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Liquid−Liquid Biopolymers Aqueous Solution Segregative Phase Separation in Food: From Fundamentals to Applications—A Review

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Abstract: As a result of the spontaneous movement of molecules, liquid-liquid biopolymer segregative phase separation takes place in an aqueous solution. The efficacy of this type of separation can be optimized under conditions where variables such as pH, temperature, and molecular concentrations have minimal impact on its dynamics. Recently, interest in the applications of biopolymers and their segregative phase separation-associated molecular stratification has increased, particularly in the food industry, where these methods permit the purification of specific particles and the embedding of microcapsules. The present review offers a comprehensive examination of the theoretical mechanisms that regulate the liquid−liquid biopolymers aqueous solution segregative phase separation, the factors that may exert an impact on this procedure, and the importance of this particular separation method in the context of food science. These discussion points also address existing difficulties and future possibilities related to the use of segregative phase separation in food applications. This highlights the potential for the design of novel functional foods and the enhancement of food properties.

Keywords: Biopolymers, Aqueous solution, Segregative phase separation, Theoretical basis, Influence factors.

1. Introduction

Primary factors contributing to macroscopic phase separation are thermodynamic incompatibilities in aqueous biopolymer mixtures. The separation of two biopolymers in a given liquid phase can be classified as either associative or segregative phase separation, of which both are studied for their membrane-less compartmentalization abilities [1, 2]. The food industry makes extensive use of phase separation, although the majority of research conducted to date has focused on associative phase separation. In this process, two solvents are applied so that both biopolymers of interest are ultimately enriched in one of these separating phases whereas the other primarily contains solvent. This method of phase separation is frequently used when dealing with mixtures of biopolymers that have opposite charges [3, 4]. On the contrary, segregative phase separation is common for mutually exclusive macromolecules, forming macromolecules that are enriched in each of the two phases. Although associative phase separation and segregative phase separation are driven by different factors, they can occur simultaneously under certain conditions. However, there have been limited investigations thus far on the segregative phase separation of natural polymers. This highlights the need to focus on the mechanisms that govern this important process.

Thermodynamic incompatibility is another term used to describe the process of segregative phase separation in aqueous biopolymer systems [5]. At the molecular level, immiscibility between macromolecular biopolymers and a thermodynamically incompatible system typically results from structural and/or chemical property differences among the biopolymers. Phase separation is enhanced when molecular

size and conformation are increased, and it is inhibited when molecular size and conformation are decreased, due to inherent differences in these properties across natural biopolymers [6, 7]. Segregative phase separation causes molecular fractionation, which can change the characteristics of natural polymers in a particular system [8].

In comparison to synthetic polymers, natural polymers are typically more complex and are often used in a relatively crude state without thorough characterization, making it difficult to accurately describe and comprehend their behavior. An understanding of the phase behaviors and structural characteristics of these natural polymers is crucial for the formulation, design, and manufacture of food and household products. It also plays a significant role in shaping the physical properties and stability of the final products [9-11].

In the last 15 years, there has been a growing focus of research interest in phase separation. This is due to advancements in compounding technologies, which have opened up new possibilities for designing innovative food additives. One such approach involves combining two or more natural polymers, based on numerous reports. Major research topics in this field include the identification of foods that can undergo phase separation, the factors that contribute to phase separation-related molecular fractionation, and the potential changes in biological activities resulting from this process. Remarkably, Brangwynne et al. [12] observed a comparable occurrence of phase separation in the nucleolus. Furthermore, a group of biochemists and structural biologists were able to replicate this phenomenon *in vitro*, demonstrating that weak forces played a role in the formation of small droplets or spherical jelly-like spots by

biological macromolecules within test tubes [13, 14]. This shows that basic biochemical approaches can replicate the phase separation process *in vitro*, and the findings have been hailed as a breakthrough in this field of study. Since then, more research has been devoted to phase separation and its potential applications in the context of the microstructural design of cosmetics [15, 16], improved emulsion stability [17], generation of complex emulsions in microfluids using aqueous phase separation [18], aqueous phase separation in biomedical applications (include design of artificial cells and to-mimetic materials within water–water(w/w) droplets, synthesis of biomaterials from w/w droplet templates, ATPS-based cell micropatterning and 3D bioprinting, as well as separation of cells and biomolecules in microfluidic channels) [19], microencapsulation [20], and protein separation and purification [21-23].

Extensive research has been conducted thus far on phase separation, including an examination of the biomacromolecules that are conducive to this process, the factors that influence the separation mechanism, and the consequences of phase separation on the biomacromolecules [24, 25]. Further research into the chemical characteristics of phase-separated molecules, as well as any corresponding alterations in their biological roles, is of continued interest. The present review provides an overview of the theoretical basis for segregative phase separation, the mechanisms that govern this process, the factors that influence its incidence, and the potential applications of it in the food industry. A summary of current challenges and prospects in this field is also provided.

2. Theoretical basis for segregative phase separation

2.1 Gibbs free energy

A mixed state is more likely to persist when the Gibbs free energy of mixing two solutes in a solution is negative. Conversely, phase separation is more likely to occur when the free energy of mixing is positive [26, 27]. The Gibbs free energy of mixing values are calculated using the mixing enthalpy and entropy:

$$\Delta G_{mix} = \Delta H_{mix} - T\Delta S_{mix}$$

where ΔH_{mix} represents mixing enthalpy, ΔS_{mix} represents mixing entropy, and T corresponds to the absolute temperature during mixing.

A positive ΔH_{mix} value is indicative of a preference for the separation of two solutes in a mixed solution. However, in the case of low molecular weight solutes, the ΔS_{mix} values are typically substantial and positive enough for the mixing of these two solutes. However, this does not apply to polymeric solutes that have significantly smaller ΔS_{mix} values, as the ΔH_{mix} term takes precedence in such cases. For these reasons, two polymeric solutes in a shared solvent are more likely to undergo separation, as it is rare for ΔH_{mix} to be sufficiently favorable for the formation of a monophasic system without being sufficiently strong that the two polymers complex with one another.

Most biopolymers possess a backbone composed of charged groups where the directionality and degree of charge for two polymers in a solution will often determine whether they interact favorably or unfavorably. If these polymers are similarly charged or uncharged, they will prefer to form a distinct two-phase system. However, if they have opposite charges, they will significantly interact with one other, resulting in the

formation of a complex that will either precipitate or develop a separate phase [28, 29]. Uncharged polymers rarely exhibit beneficial interactions with each other to form complexes, however, this phenomenon may occur in some instances.

2.2 Flory-Huggins theory

A lattice model, the Flory-Huggins solution theory computes the distributions of molecules on a mean lattice field [3, 30]. The first iteration of this model was used to describe the distribution of one polymer in a given solution, but more recent extensions of it have been employed to describe the distributions of multiple polymer species within a solution. This model assumes that each site in the lattice is occupied by either a solvent molecule or a polymer segment, that polymers are flexible, and that all interactions within the lattice are limited to nearest-neighbor pair interactions [31]. To gain physical insight into the numerous factors that govern the phase behavior of polymers, the Flory-Huggins theory is quite helpful.

The Flory-Huggins mean-field lattice model [32, 33] defines pair interaction parameters according to the following formula:

$$X_{ij}=(z/kT)[w_{ij}-(w_{ii}+w_{jj})/2]$$

where z denotes the number of nearest neighbors adjacent to a given lattice site, T is absolute temperature, k is the gas constant, and w values represent the free energy of interaction between the segments of species i and j when occupying neighboring positions within the modeled lattice. The free energy of mixing is used to calculate the phase diagram. In multi-component systems that include polymers and solvents, the Gibbs free energy of mixing Amix per lattice site is represented by the following formula:

$$\Delta G_{mix}/kT = \sum_i (\varphi_i/V_i)\ln \varphi_i + (1/2) \sum_i \sum_j X_{ij}\varphi_i\varphi_j$$

where T is absolute temperature, k is the gas constant, φ_i represents the volume fraction for component i, V_i represents the volume of component i, and X_{ij} is the Flory-Huggins pair interaction parameter, which is determined by the energies of the component segments i and j that occupy adjacent lattice positions. This interaction parameter has an inverse relationship with temperature when the system is fully entropic. The initial component in the equation for $\Delta G_{mix}/RT$ corresponds to combinatorial entropy, while the second component relates to the interaction. A binary mixture phase separates if X_{ij} exceeds the critical value.

