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REVIEW

Some Physicochemical Approaches to the Problem of Protein Texturization

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This paper is concerned with the research on the physicochemical problems of protein texturization, which is carried out at the laboratory of new food forms at the A. N. Nesmeyanov Institute of Organo-Element Compounds of the USSR Academy of Sciences. From studies of liquid- and gel-like aqueous systems containing proteins and polysaccharides, we were able to develop methods for controlling the composition, structure, phase states, and physicochemical properties of food systems. In the processing of proteins into new forms of food, of paramount importance is the information on the interaction and compatibility of proteins and polysaccharides as well as of various proteins in aqueous media. It is also essential to know the formation conditions and the properties of filled, complex, mixed, and anisotropic gels. Examples are given of the implementation of this concept for processing proteins into some new food products, including meat and milk analogues.

This review paper is devoted to the main trends of research work conducted at the laboratory of new food forms at the A. N. Nesmeyanov Institute of Organo-Element Compounds of the USSR Academy of Sciences. The general objective of this research is to provide a scientific basis for the processing of proteins into various food products.

The problems pertaining to food protein processing may fall into two groups: (1) structurizing proteins in mixtures with other food components to finish with a food product of a required texture; (2) imparting certain flavors and appearance to products by means of dyeing agents, nutritive and flavor additives, vitamins, etc. The distinction between the two groups is rather provisional, since the flavor and color of the product are determined by its physicochemical properties specified by the texturization process.

This paper takes up only the first group of problems, i.e., physicochemical aspects of protein structurization and texturization.

In developing a general physicochemical approach to the problem of protein processing, we started from two obvious concepts. First, food systems represent basically multicomponent systems. Second, food products are generally solid systems containing much (over 50%) water. Such a composition and properties are typical of gels. In other words, from the standpoint of physical chemistry, most solid foodstuffs can be treated as gels of proteins and other food substances. Hence, the main problem encountered here is to produce gels of required composition, structure, and physicochemical properties (Tolstoguzov, 1978). This physicochemical problem involves, however, two other relevant problems. First, we naturally wish to process a

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given protein into a wide range of food products. To this end, we need liquid- and gel-like systems exhibiting various properties. So, another problem arises—to control the structure-forming functions of a protein, its solubility, and its ability to stabilize dispersion systems—to form gels, etc. This intricate combination of physicochemical characteristics, determining the structural functions of proteins in processed systems, has been termed "protein functional properties" (PFPs). A second task is to develop methods of processing that might be applicable to any protein. It is evident that more reliable, efficient, and simple processes must be those involving fundamental physicochemical phenomena. Proceeding from these considerations, attention has been centered on the following: (1) limited thermodynamic compatibility (TC) of proteins with polysaccharides in aqueous media (Grinberg and Tolstoguzov, 1972); (2) limited TC of various proteins with each other, which was discovered later (Polyakov et al., 1979, 1984); (3) nonequilibrium phenomena involved in protein complexing with anionic polysaccharides (Gurov et al., 1977, 1984a).

Complexing of Proteins and Anionic Polysaccharides and Application of Complexes to PFP Control. Protein interaction with polysaccharides has been investigated by many researchers, and some of the aspects are generalized in a number of review papers (Boojy and Bungenberg de Jong, 1956; Evreinova, 1966; Serebrovskaya, 1971; Ledward, 1979). Even the earliest systematic studies, largely on the nature of phase separation taking place in protein and anionic polysaccharide solutions (i.e., of the formation of a complex coacervate or water-soluble complexes), have revealed that these complexes are stabilized mainly by electrostatic forces. On the other hand, the complexing of anionic polysaccharides and proteins at pH values above the protein isoelectric point (IEP) (for similarly charged macroagents), observed in some studies (Sasaki and Noguchi, 1959; Noguchi, 1960; Thompson and McKernan, 1961; Snoeren et al., 1975; Rosenfeld et al., 1975), and the complexing of neutral polysaccharides and proteins (other than lectins) (Ponder and Ponder, 1960; Comper and Laurent, 1978) indicate that secondary nonelectrostatic interactions are significant in the stabilization of protein-anionic polysaccharide complexes (PPCs).

