yogurt gels^[130].

6 Conclusions

During the production and storage of yogurt, yogurt is prone to undesirable phenomena such as whey separation and tissue delamination. This paper provided a review of the gelling mechanism of yogurt, factors contributing to textural defects in yogurt, and the application of hydrocolloids to improve the textural stability of yogurt. Polysaccharides and proteins have gelling and thickening stabilizing properties, as well as some nutritional value. Many studies have been conducted to show that single polysaccharides or proteins as thickening stabilizers of yogurt can improve the texture of yogurt. However, there are fewer studies on how the mixture of multiple polysaccharides or proteins and polysaccharide-protein composite systems can improve yogurt, and the study of the gelling mechanism of vegetable protein-based yogurt is still unclear. Sensory perception is an important factor influencing consumers to buy yogurt, and it is necessary to carry out research on the effect of hydrocolloids on the taste and odor of yogurt. In the future, by optimizing production processes and technological means, adjusting the temperature, pH, fermenting agents in the processing process, and adopting new technologies such as pulsed electric fields, and ultrasonic treatment, the stability of yogurt gel can be improved. Develop suitable encapsulation techniques to enhance the activity of bioactive substances (such as probiotics) in yogurt, so that yogurt has good texture and functionality. The influence and mechanism of polysaccharides and proteins of different types and concentrations on the texture of yogurt can be studied in depth, and a variety of plant protein yogurts and algal protein yogurts can be developed, so as to provide theoretical support for guiding merchants to produce a wide range of yogurts with good texture, high quality taste, and rich nutrition.

Conflict of interest

The authors declare no conflict of interest.

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