2.3 Depletion

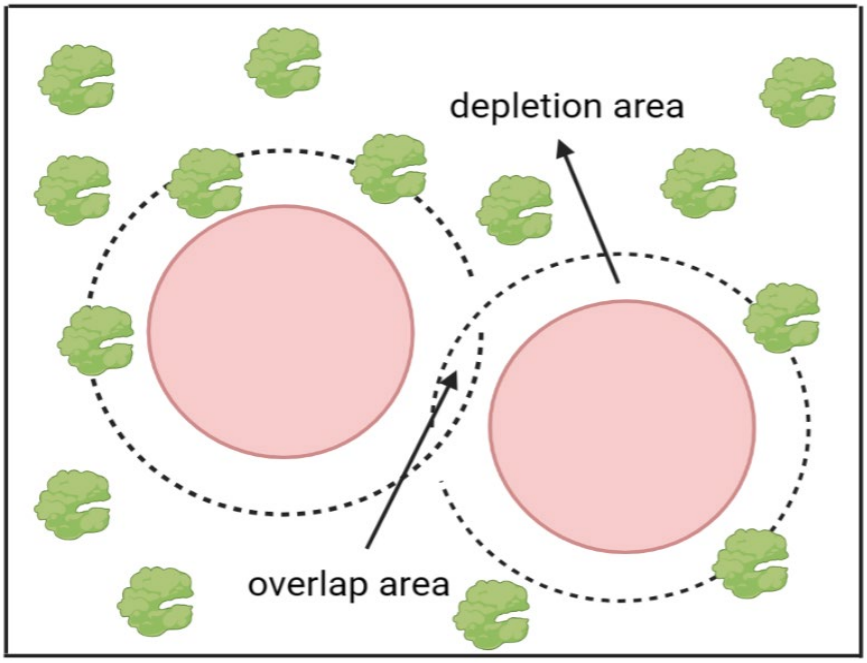
The depletion force, commonly referred to as the entropy force, is a significant factor that regulates interactions within a colloidal system [34]. The repelling volume approach was initially introduced by Asakura and Oosawa during the 1950s to explain the polymer-induced attractive depletion interactions among colloidal globules [35]. In this system, polymers in a solution are regarded as small balls capable of penetrating one another while interactions between colloidal balls and these polymer balls are referred to as hard-sphere interactions. The principle of hard-sphere interactions states that there is no mutual attraction between particles. When the distance between two hard-sphere particles is more than their diameter, the interaction is zero. Conversely, when the distance is smaller than the diameter, the interaction is infinite. Gum Arabic, pectin, gelatin, and other natural polymers are frequently employed in food science. These spherical colloidal particles can also be regarded as a system of nearly hard

spheres. Real colloidal molecules have both repulsive and attractive interactions, with repulsive interactions being more prominent at shorter distances and attractive interactions prevailing at longer distances. The repulsive interaction exhibits an abrupt increase as the distance decreases, therefore making it possible to substitute it with a hard-sphere potential. The interactions between hard spheres are strongly associated with the molecular motion in phase separation. The calculation of density levels for depletion interactions between mixtures can be performed using models of hard-sphere interactions. This provides a more comprehensive understanding of the mechanisms that regulate phase separation.

The depletion model provides a thermodynamic depiction of the excluded volume-based phase separation of colloids and non-adsorbing polymers [36]. A model of this process is shown in Fig. 1. The depletion area surrounding colloid particles refers to the region from which polymer centroids are expelled. When polymers are larger in diameter than the distance between two colloids, polymers in the overlapping region will either be consumed or expelled from this area, imposing surface osmotic pressure on these colloids. Osmotic pressure in colloids results in the formation of an attractive force between the colloidal particles, causing the volume of the polymer to increase as the overlapping region expands. The increase in the overlap region leads to a decrease in the free energy ΔG of the system. This depletion model provides a theoretical basis for hybrid systems to move from a phase-separated state to a phase-equilibrium state.

Fig. 1 illustrates a situation in which colloidal particles are larger than polymer chains; however, this model has also been applied to phase situations in which the opposite is

192 true [37].



193

194 **Fig. 1.** Depletion interaction mechanisms

195 **2.4 Nucleation, growth, and spinodal decomposition**

196 Phase separation is a frequently observed phenomenon in high molecular-weight
197 polymers [38]. The mixing of two separate high molecular polymers will lead to the
198 transfer of the components of one polymer to the other polymer, frequently causing
199 changes in the characteristics of these polymers during the procedure. Several
200 applications in the food industry take advantage of this phase separation-related shift in
201 polymer behaviors [39].

202 The first stages of phase separation can be described by kinetic models such as the
203 nucleation and growth model and the spinodal decomposition model [40, 41].
204 Nucleation and growth (NG) lead to the production of random polydispersed droplet
205 arrays distinguished by sharp interfaces, whereas spinal decomposition (SD) is
206 characterized by droplets or other continuous morphological features [42]. NG is

characterized by short-range/strong-amplitude concentration fluctuations, whereas SD is formed by long-range/small-amplitude fluctuations. As shown in Fig. 2, they lead to different initial geometries due to different mechanisms. During NG, the nucleus components remain unchanged and increase in size, eventually forming a series of random polydisperse spherical droplets dispersed in a continuous phase, comparable to a "island." During SD, the components grow but the volume remains constant, resulting in a microscopic droplet or bicontinuous geometry with a more homogenous phase structure. Initial kinetic modelling of phase separation is generally described in terms of the NG mechanism and the SD mechanism. In the subsequent stages of the phase separation, the coarsening of the microstructure is influenced by two processes: Ostwald ripening and droplet coalescence. The latter of which results from collisions and coalescence between droplets [43]. Ostwald ripening refers to the continuous growth of larger droplets and the redissolution of smaller droplets. If a system continues in a permanently liquid condition, bulk phase separation will inevitably occur over time.

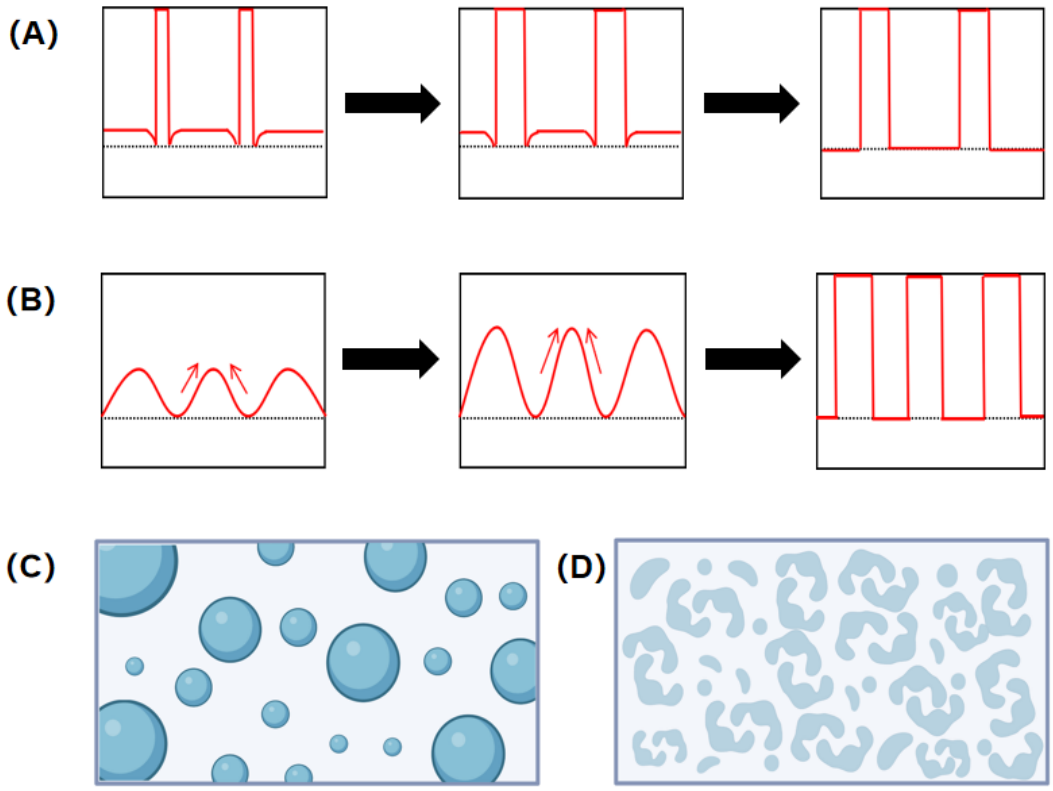


Fig. 2. (A) Nucleation and growth. (B) Spinodal decomposition. (C) droplet, that is obtained via nucleation and growth and spinodal decomposition of mixtures. (D) bicontinuous, which is obtained via the spinodal decomposition of mixtures.