The functional properties of complexes, e.g., solubility, surface activity, and rheological properties, differ markedly from the corresponding properties of the initial macroagents and, more importantly, they are controllable over a very wide range. Of special interest to food technology may be soluble PPCs. But the mechanisms of their formation and properties have not been studied very much so far. It seems quite appropriate to study the nonequilibrium phenomena involved in the PPC formation, the nature of forces stabilizing these complexes, their molecular structure and functional properties, and the structure of bound proteins. In view of this, we investigated PPCs bound both by strong (particularly, sulfated) and by weak (carboxyl-containing) anionic polysaccharides.

Investigation of Nonequilibrium Complexes. The study of the PPC formation process has revealed that at rather low pH values and moderate ionic strengths, complexes are not at equilibrium: their properties, say, solubility (the use of the terms "soluble" and "insoluble" with nonequilibrium complexes is rather provisional, since, strictly speaking they should refer to a stable thermodynamic equilibrium), are determined by the routes of their production (Gurov et al., 1977, 1982, 1984a). Thus, when the macroagent solutions are mixed under the conditions of intense complexing (at pH values below the protein IEP), mixing complexes (M-complexes) are obtained, which will hardly dissolve in water. On the contrary, when the macroagent mixture is slowly titrated and the conditions of the medium are changed, thereby gradually increasing the interaction, titration complexes (T-complexes) are obtained, which dissolve in water fairly well.

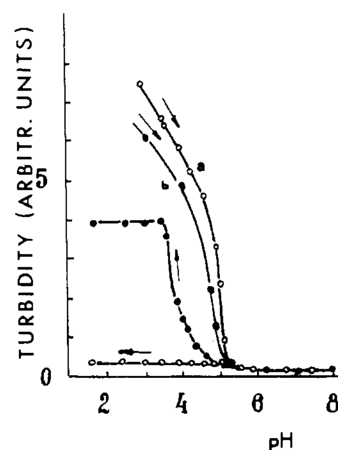


Figure 1. Curves of nephelometric titration of BSA–dextran sulfate mixtures with alkali and acid, illustrating an aggregation hysteresis. The weight protein:polysaccharide ratios are 1:1 (a) and 2:1 (b); ionic strength is 0.3 (Gurov et al., 1984a).

The difference in the solubilities of M- and T-complexes is clearly seen in the nephelometric titration of protein–polysaccharide mixtures with alkali and acid (Figure 1). In this case, turbitidy serves as a semiquantitative characteristic of the yield of insoluble PPCs. The titration of a solution with an alkali and later with an acid involves an aggregation hysteresis, which is particularly pronounced with PPCs of highly sulfated polysaccharides, e.g., dextran sulfate and heparin. For example, under the same conditions (pH values below the protein IEP), protein–dextran sulfate complexes may yield a solution of T-complexes with a high (say, 20%) protein concentration. On the contrary, the protein may be completely precipitated in the form of M-complexes out of dilute protein solutions. Solutions and suspensions of protein–dextran sulfate complexes are highly resistant to aggregation: the light scattering and the wave exponent (sensitive to a varying size of scattering particles) can remain constant for several days at room and elevated temperatures (40–50 °C).

Nonequilibrium PPCs have been carefully studied on complexes of high molecular weight dextran sulfate with proteins, in particular with bovine serum albumin (BSA) (Gurov et al., 1982, 1984a). It has been established that the nature of BSA interaction with dextran sulfate is radically changed, depending on the pH. For pH $\gtrsim$5.6 (the range of a relatively weak interaction), the PPC properties are independent of the way in which they have been produced. This indicates an equilibrium nature of complexing. An analysis of isotherms of BSA binding at pH 5.6 has shown that sorption is a noncooperative process (i.e., protein–protein interaction has no appreciable effect on the protein binding process). In this case, PPCs are not aggregated: each particle contains a single dextran sulfate molecule.