3. The mechanics of segregative phase separation

Molecules suspended within a droplet are in constant motion and frequently collide during the segregative phase separation process, which also coincides with changes in droplet morphology [44]. In this context, a comprehensive understanding of the characteristics of molecules contained within droplets can facilitate the enhanced implementation of segregative phase separation in the food industry. This, in turn, can support the development of more stable functional foods with superior taste and quality.

3.1 Fusion and infiltration

The process of segregative phase separation occurs in supersaturated solutions. The molecules in the solution initially nucleate and expand to form droplets, which then move freely through the solution until they achieve equilibrium. The microstructural features of these droplets change due to Ostwald ripening and coalescence. Smaller droplets fuse when they collide, whereas larger droplets split into smaller droplets due to oscillation [45, 46]. The fusion and infiltration of these droplets are an important aspect of the segregative phase separation process, and research focused on understanding liquid droplet behaviors can thus allow for more effective characterization of the phase separation process.

Butler et al. [40] investigated molecular conformational changes associated with the segregative phase separation of solutions containing gelatin and maltodextrin/dextran. When these systems were compared, distinct nucleation and growth mechanisms were observed. When assessing the scattering structure, an initial monotonic function was observed followed by movement to higher scattering vectors and a gradual S-shaped growth in intensity. They further determined that the hydrodynamic effects of larger droplet diameters affected coarsening rates consistent with droplet coalescence, thereby preserving the fluidity of the system. Their findings provide an understanding of the process by which droplets change from a state of freely moving equilibrium to Ostwald ripening and coalescence during segregative phase separation.

3.2 Molecule mobility within droplets

The compounds contained in droplets are capable of unrestricted movement,

allowing for the exchange of molecules between these droplets and exterior ones. The dynamic motion of these molecules can significantly influence the composition and viscosity of droplets. By employing ultrafast scanning fluorescence correlation spectroscopy, Wei et al. [47] evaluated the mobility of the Caenorhabditis elegans protein (LAF-1), molecular interactions, and associated binodal coexistence curves within droplets. The results of these analyses indicated that the quantities of LAF-1 in droplets produced by the combined action of LAF-1 and poly-rA3k long-chain RNA were considerably reduced in comparison to droplets containing only LAF-1. However, a substantial increase in droplet viscosity was observed in response to this change. These results are consistent with the ability of intrinsically disordered proteins to form permeable low-density liquid structures with properties that impact biological functions. Reichheld et al. [48] utilized NMR spectroscopy to assess the specific structural features and dynamics of self-assembled elastin polypeptides during the self-assembly process. They observed the mobility of elastin-like polypeptides (ELPs) during phase separation and highlighted the structural alterations resulting from the continuous movement of these molecules.

3.3 Molecular permeability

Molecules exhibiting non-specific permeating activity can penetrate droplets via accessible pores, with the extent of such penetration being determined by molecule size. In general, smaller molecules have a greater propensity to enter the interior of a droplet. Droplet structure and viscosity can also be substantially impacted by the penetration of these molecules [47].

Schuster et al. [49] conducted a study in which they mixed RGG-RGG (By multi-merizing the RGG domain from LAF-1) protein droplets with small dextran molecules of varying molecular weights and then analyzed droplet permeability based on fluorescence microscopy-mediated assessments of solute partitioning. A negative correlation was observed between molecular size and RGG-RGG droplet partitioning; dextran particles labeled with rhodamine demonstrated escalating rates of droplet exclusion as their size increased. The RGG-RGG droplets exhibited a marginal increase in the concentration of the 30 kDa green fluorescent protein (GFP), which was approximately 1.6-fold greater than the concentration of GFP in the continuous phase. Conversely, the monomeric ~30 kDa red fluorescent protein was excluded from these droplets, as was the ~55 kDa glutathione-transferase-fused red fluorescent protein (RFP). An intermediate phenotype was identified for Texas Red-labeled bovine serum albumin with a molecular weight of around 68 kDa. In this phenotype, the fluorescence intensity was found to be similar in both the droplets and the continuous phase. These results offer strong evidence that while molecular size is an important determinant of permeability, other protein-specific properties also make their contributions.

4. Factors that influence segregative phase separation

Various factors can affect the process of segregative phase separation and molecular fractionation, such as temperature, pH, ionic strength, mixing ratios, macromolecule concentrations, and molecular weight. These factors have an impact on the properties of functional food products that are made using this method [50-52]. Efforts to control

299 the phase separation process and to identify the most effective conditions under which
300 this process can occur will ultimately enable the more reliable preparation of improved
301 functional foods. The influencing factors of segregative phase separation are detailed
302 in Table 1.
303

304 Table 1: Summary of factors affecting segregative phase separation.

Factor	mechanism	Conclusions	Reference
Temperature	Gibbs free energy, etc.	The appropriate temperature promotes phase separation; too high or too low a temperature can inhibit phase separation.	[8, 53, 54]
pH	Electrostatic effect, etc.	In general, an increase in pH promotes phase separation.	[55-57]
Ionic strength	Electrostatic effect, etc.	Increased ionic strength is not generally conducive to phase separation.	[58, 59]
Mixing ratio and macromolecule concentrations	Flory-Huggins theory, etc.	Increased mixing ratios and macromolecule concentrations generally promote phase separation.	[58, 60]
Molecular weight	Flory-Huggins theory, etc.	Increased molecular weight generally promotes phase separation.	[61, 62]

305

306 **4.1 Temperature**

307 The temperature can significantly influence the phase separation, which is a result of
308 the impact of entropy on this process. Biomolecular phase separation is typically
309 accompanied by a reduction in the overall system's entropy. Increasing the temperature
310 of the system can be counterproductive as it hinders the progression of phase separation
311 and may negatively affect the structural characteristics of natural polymers at
312 excessively high temperatures [63-65].

313 When a multi-component polymer-containing system approaches the phase
314 separation critical point, it exhibits a high degree of sensitivity to temperature gradients.
315 The kinetic properties of polymer chain diffusion at this crucial point can cause
316 significant changes in the density distributions of system components, even with a
317 minor temperature gradient [53]. Hu et al. [8] analyzed the effects of temperature on
318 the phase separation of an aqueous mixture of gum Arabic (GA) and hyaluronan (HA).
319 The results indicated that variations in the temperature gradient affected the
320 concentration of arabinogalactan-protein (AGP) in the GA separation phase. In
321 particular, they discovered that at 40°C, where the highest AGP concentration was
322 detected, optimal fractionation was obtained. As temperatures were further increased to
323 80°C, each mixture exhibited a nearly 50% reduction in molecular weight, confirming
324 the absence of phase separation activity. As a result, the concentration of APG
325 decreased as temperatures rose within this elevated temperature range. Another study
326 looked at the effect of temperature on the atypical phase behavior of a quinoa protein
327 isolate (QPI) and maltodextrin mixture [54]. Almond proteins will still undergo

denaturation and aggregation to some extent when thermally treated at a moderate temperature below the temperature at which denaturation occurs. At higher temperatures above the denaturation temperature, almond protein will instead undergo gelation. They discovered that to obtain better phase separation during QPI, it is necessary to select an optimal intermediate preheating temperature based on the reaction of almond proteins to thermal incubation.

4.2 pH

The pH of a solution can significantly affect the occurrence of phase separation, causing certain natural polymers to be unable to form gels at low pH levels [66, 67]. When the pH of a solution containing polyelectrolytes like poly(acrylic acid) is higher than the pKa, the carboxylate groups will become ionized, causing the polymer to display polyelectrolyte characteristics. On the other hand, at lower pH levels, the carboxyl groups will not be ionized, and the polymer will behave more like a neutral macromolecule [68].

The protein charge and charge density are influenced by the extent of deviation between the pH of a solution containing the protein and its isoelectric point (pI) value. This, in turn, affects the interactions between proteins and other charged substances. When the pH level is higher than the pI of a specific protein, the protein will have a net negative charge. As a result, it will repel other macromolecules that also have a negative charge. Conversely, when the pH is lower than the pI, the protein will carry a net positive charge [55, 56]. Ji et al. [57] studied the impact of pH on the gelation of a mixture of gelatin (G) and hydroxypropyl methylcellulose (HPMC) in an aqueous

solution, and they determined that at a pH below 4.75, HPMC remained in a continuous phase while G was distributed in small spheres throughout the HPMC matrix. When the pH reached 4.75 or above, there was a reduction in the volume of G and the start of phase separation, resulting in a drop in the ultimate volume of gelatin as the pH increased. Thus, at a reduced pH, G and HPMC are compatible with one another. Researchers observed that segregative phase separation was easily facilitated and all acid residues were ionized at high pH levels in a solution of 1M NaCl containing mixtures of poly(acrylic acid) and poly(styrene sulfonate) [3]. Conversely, at a lower pH, the poly(acrylic acid) did not undergo ionization, resulting in the occurrence of phase separation. Previous studies indicate that natural polymers, which have a mutual repulsion, can undergo segregative phase separation. This separation is more likely to occur at higher pH levels.

4.3 Ionic strength

Several investigations have recorded the impact of ionic strength on the segregative phase separation of natural polymers. In this setting, salts are often distributed evenly between phases to ensure a balanced chemical and electrical potential. Although not universally applicable, it is worth noting that higher ionic strength in a mixture of polyelectrolytes can mitigate electrostatic effects. Additionally, polyelectrolytes with the same charge may experience diminished repulsion, resulting in decreased chances of separation or an increased concentration threshold required for separation to take place [59]. When polyelectrolytes and uncharged polymers are combined, the addition of salts can help address problems associated with electroneutrality. This is because salt

372 ions can reduce the density of charges and weaken polymer-polymer interactions due
373 to the shielding of charged groups [69, 70].

374 Bahraseman et al. [58] examined the correlation between the ionic strength and the
375 process of phase separation between gelatin (G) and tragacanth gum (TG). Significant
376 alterations in the performance of the G-TG system were detected in their assay system
377 when NaCl was introduced; this led to a substantial expansion of the two-phase regions.
378 Under these conditions, the TG-containing solution had a low volume fraction and
379 settled at the bottom of the test tubes, but the gelatin-containing solution had a higher
380 volume fraction and remained at the top of the tube. The presence of NaCl in this system
381 is believed to be responsible for the screening effects of the salt ions on the TG
382 backbone. As a result of this charge screening, the interactions between G-TG become
383 more dominant compared to the interactions between TG and water. This leads to a
384 decrease in the quality of the solvent, producing conditions that promote phase
385 separation. According to Yang et al. [71] lower salt concentrations hinder the process
386 of associative phase separation due to intermolecular repulsion and the resulting
387 elongation of chains. Other studies have also shown that the addition of small amounts
388 of salt can assist in balancing molecular complexes that lack electrical neutrality,
389 thereby partially facilitating electrostatic recombination and associative phase
390 separation [72]. According to a study, the existence of NaCl was enough to modify the
391 intramolecular electrostatic forces linked to β-lactoglobulin [73]. NaCl in this system
392 demonstrated charge-shielding properties that inhibited interactions between the
393 protein and oppositely charged dextran sulfate while promoting stronger molecular

associations between β-lactoglobulin molecules through charge and hydrophobic interactions. Higher concentrations of NaCl in this system led to accelerated β-lactoglobulin aggregation and phase separation. Several reports have demonstrated that raising the salt concentration in a given system can reduce the strength of electrostatic interactions. Therefore, higher salt concentrations usually do not promote phase separation, except in situations where hydrophobic interactions are the main factor influencing protein interactions between molecules [74, 75].

4.4 Mixing ratio and macromolecule concentrations

Segregative phase separation can be significantly affected by changes in mixing ratios or macromolecule concentrations. A change in the mixing ratio can affect the structures of complexes in a solution, the bonding between those complexes, and the overall viscosity of the solution [76]. Phase separation is more likely to occur at unequal mixing ratios and there is a positive correlation between macromolecule concentrations and the incidence of phase separation [77, 78].

Antonov & Gonçalves [79] investigated the phase separation properties of gelatin (G) and k-carrageenan. They found that the two biopolymers were compatible when the total biopolymer concentration was below 0.2% (w/w). However, as the total biopolymer concentration increased, they formed an increasingly water-insoluble mixture. The extent to which charge is redundant in this particular context is ultimately determined by complex mixture ratio values. The volume fraction of each phase following the completion of phase separation can also be used to assess the results [60]. Bahraseman et al. [58] analyzed the phase separation of gelatin (G) and tragacanth gum

(TG). They found that after initially combining equal volumes of TG and G at various mixing ratios, the volume fractions of the separated biopolymers were not equal. In particular, the volume of the G-enriched phase was found to be greater than that of the TG-enriched phase. This disparity in volume was observed to increase as the initial TG concentration decreased, while the G concentration remained constant. Phase separation occurs when the solvent is redistributed between biopolymers, and a higher volume fraction is generally observed due to increased solvent absorption caused by enhanced hydrophilicity. Segregative phase separation is typically favored by higher polymer concentrations, although this is not always true because increased solution viscosity might hinder this process. Thus, viscosity and thermodynamic forces must both be considered when assessing the phase separation process. Concentrations that accelerate phase separation emphasize the importance of thermodynamic forces above viscosity-related forces. The decrease in the occurrence of phase separation, despite increasing concentrations, can be attributed to the predominance of viscosity-related forces [80, 81].

4.5 Molecular weight

The molecular weight directly influences the process of segregative phase separation. The relative molecular weights of the polymers are considered to be an essential factor in the thermodynamics, kinetics, and morphology of polymer blends [82]. Jiang et al. investigated the impact of molecular weight on the kinetics of phase separation in Poly (methyl methacrylate) (PMMA)/Poly (styrene-co-acrylonitrile) (SAN) copolymers [62]. It was observed that an increase in the molecular weight of PMMA corresponds

to a decrease in miscibility and an acceleration of the phase separation phenomenon. A study conducted by Ott et al. examined the impact of molecular weight on the phase separation behavior of the poly(vinyl alcohol)/poly(4-styrene sulfonic acid) system [61]. It was discovered that increasing the molecular weight of the polymers, while keeping a constant ratio between the monomers of polymer A and polymer B, leads to phase separation in smaller particles with a more prominent effect. It has been extensively documented that an increase in molecular weight favors the occurrence of segregative phase separation.

5 Applications

After the incidence of segregative phase separation, the resultant individual phases are enriched for particular macromolecules while containing low levels of other macromolecules. By modulating the mixing ratio of the utilized natural polymers, it is possible to control the degree of phase separation, yielding different upper and lower phase volumes and altering the makeup of each phase to some degree. Substances with a greater molecular weight tend to remain in one phase, whereas those with a reduced molecular weight undergo molecular fractionation simultaneously with the segregative phase separation process [39, 83]. The application of segregative phase separation is detailed in Table 2.

458 Table 2: Application Summary for segregative Phase Separatio.

Application	approach	Reference
Polymer separation and purification	As molecular grading occurs, small molecules move into another phase, while large molecules tend to remain in their phase. Segregative phase separation occurs as the molecules are separated and purified.	[54, 84, 85]
Design of food microstructural properties	Segregative phase separation between natural macromolecules and transformation of molecular conformations can enrich the structure of food systems	[86-88]
Improve food quality	The molecular grading induced by segregative phase separation can prolong the storage duration or enhance the emulsification and gel stability of a given food product.	[89, 90]

459 As illustrated in Fig. 3, thermodynamic immiscibility can be achieved by combining
460 two or more natural polymer solutions with comparable charge levels, no charge levels,
461 or one charged and one uncharged solutions. Repulsive forces will cause the solution
462 to separate into phases segregatively if the mixing concentration of the two solutions
463 exceeds their respective critical concentrations. Segregative phase separation-related
464 molecular fractionation causes smaller molecules in a given phase to move to a separate
465 phase while the larger molecules remain in the initial phase. The process of molecular
466 classification can modify the characteristics of natural polymer solutions, allowing for
467 the extraction of enhanced natural polymers that possess superior properties. These
468 modified polymers can be utilized in the advancement of the food industry [91, 92].

The food industry can benefit from the advantageous properties of segregative phase separation to regulate the composition of food products and enhance their overall quality[93, 94].