Around the IEP of BSA ($5.0 < pH < 5.6$), the composition of soluble PPCs is also independent of the way in which they are produced but it is very dependent on the pH of a solution. Of special concern is the fact that over this pH range a uniform distribution of protein molecules among the dextran sulfate chains goes over into a nonuniform distribution. Two discrete fractions of a complex

are formed in a solution—a rapidly sedimenting α-fraction and a slowly sedimenting β-fraction. An analysis of their compositions and amounts has revealed that their ratios change regularly. The higher the BSA content in a solution, the higher the proportion of the α-fraction and the lower the proportion of the β-fraction. At the same time, the sedimentation coefficients of these fractions increase. The two fractions are a BSA–dextran sulfate complex with differing compositions. The α-fraction has a high protein content and the β-fraction has a low protein content. The nonuniform distribution of protein among polysaccharide chains is attributed to the cooperative nature of its sorption (Olins et al., 1967; McGhee and von Hippel, 1974). The transition from noncooperative sorption of BSA to cooperative sorption may be due to increasing interactions between protein molecules in PPC particles as the pH decreases. This, in turn, can be accounted for by a decreasing total charge on the protein, which prevents, at higher pH values, protein molecules from approaching each other (Kabanov et al., 1977, 1978).

For pH <5.0, the sorption of BSA by dextran sulfate becomes irreversible. In these conditions, the α-fraction of M-complexes of any composition precipitates. On the contrary, the α-fraction of T-complexes is highly soluble if the weight ratio of BSA to dextran sulfate does not exceed 4:1 (critical composition). In the latter case, there is no β-fraction, and the α-fraction precipitates completely. The β-fraction dissolves fairly well, no matter how a complex has been produced.

Aggregation of protein–dextran sulfate complexes and aggregation hysteresis are also observed in concentrated urea solutions. Since protein aggregation is inhibited in this medium, it can be claimed that secondary interactions, i.e., hydrogen bonds and hydrophobic interactions, are of little importance for the stabilization of aggregated (equilibrium and nonequilibrium) protein–dextran sulfate complexes. The nonequilibrium aggregates form, probably, due to electrostatic interactions. At the same time, the number of ionic bonds between macroagents is independent of the way of their complexing, since there is no proton sorption hysteresis in the potentiometric titration of BSA–dextran sulfate solutions. Hence, the differences between M- and T-complexes may be accounted for by different distributions of protein molecules among polysaccharide chains: T-complexes are particles with a single dextran sulfate chain (monomatrix complexes), whereas M-complexes (α-fraction) represent entangled networks of dextran sulfate chains cross-linked by the protein (polymatrix complexes).

Thus, when the BSA and dextran sulfate solutions are mixed at pH <5.0 (for all studied protein to polysaccharide ratios), along with a highly soluble β-fraction, a highly aggregated α-fraction is formed as a result of protein cross-linking the network of dextran sulfate chains. In T-complexes formed by "freezing" the equilibrium of the complexing process at pH ≃5.0 and by subsequent transition into the same conditions under which M-complexes exist, the α-fraction remains soluble up to the protein: dextran sulfate ratio of 4:1, owing to the electrostatic repulsion of similarly charged particles.

Effect of Temperature on the Structure of Protein Bound in a Complex. Complexing with anionic polysaccharides makes it possible to prevent thermal coagulation of proteins. This may be explained by immobilization of protein molecules in a complex and, hence, by the impossibility of formation of large aggregates in the denaturation. For example, in BSA–dextran sulfate complexes, no protein coagulation was observed up to 100 °C

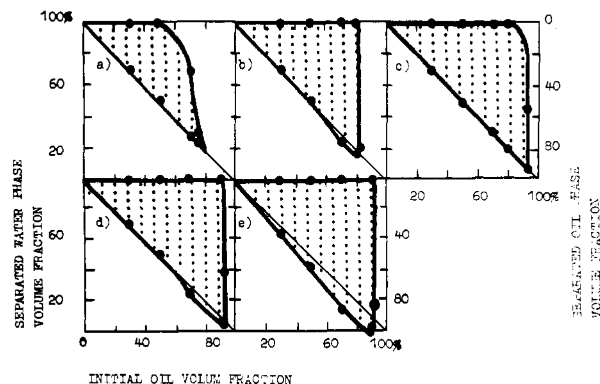


Figure 2. Diagrams of stability of corn oil emulsions in solutions of human serum albumin and its complexes with dextran sulfate. The protein concentration is 5.0 g/L. The polysaccharide concentrations are 0 (a), 0.37 (b), 1.93 (c), 3.3 (d), and 5.0 (e) g/L. (Gurov et al., 1984b).

(pH 5.6). Using the circular dichroism method, we observed denaturation of a bound protein, which was intensive as with a freely accessible protein. But unlike free protein denaturation, the denaturation of a bound protein is a noncooperative process and it does not display a maximum heat absorption around the denaturation temperature (Gurov et al., 1978), which is typical of protein thermal denaturation.

Application of PPCs for Controlling Protein Surface Properties. Protein–anionic polysaccharide complexes are superior in some respects to native proteins as surface active substances. For example, they may ensure a higher stability of oil in water emulsions (Larichev et al., 1983). Figure 2 gives stability diagrams of corn oil emulsions in solutions of human serum albumin and its complexes with dextran sulfate (the latter is not surface active and by itself it does not stabilize emulsions). As is seen, higher concentrations of dextran sulfate lead to more stable emulsions and larger emulsifying capacities per gram of protein.

A similar increase in the emulsion stability was observed in casein–carrageenan, casein–pectin, and some other complexes used in place of protein (Tokayev et al., 1981, 1984).

PPCs make it easy to control the rheological characteristics of emulsions over a wide range. A high-viscosity emulsion is used in the manufacture of cooked sausages (Tokayev et al., 1981).

It is of no less interest to consider the behavior of complexes at the water solution–air interface. Figure 3 plots the concentration dependence of the half-life of foams stabilized by sodium caseinate and by its PPC with dextran sulfate. Unlike the free protein case, in a complex this dependence does not have a maximum. Hence, with normally used protein concentrations, the foam can be made much more stable.

Properties of interphase adsorption layers, formed by PPCs, have remained virtually unstudied up to now. We can expect, however, that the structural organization of PPCs at the interface is similar to that of complex gels.

Gels of Protein-Anionic Polysaccharide Complexes. Systematic investigations of PPC gelation were carried out for gelatin–sodium alginate (Muchin et al., 1976, 1978, 1984) and gelatin–pectin systems (Tschumak et al., 1976), which proved to be very different from gelatin gels. For example, unlike the gelatin gels, the gelatin–alginate gels do not melt at 30–40 °C. Their compliance jumps in a steplike manner, with the elasticity preserved up to 100 °C. Casein–alginate complexes undergo a thermally reversible gelation at the conditions where individual macroagents will not form gels (Figure 4) (Muchin, 1984).

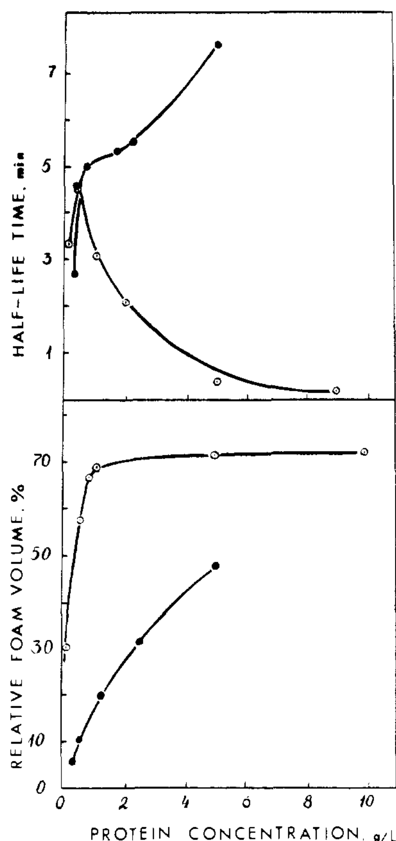


Figure 3. Half-life (a) and relative volume (b) of foam created by shaking solutions of casein (◎) and its complexes (●) with dextran sulfate vs. protein concentration. The protein:polysaccharide ratio in a complex is 1.5:1, pH 4.5; the pH of casein solution is 9.0. (Gurov et al., 1984b).