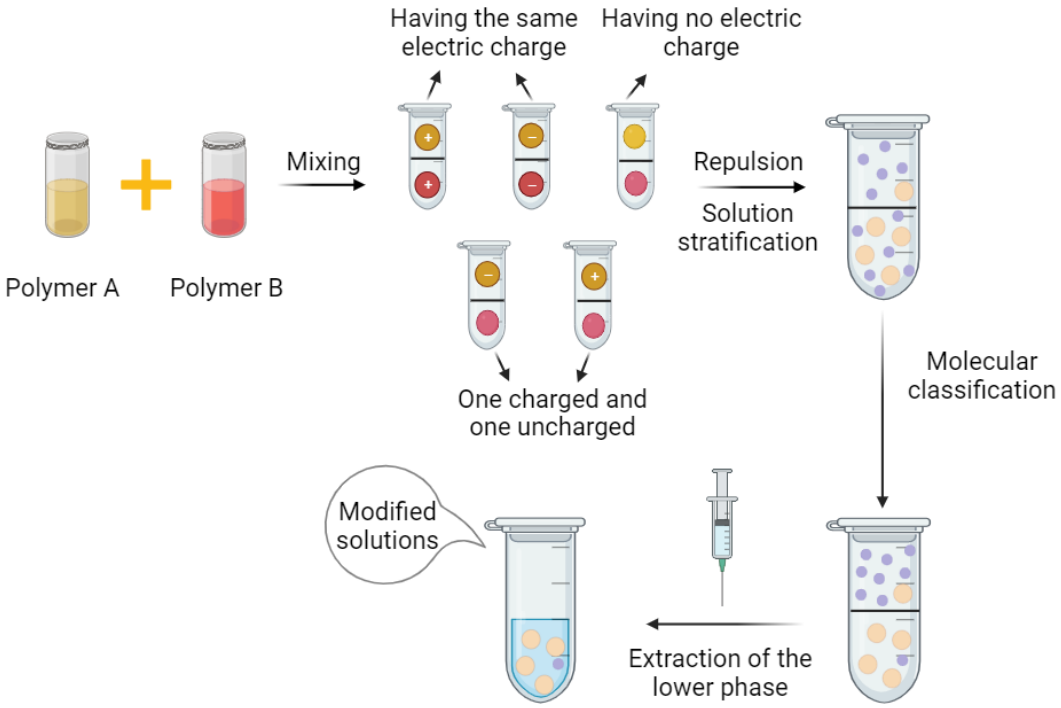


Fig. 3. An overview of segregative phase separation under repulsive forces.

5.1 Polymer separation and purification

Segregation phase separation often coincides with molecular fractionation. Different polymers are ultimately enriched in distinct phases as a result of osmotic pressure-induced phase transitions between molecules in a mixed solution. Specific substances of interest can be efficiently separated and purified by employing this method [84]. After two solutions are combined, a layering phenomenon takes place, which is subsequently influenced by segregative phase separation-induced molecular fractionation. This modification impacts both the properties of the observed layer and the entire mixed solution.

Applying segregative phase separation for the separation and purification of particular substances in the food industry can improve the properties of associated complexes, thereby yielding foods of superior quality. Mao et al. [85] examined the process of segregative phase separation between gum arabic (GA) and sugar beet pectin (SBP). They found that this process leads to the transfer of lower molecular weight glycoprotein and arabinogalactan compounds from the GA phase to the SBP phase, while higher molecular weight arabinogalactan-protein (AGP) remains in the GA phase. AGP plays a crucial role in the process of emulsifying GA, leading to the formation of GA with exceptional emulsification capabilities through a phase separation process. de Amarante et al. [54] aimed to utilize molecular fractionation to modify the structural characteristics of quinoa protein isolate (QPI) by combining QPI with maltodextrin. Their objective was to establish effective methods for substituting animal protein with QPI. However, the findings of these analyses indicate that developing plant-based products with reliable properties may present other obstacles that need to be addressed.

5.2 Design of food microstructural properties

The process of segregative phase separation of natural biopolymers can significantly influence the macroscopic structural properties of foods, hence affecting the taste, sensory qualities, and overall quality of the food products. The phase separation processes, influenced by the concentrations and ratios of natural polymers, will ultimately affect the microstructure of the mixed system, resulting in foods with distinct structural and nutritional properties [86].

Wassén et al. [87] examined the combination of gellan gum and whey protein isolate

505 to assess how confinement affects the rate at which the compounds separate and the
506 structure of the resulting compounds. They discovered that changing the proportion of
507 these two polymers led to significant changes in the microstructural properties of the
508 resulting mixture. The microstructures found in large droplets were similar to those in
509 bulk materials, but a core-shell structure was detected in the microstructures of small
510 droplets. This highlights the impact of different levels of phase separation on the
511 properties of natural polymers. The phenomenon of phase separation frequently plays
512 a role in inducing diverse phase transitions and alterations in the molecular
513 conformations of these substances, including gelation and liquid crystal transitions [88,
514 95]. By coupling phase separation with these transitions, it is possible to enhance the
515 structural properties of food in a controlled manner, thereby facilitating the
516 development of a wider range of functional food products.

517 **5.3 Improve food quality**

518 Segregative phase separation, when appropriately applied, can extend the shelf life
519 or improve the emulsification and gel stability of a certain food product. Under acidic
520 conditions, the negatively charged groups of polysaccharides can interact with
521 positively charged residues of enzymes, causing the enzymes to undergo
522 conformational changes [90]. Many polysaccharides can operate as competitive
523 inhibitors of enzyme activity. The addition of ionic polymers can often deactivate
524 enzymes in food, hence extending its shelf life [89]. Fig. 4 provides a comprehensive
525 explanation of the procedure for enhancing the quality of food products via segregative
526 phase separation. As shown in the figure, molecular classification occurs after the

extraction of modified polymers, which have many applications in improving the storage stability of food products, improving gel strength, and improving the stability of emulsions.

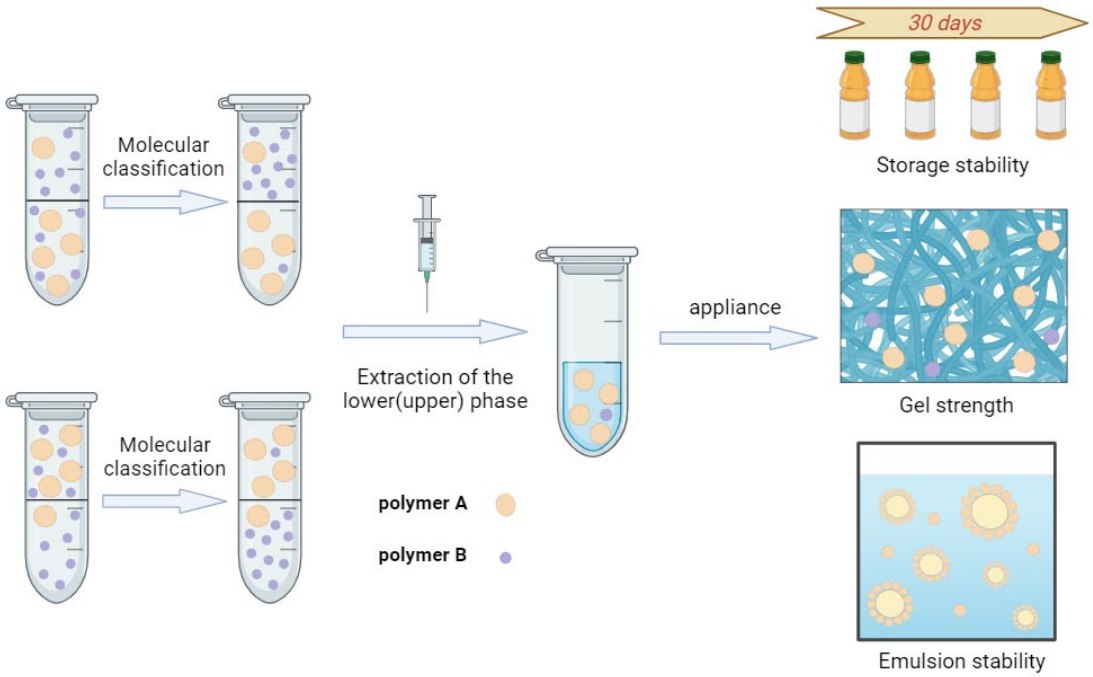


Fig. 4. Schematic overview of the process of molecular classification.

The authors of a previous analysis on the phase separation of k-carrageenan and whey protein isolate mixtures determined that the concentrations of salt and k-carrageenan influenced the ultimate structure of the resulting gel. The products exhibited a range of microstructural conformations, from granular to stranded [39]. Phase separation contributed to increased local protein concentrations at low k-carrageenan concentrations, which resulted in the dispersion of k-carrageenan-enriched droplets within a continuous proteinaceous matrix. This property enhanced the rigidity and strength of the resulting gel. However, as the concentration of k-carrageenan increased, the resulting gel became more stiffer, less deformable, and weaker. This indicates that phase separation could impact the structural characteristics of this prepared gel. Mao et

al. [85] reported comparable enhancements in emulsion stability by using gum Arabic (GA) with segregative phase separation into an emulsion. The process of segregative phase separation can significantly affect the texture of food, which has important implications for the preparation of oil-free and low-calorie emulsions.