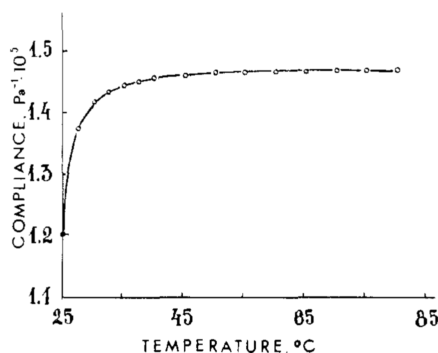


Figure 4. Thermomechanical curve of a complex gel of casein–sodium alginate. The weight protein:polysaccharide ratio is 2:1; the bulk concentration of macroagents is 100 g/L, pH 4.5 (Muchin, 1984).

Thermodynamic Compatibility of Proteins with Polysaccharides. Investigations of several systems containing albumins and neutral polysaccharides have revealed a limited thermodynamic compatibility (TDC) of proteins with polysaccharides (Grinberg and Tolstoguzov, 1972). Based on the microrheology of model two-phase liquid systems, it has been suggested that they could be used to generate fibers and form anisotropic gels without spinnerets (Tolstoguzov et al., 1973; Antonov et al., 1980; Suchkov et al., 1980).

Later workers started to examine systematically a limited TDC of the main classes, according to Osborne classification, of proteins with polysaccharides of various chemical compositions, structures, and molecular masses, as well as a limited protein–protein TDC (Polyakov et al., 1979, 1980, 1984). More than 80 systems were used to

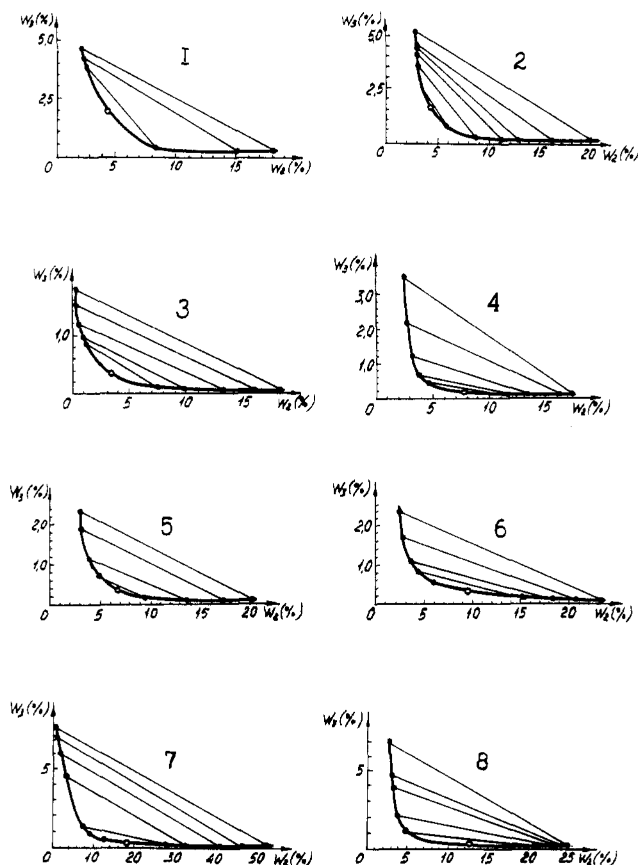


Figure 5. Phase diagrams for some protein–anionic polysaccharide–water systems at 20 °C. (1) Casein–sodium alginate–water, 0.1 M NaOH. (2) Casein–carboxymethylcellulose (CMC)–water, 0.1 M NaOH. (3) Casein–gum arabic–water, 0.1 M NaOH. (4) Soyabean globulin–pectin–water, pH 9.0. (5) Soybean globulins–sodium alginate–water, pH 9.0. (6) Soybean globulins–CMC–water, pH 9.0. (7) Soybean globulins–dextran sulfate–water, pH 9.0. (●) The points of binodal; (○) the critical point; w_2 and w_3 = protein and polysaccharide concentrations, respectively.

ascertain that the phenomenon of a limited TDC is common to all protein–polysaccharide systems. All the systems studied, with the macrocomponent concentrations over 4%, have separated into the two phases, each containing primarily one macrocomponent.