6 Conclusions and Outlook

Since the initial introduction of the concept of liquid-liquid phase separation for biological macromolecules, this topic has attracted substantial research and industrial interest. Phase separation effectively combines various physiological processes within the context of the distinctive organizational characteristics of biopolymers, offering a more comprehensive approach to investigating and implementing biological processes of interest. Segregative phase separation is a common process found in natural polymers. It leads to the formation of molecular classifications, which can be utilized to develop well-designed food structures and produce high-quality functional foods with enhanced flavor. This article offers a comprehensive explanation of the theoretical and mechanical foundations underlying the occurrence of segregative phase separation. The efficacy of this separation process can be influenced by various characteristics of the mixed solution, such as its pH, ionic strength, molecular weight, and temperature. Additionally, the mixing ratio and concentrations of natural polymers present in the solution can also have an impact. Furthermore, it describes the application of segregative phase separation in the food industry

Despite significant advancements in research concerning the application of

segregative phase separation in the food industry in recent years, there are still several obstacles that necessitate further investigation. The separation process is significantly impacted by alterations in internal parameters; therefore, it is critical to establish particular conditions that permit the greatest molecular fractionation of the segregative phase. Simultaneously, the degree and state of segregative phase separation that takes place among various natural polymers differ, posing a challenge in identifying polymers whose enhanced properties will contribute more value to the food industry.

The development and implementation of segregative phase separation have led to its utilization in microencapsulation, emulsion use, and bio-cells, which warrant further comprehensive investigation. Efforts to further apply segregative phase separation in the food industry have the potential to enable the design of novel functional foods and to further enhance the properties of extant foods, highlighting this as a major focus for future research.

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Conflict of Interest

The authors declare that there is no conflict of interest.

References

[1] L. Piculell, B. Lindman, Association and segregation in aqueous polymer/polymer, polymer/surfactant, and surfactant/surfactant mixtures: similarities and differences, Adv. Colloid Interface Sci. 41 (1992) 149–178

[2] T. Yao, J. Song, H. Yan, S. Chen, Functionalized aqueous biphasic system coupled with HPLC for highly sensitive detection of quinolones in milk, Lwt-Food Sci. Techonl. 173 (2023) 114398.

[3] K. Bergfeldt, L. Piculell, P. Linse, Segregation and Association in Mixed Polymer Solutions from Flory−Huggins Model Calculations, j.phys.chem. 100 (1996) 3680-3687.

[4] X. Chen, J. Xiao, J. Cai, H. Liu, Phase separation behavior in zein-gelatin composite film and its modulation effects on retention and release of multiple bioactive compounds, Food Hydrocoll. 109 (2020) 106105.

[5] Y. Fang, L. Li, C. Inoue, L. Lundin, I. Appelqvist, Associative and Segregative Phase Separations of Gelatin/κ-Carrageenan Aqueous Mixtures, Langmuir 22 (2006) 9532-9537.

[6] T. Yao, J. Song, Y. Gan, L. Qiao, K. Du, Preparation of cellulose-based chromatographic medium for biological separation: A review, J. Chromatogr. A 1677 (2022) 463297.

[7] T. Yao, Y. Liao, S. Li, L. Qiao, K. Du, Bisphosphonated-immobilized porous cellulose monolith with tentacle grafting by atom transfer radical polymerization for selective adsorption of lysozyme, J. Chromatogr. A 1651 (2021) 462337.

[8] B. Hu, L. Han, Z. Gao, K. Zhang, S. Al-Assaf, K. Nishinari, G.O. Phillips, J. Yang, Y. Fang, Effects of temperature and solvent condition on phase separation induced molecular fractionation of gum arabic/hyaluronan aqueous mixtures, Int. J. Biol. Macromol. 116 (2018) 683-690.

[9] W.J. Frith, Mixed biopolymer aqueous solutions--phase behaviour and rheology, Adv. Colloid Interface Sci. 161(1-2) (2010) 48-60.

[10] I. Dammak, P. Sobral, Effect of different biopolymers on the stability of hesperidin-encapsulating O/W emulsions, J. Food Eng. 237 (2018) 33-43.

[11] T. Yao, J. Song, Y. Hong, Y. Gan, X. Ren, K. Du, Application of cellulose to chromatographic media: Cellulose dissolution, and media fabrication and derivatization, J. Chromatogr. A 1705 (2023) 464202.

[12] C.P. Brangwynne, T.J. Mitchison, A.A. Hyman, Active liquid-like behavior of nucleoli determines their size and shape in Xenopus laevis oocytes, P. Natlacad. Sci. Usa. 108(11) (2011) 4334-4339.

[13] P. Li, S. Banjade, H.-C. Cheng, S. Kim, B. Chen, L. Guo, M. Llaguno, J. Hollingsworth, D. King, S. Banani, P. Russo, Q.-X. Jiang, B. Nixon, M. Rosen, Phase Transitions in the Assembly of Multi-Valent Signaling Proteins, Nature 483 (2012) 336-340.

[14] M. Kato, T. Han, S. Xie, K. Shi, X. Du, L. Wu, H. Mirzaei, E. Goldsmith, J. Longgood, J. Pei, N. Grishin, D. Frantz, J. Schneider, S. Chen, L. Li, M. Sawaya, D. Eisenberg, R. Tycko, S. McKnight, Cell-free Formation of RNA Granules: Low Complexity Sequence Domains Form Dynamic Fibers within Hydrogels, Cell 149 (2012) 753-767.

[15] A. Lazaridou, C. Biliaderis, Concurrent phase separation and gelation in mixed oat β-glucans/sodium caseinate and oat β-glucans/pullulan aqueous dispersions, Food Hydrocoll. 23 (2009) 886-895.

[16] O. Pérez, V. Wargon, A. Pilosof, Gelation and structural characteristics of incompatible whey

626 proteins/hydroxypropylmethlcellulose mixtures, Food Hydrocoll. 20 (2006) 966-974.
627 [17] J. Esquena, Recent advances on water-in-water emulsions in segregative systems of two water-
628 soluble polymers, Curr. Opin. Food Sci. 51 (2023) 101010.
629 [18] S. Keller, S.P. Teora, M. Boujemaa, D.A. Wilson, Exploring New Horizons in Liquid
630 Compartmentalization via Microfluidics, Biomacromolecules 22(5) (2021) 1759-1769.
631 [19] Y. Chao, H.C. Shum, Emerging aqueous two-phase systems: from fundamentals of interfaces to
632 biomedical applications, Chem. Soc. Rev. 49(1) (2020) 114-142.
633 [20] A. Matalanis, U. Lesmes, E. Decker, D. McClements, Fabrication and characterization of filled
634 hydrogel particles based on sequential segregative and aggregative biopolymer phase separation, Food
635 Hydrocoll. 24 (2010) 689-701.
636 [21] F. Jara, A. Pilosof, Partitioning of α-lactalbumin and β-lactoglobulin in whey protein
637 concentrate/hydroxypropylmethylcellulose aqueous two-phase systems, Food Hydrocoll. 25 (2011)
638 374-380.
639 [22] T. Yao, C. Feng, W. Chen, S. Chen, Selective separation and simultaneous recoveries of amino acids
640 by temperature-sensitive magnetic ionic liquid aqueous biphasic system, J. Mol. Liq. 371 (2023) 121099.
641 [23] T. Yao, Q. Li, H. Li, L. Peng, Y. Liu, K. Du, Extractive resolution of racemic phenylalanine and
642 preparation of optically pure product by chiral magnetic ionic liquid aqueous two-phase system, Sep.
643 Purif. Technol. 274 (2021) 119024.
644 [24] C. Loret, S. Schumm, P. Pudney, P. Fryer, Phase separation and molecular weight fractionation
645 behavior of maltodextrin/agarose mixtures, Food Hydrocoll. 19 (2005) 557-565.
646 [25] E. Hasanvand, A. Rafe, B. Emadzadeh, Phase separation behavior of flaxseed gum and rice bran
647 protein complex coacervates, Food Hydrocoll. 82 (2018) 412-423.
648 [26] Y. Fang, S. Al-Assaf, G. Phillips, K. Nishinari, T. Funami, L. Li, Multiple Steps and Critical Behaviors of
649 the Binding of Calcium to Alginate, J. Phys. Chem. B 111 (2007) 2456-62.
650 [27] T. Yao, J. Song, Y. Gan, C. Feng, L. Peng, Liquid-liquid equilibria for (polypropylene glycol 400 based
651 magnetic ionic liquids + inorganic salts) aqueous two-phase systems at 298.15 K, J. Mol. Liq. 349 (2022)
652 118203.
653 [28] D. McClements, Non-covalent interactions between proteins and polysaccharides, Biotechnol. Adv.
654 24 (2006) 621-5.
655 [29] T. Yao, H. Li, J. Yang, X. Shi, H. Yan, L. Peng, Determination and correlation of phase equilibria of
656 chiral magnetic ionic liquid aqueous two-phase systems with different inorganic salts at 298.15 K, J. Mol.
657 Liq. 345 (2022) 116983.
658 [30] S. Turgeon, M. Beaulieu, C. Schmitt, C. Sanchez, Protein-polysaccharide interactions: Phase-
659 ordering kinetics, thermodynamic and structural aspects, Curr. Opin. Colloid In 8 (2003) 401-414.
660 [31] H.X. Zhou, V. Nguemaha, K. Mazarakos, S. Qin, Why Do Disordered and Structured Proteins Behave
661 Differently in Phase Separation?, Trends Biochem. Sci. 43(7) (2018) 499-516.
662 [32] P.J. Flory, Thermodynamics of High Polymer Solutions, J. Chem. Phys. 10(1) (1942) 51-61.
663 [33] M. Huggins, Some Properties of Solutions of Long-Chain Compounds, J. Chem. Phys. 46 (1942).
664 [34] B.G.P. van Ravensteijn, P.A. Hage, I.K. Voets, Framed by depletion, Nat. Mater. 19(12) (2020) 1261-
665 1263.
666 [35] F. Oosawa, The history of the birth of the Asakura-Oosawa theory, J. Chem. Phys. 155(8) (2021)
667 084104.
668 [36] C.M. Marques, D. Mukherji, K. Kremer, T. Stuehn, Depleted Depletion Drives Polymer Swelling in
669 Poor Solvent Mixtures, Nat. Commun. 8 (2017) 1374.