The protein–polysaccharide–water systems were classified according to the conditions of their separation into two liquid phases (Tolstoguzov, 1978). These conditions are specific for neutral, carboxyl-containing, and sulfated polysaccharides and determine their interactions with proteins in solutions.

Two-phase liquid systems are obtainable under the conditions in which the attraction of one-type macromolecules is predominant, whereas the attraction of different-type macromolecules is inhibited. Such conditions are met by anionic polysaccharides, whose complexing was considered above. The formation of complexes composed of proteins and neutral polysaccharides has been studied far less. In view of this, attempts were made to get a better insight into the observed effect of salts on the TDC of proteins with polysaccharides. It is evident from rheological measurements on single-phase protein–neutral polysaccharide–water systems, made for various ionic strengths corresponding to a complete and a limited TDC of macrocomponents, that at low ionic strengths proteins and neutral polysaccharides form weak complexes. At higher ionic strengths, these complexes break down, thereby reducing the TDC of proteins with neutral poly-

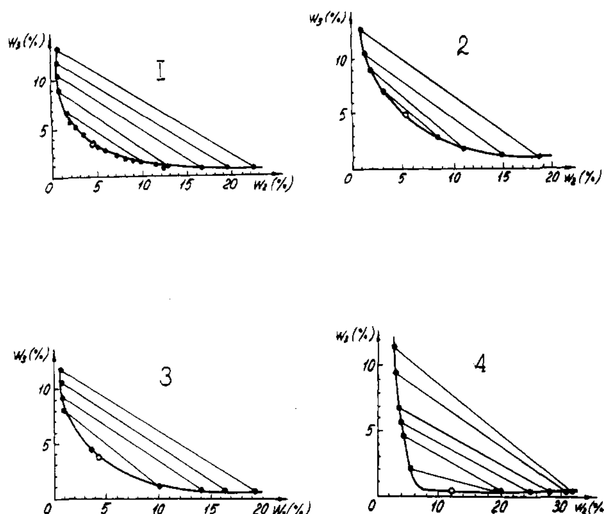


Figure 6. Phase diagrams for some protein-neutral polysaccharide-water systems at 20 °C. (1) Casein-dextran T-2000-water, pH 6.5, 0.15 M NaCl. (2) Casein-Ficoll-water, pH 6.5, 0.15 M NaCl. (4) Soybean globulins-dextran T-500-water, pH 9.0, 0.15 M NaCl. The designations are the same as in Figure 5.

saccharides (Varfolomeyeva et al., 1980).

The limited TDC of proteins with polysaccharides was examined in greater detail on casein (Antonov et al., 1975, 1977) and soybean globulins (Antonov et al., 1979). Figures 5 and 6 represent phase diagrams for several protein-acidic polysaccharide-water and protein-neutral polysaccharide-water systems. These diagrams are notable for a strong asymmetry and for a low compatibility of macrocomponents.

Let us briefly discuss the results on the effect of the basic thermodynamic parameters on the protein-polysaccharide TDC. Figure 7 demonstrates the effect of pH. If the criterion of a protein-polysaccharide TDC is a magnitude of separation interval, then for carboxyl-containing polysaccharides (diagrams 1 and 2), the TDC is decreased around the protein IEP. For sulfated polysaccharides (diagrams 3 and 4), the TDC is, on the contrary, decreased away from the protein IEP and it is complete near the IEP.

Figure 8 shows the effects of NaCl and temperature on the protein-ionic polysaccharide TDC. In all cases, the TDC is reduced with increasing concentration of NaCl (diagrams 1-3). As the temperature rises, the protein-anionic polysaccharide TDC is generally decreased (diagram 4).