[37] S. Wang, J. Dijk, T. Odijk, J. Smit, Depletion-Induced Demixing in Aqueous Protein−Polysaccharide Solutions, Biomacromolecules 2 (2001) 1080-8.
[38] L. Tang, Liquid phase separation, Nat. Methods 16(1) (2019) 18-18.
[39] E. Çakır, E. Foegeding, Combining protein micro-phase separation and protein–polysaccharide segregative phase separation to produce gel structures, Food Hydrocoll. 25 (2011) 1538-1546.
[40] M.F. Butler, M. Heppenstall-Butler, Phase separation in gelatin/dextran and gelatin/maltodextrin mixtures, Food Hydrocoll. 17(6) (2003) 815-830.
[41] A. Manisekaran, P. Grysan, B. Duez, D.F. Schmidt, D. Lenoble, J.-S. Thomann, Solvents drive self-assembly mechanisms and inherent properties of Kraft lignin nanoparticles (<50 nm), J. Colloid Interf. Sci. 626 (2022) 178-192.
[42] S. Das, K.M. Tadepalli, S. Roy, R. Kumar, A review of clathrate hydrate nucleation, growth and decomposition studied using molecular dynamics simulation, J. Mol. Liq. 348 (2022) 118025.
[43] J. Santos, N. Calero, L.A. Trujillo-Cayado, M.C. Garcia, J. Muñoz, Assessing differences between Ostwald ripening and coalescence by rheology, laser diffraction and multiple light scattering, Colloids Surf. B Biointerfaces 159 (2017) 405-411.
[44] C. Li, X. Fang, Phase Separation as a Molecular Thermosensor, Dev. Cell 55(2) (2020) 118-119.
[45] J. Wang, J.-M. Choi, A. Holehouse, H. Lee, X. Zhang, M. Jahnel, S. Maharana, R. Lemaitre, A. Pozniakovsky, D. Drechsel, I. Poser, R. Pappu, S. Alberti, A. Hyman, A Molecular Grammar Governing the Driving Forces for Phase Separation of Prion-like RNA Binding Proteins, Cell 174 (2018) 688-699 .
[46] A. Patel, H. Lee, L. Jawerth, S. Maharana, M. Jahnel, M. Hein, S. Stoynov, J. Mahamid, S. Saha, T. Franzmann, A. Pozniakovsky, I. Poser, N. Maghelli, L. Royer, M. Weigert, E. Myers, S. Grill, D. Drechsel, A. Hyman, S. Alberti, A Liquid-to-Solid Phase Transition of the ALS Protein FUS Accelerated by Disease Mutation, Cell 162 (2015) 1066-1077.
[47] M.T. Wei, S. Elbaum-Garfinkle, A.S. Holehouse, C.C. Chen, M. Feric, C.B. Arnold, R.D. Priestley, R.V. Pappu, C.P. Brangwynne, Phase behaviour of disordered proteins underlying low density and high permeability of liquid organelles, Nat. Chem. 9(11) (2017) 1118-1125.
[48] S.E. Reichheld, L.D. Muiznieks, F.W. Keeley, S. Sharpe, Direct observation of structure and dynamics during phase separation of an elastomeric protein, Proc Natl Acad Sci U S A 114(22) (2017) E4408-E4415.
[49] B.S. Schuster, E.H. Reed, R. Parthasarathy, C.N. Jahnke, R.M. Caldwell, J.G. Bermudez, H. Ramage, M.C. Good, D.A. Hammer, Controllable protein phase separation and modular recruitment to form responsive membraneless organelles, Nat. Commun. 9(1) (2018) 2985.
[50] V. Tolstoguzov, Some thermodynamic considerations in food formulation, Food Hydrocoll. 17(1) (2003) 1-23.
[51] R.A. Kapelner, A.C. Obermeyer, Ionic polypeptide tags for protein phase separation, Chem. Sci. 10(9) (2019) 2700-2707.
[52] Y. Zhu, X. Chen, D. McClements, L. Zou, W. Liu, PH-, ion- and temperature-dependent emulsion gels: Fabricated by addition of whey protein to gliadin-nanoparticle coated lipid droplets, Food Hydrocoll. 77 (2017) 870-878.
[53] E. Tarabukina, E. Seyednov, A. Filippov, M. Constantin, V. Harabagiu, G. Fundueanu, Thermoresponsive properties of N-isopropylacrylamide with methacrylic acid copolymer in media of different acidity, Macromol. Res. 25 (2017) 1-9.
[54] M. Campos Assumpção de Amarante, T. MacCalman, S.E. Harding, F. Spyropoulos, S. Gras, B. Wolf, Atypical phase behaviour of quinoa protein isolate in mixture with maltodextrin, Food Res. Int. 162 (2022) 112064.

[55] S. Das, A. Eisen, Y.-H. Lin, H. Chan, A Lattice Model of Charge-Pattern-Dependent Polyampholyte Phase Separation, J. Phys. Chem. B 122 (2018) 763: □7653.
[56] Y. Wang, H. Zhou, X. Sun, Q. Huang, S. Li, Z. Liu, C. Zhang, L. Lai, Charge Segregation in the Intrinsically Disordered Region Governs VRN1 and DNA Liquid-like Phase Separation Robustness, J. Mol. Biol. 433(22) (2021) 167269.
[57] Z. Ji, H. Liu, L. Yu, Q. Duan, Y. Chen, L. Chen, pH controlled gelation behavior and morphology of gelatin/hydroxypropylmethylcellulose blend in aqueous solution, Food Hydrocoll. 104 (2020) 105733.
[58] N. Molaahmadi Bahraseman, H. Shekarchizadeh, S.A.H. Goli, Segregative phase separation of gelatin and tragacanth gum solution and Mickering stabilization of their water-in-water emulsion with microgel particles prepared by complex coacervation, Int. J. Biol. Macromol. 237 (2023) 124250.
[59] C. Zhu, B.J. Pedretti, L. Kuehster, V. Ganesan, G.E. Sanoja, N.A. Lynd, Ionic Conductivity, Salt Partitioning, and Phase Separation in High-Dielectric Contrast Polyether Blends and Block Polymer Electrolytes, Macromolecules 56(3) (2023) 1086-1096.
[60] F. Zhang, R. Roth, M. Wolf, F. Roosen-Runge, M. Skoda, R. Jacobs, M. Stzucki, F. Schreiber, Charge-controlled metastable liquid-liquid phase separation in protein solutions as a universal pathway towards crystallization, Soft Matter 8 (2012) 1313-1316.
[61] E.-J.E. Ott, M.A. Freedman, Inhibition of Phase Separation in Aerosolized Water-Soluble Polymer–Polymer Nanoparticles at Small Sizes and the Effects of Molecular Weight, J. Phys. Chem. B 124(34) (2020) 7518-7523.
[62] X. Jiang, Y. Huang, T. Xia, Q. Yang, G. Li, Unusual Phase Separation Kinetics in Poly(Methyl Methacrylate)/Poly(Styrene-co-Acrylonitrile) (PMMA/SAN) Blends with PMMA of Different Molecular Weights, J. Macromol. Sci. B. Phys. 54(10) (2015) 1233-1247.
[63] E. Tarabukina, A. Rozanova, A. Filippov, M. Constantin, V. Harabagiu, G. Fundueanu, Thermo- and pH-responsive phase separation of N-isopropylacrylamide with 4-vinylpyridine random copolymer in aqueous solutions, Colloid. Polym. Sci. 296 (2018) 557-565.
[64] M. Pascual, A. Poquet, A. Vilquin, M.-C. Jullien, Phase separation of an ionic liquid mixture assisted by a temperature gradient, Phys. Rev. Fluids 6 (2021) 024001.
[65] T. Yao, H. Li, Y. Ren, M. Feng, Y. Hu, H. Yan, L. Peng, Extraction and recovery of phenolic compounds from aqueous solution by thermo-separating magnetic ionic liquid aqueous two-phase system, Separation and Purification Technology 282 (2022) 120034.
[66] C. Michon, K. Karidia, G. Cuvelier, b. Launay, Gelatin/carrageenan interactions in coil and ordered conformations followed by a methylene blue spectrophotometric method, Food Hydrocoll. 16 (2002) 613-618.
[67] Q. Wang, Z. Han, B. Cui, C. Yuan, J. Li, K. Nishinari, M. Zhao, The pH-dependent phase separation kinetics of type-A gelatin and dextran with different initial microstructures, Food Hydrocoll. 134 (2023) 108006.
[68] N. Velychkivska, O. Sedláček, A.B. Shatan, M. Spasovová, S.K. Filippov, M.K. Chahal, L. Janisova, J. Brus, L. Hanyková, J.P. Hill, F.M. Winnik, J. Labuta, Phase Separation and pH-Dependent Behavior of Four-Arm Star-Shaped Porphyrin-PNIPAM4 Conjugates, Macromolecules 55(6) (2022) 2109-2122.
[69] X. Li, Y. Cheng, C. Yi, Y. Hua, C. Yang, S. Cui, Effect of ionic strength on the heat-induced soy protein aggregation and the phase separation of soy protein aggregate/dextran mixtures, Food Hydrocoll. 23(3) (2009) 1015-1023.
[70] L. Guo, Y.-h. Li, S. Fang, Y. Pan, J. Chen, Y.-c. Meng, Characterization and interaction mechanism of selective protein separation by epsilon-polylysine: The role of hydrophobic attraction, Food Hydrocoll.

130 (2022) 107710.
[71] Q. Yang, D. Ke, M. Yang, J. Hong, Q. Ran, X. Wang, Effect of salt concentration on the phase separation of bitumen emulsions, Colloids Surf. A Physicochem. Eng. Aspects 425 (2013) 1-5.
[72] C.G. De Kruif, F. Weinbreck, Vries, Complex Coacervation of Proteins and Anionic Polysaccharides, Curr. Opin. Colloid Interface Sci. 9 (2004) 340-349.
[73] B. Vardhanabhuti, E. Foegeding, M. McGuffey, C. Daubert, H. Swaisgood, Gelation properties of dispersions containing polymerized and native whey protein isolate, Food Hydrocoll. 15 (2001) 165-175.
[74] M. Du, Z.J. Chen, DNA-induced liquid phase condensation of cGAS activates innate immune signaling, Science 361(6403) (2018) 704-709.
[75] K.A. Burke, A.M. Janke, C.L. Rhine, N.L. Fawzi, Residue-by-Residue View of In Vitro FUS Granules that Bind the C-Terminal Domain of RNA Polymerase II, Mol. Cell 60(2) (2015) 231-241.
[76] P. Moll, H. Salminen, C. Roeth, C. Schmitt, J. Weiss, Concentrated pea protein – apple pectin mixtures as food glue: Influence of biopolymer concentration and pH on stickiness, Food Hydrocoll. 130 (2022) 107671.
[77] J. Liu, C. Jiale, T. Zhang, Y. Yixin, R.K. Saini, M. Xu, S. Li, X. Shang, Phase behavior, thermodynamic and rheological properties of ovalbumin/dextran sulfate: Effect of biopolymer ratio and salt concentration, Food Hydrocoll. 118 (2021) 106777.
[78] Y. Lan, B. Chen, J. Rao, Pea protein isolate–high methoxyl pectin soluble complexes for improving pea protein functionality: Effect of pH, biopolymer ratio and concentrations, Food Hydrocoll. 80 (2018) 245-253.
[79] Y. Antonov, M. Gonçalves, Phase separation in aqueous gelatin–κ-carrageenan systems, Food Hydrocoll. 13 (1999) 517-524.
[80] J.-Y. Chun, G.-P. Hong, S. Surassmo, J. Weiss, S.-G. Min, M.-J. Choi, Study of the phase separation behaviour of native or preheated WPI with polysaccharides, Polymer (2014) 4379-4384.
[81] C.F. Rediguieri, O. de Freitas, M.P. Lettinga, R. Tuinier, Thermodynamic Incompatibility and Complex Formation in Pectin/Caseinate Mixtures, Biomacromolecules 8(11) (2007) 3345-3354.
[82] W. Liu, L. Zhang, Y. Wei, The Influence of Annealing Atmosphere, Blending Ratio, and Molecular Weight on the Phase Behavior of Blend Materials, Processes 9(9) (2021) 100049.
[83] Y. Chu, Y. Jo, L. Chen, Size-controllable core/shell whey protein microgels with narrow particle size distribution fabricated by a facile method, Food Hydrocoll. 124 (2022) 107316.
[84] B. Yang, W. Su, S. Deng, L. Zhao, P. Lu, State-of-art of impacting T-junction : Phase separation, constituent separation and applications, Int. J. Heat Mass Transf. 148 (2020) 119067.
[85] P. Mao, M. Zhao, F. Zhang, Y. Fang, G.O. Phillips, K. Nishinari, F. Jiang, Phase separation induced molecular fractionation of gum arabic--sugar beet pectin systems, *Carbohydr. Polym*. 98(1) (2013) 699-705.
[86] M. Nejatian, S. Abbasi, F. Azarikia, Gum Tragacanth: Structure, characteristics and applications in foods, Int. J. Biol. Macromol. 160 (2020) 846-860.
[87] S. Wassén, N. Lorén, K. Bemmel, E. Schuster, E. Rondeau, A.-M. Hermansson, Effects of confinement on phase separation kinetics and final morphology of whey protein isolate–gellan gum mixtures, Soft Matter 9 (2013) 2738-2749.
[88] P. Azzari, M. Bagnani, R. Mezzenga, Liquid-Liquid Crystalline Phase Separation in Biological Filamentous Colloids: Nucleation, Growth and Order-Order Transitions of Cholesteric Tactoids, Soft Matter 17 (2021) □□□□ □□□□.
[89] B. O' Flynn, T. Mittag, The role of liquid–liquid phase separation in regulating enzyme activity, Curr.

802 Opin. Cell Biol. 69 (2021) 70-79.
803 [90] B. Xiao, F. Hébert, G. Batrouni, R. Scalettar, Competition between phase separation and spin density
804 wave or charge density wave order: Role of long-range interactions, Phys. Rev. B 99 (2019) 205145.
805 [91] D. Shapiro, M. Ney, A. Eghtesadi, A. Chilkoti, Protein Phase Separation Arising from Intrinsic Disorder:
806 First-Principles to Bespoke Applications, J. Phys. Chem. B 125 (2021) 8962□897; .
807 [92] Y. Sun, Z. Lim, Q. Guo, J. Yu, A. Miserez, Liquid–liquid phase separation of proteins and peptides
808 derived from biological materials: Discovery, protein engineering, and emerging applications, MRS Bull.
809 45 (2020) 1039-1047.
810 [93] S. Xu, H. Zhang, B. Qiao, Y. Wang, Review of Liquid–Liquid Phase Separation in Crystallization: From
811 Fundamentals to Application, Cryst. Growth Des. 21(12) (2021) 7306-7325.
812 [94] R. Rarima, G. Unnikrishnan, Poly(lactic acid)/gelatin foams by non-solvent induced phase
813 separation for biomedical applications, Polym. Degrad. Stabil. 177 (2020) 109187.
814 [95] P. Rohini, M.L.N. Madhu Mohan, N. Pongali Sathya Prabu, Detection of phase transitions in liquid
815 crystals through optical, thermal and electrical techniques, Optik 258 (2022) 168951.