The temperature and pH effects on protein-neutral polysaccharide TDC are illustrated in Figure 9. As a glance at diagrams 1 and 2 shows, the TDC is made poorer as the pH reaches the protein IEP. As the temperature rises, the protein-neutral polysaccharide TDC is destroyed (diagram 4). Of definite interest are the results on the impact of the protein-neutral polysaccharide TDC by polysaccharide molecular mass, concentration, and nature of a low molecular weight salt (Figure 10). It follows from diagrams 1-3 that the higher the molecular mass of a polysaccharide and the stronger the low molecular weight salt concentration, the worse the TDC. We should like to note that when the salt concentration is below a critical value, C_4*, the macrocomponents are completely compatible (diagrams 2 and 3). This salt concentration depends on the pH and the nature of the composition (diagram 4). The effect of the type of salt on the protein-neutral polysaccharide TDC does not generally correlate with the lyotropic series. It is apparently important that a complete TDC of these systems be observed in pure

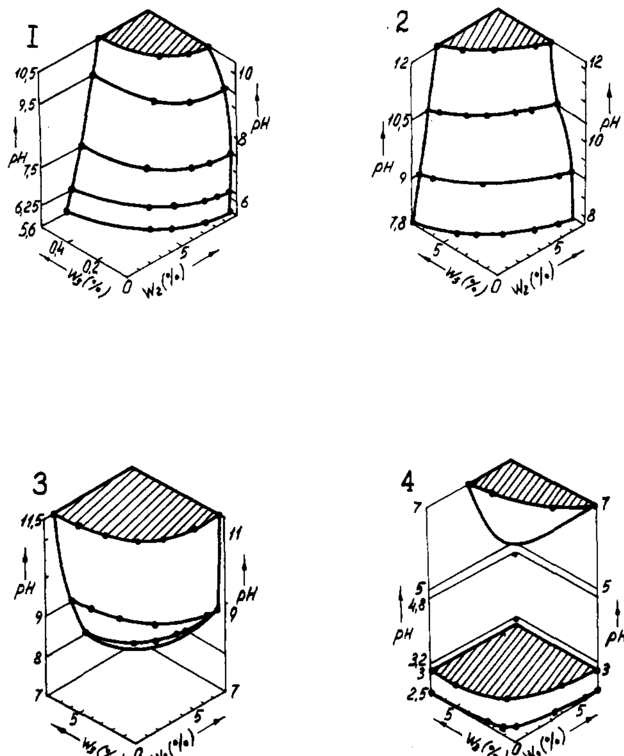
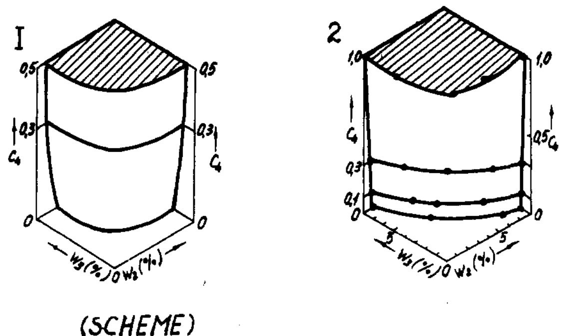


Figure 7. Effect of pH on the protein-anionic polysaccharide TDC at 20 °C. (1) Casein-sodium alginate-water. (2) Soybean globulins-gum arabic-water. (3) Soybean globulins-dextran sulfate-water, 1 M NaCl. (4) Soybean globulins-dextran sulfate-water, 1 M NaCl, 6 M urea. w_2 and w_3 = protein and polysaccharide concentrations. Demixing gaps are shaded.



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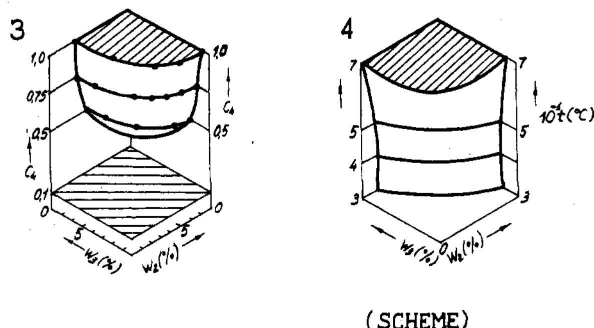

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Figure 8. Effect of NaCl concentration (C_4, mol/L) and temperature (t) on the protein-anionic polysaccharide TDC at pH 9.0. (1) Casein-sodium alginate-water, 20 °C. (2) Soybean globulins-gum arabic-water, 20 °C. (3) Soybean globulins-dextran sulfate-water, 20 °C. (4) Casein-gum arabic-water. w_2 and w_3 = protein and polysaccharide concentrations. Demixing gaps are shaded.

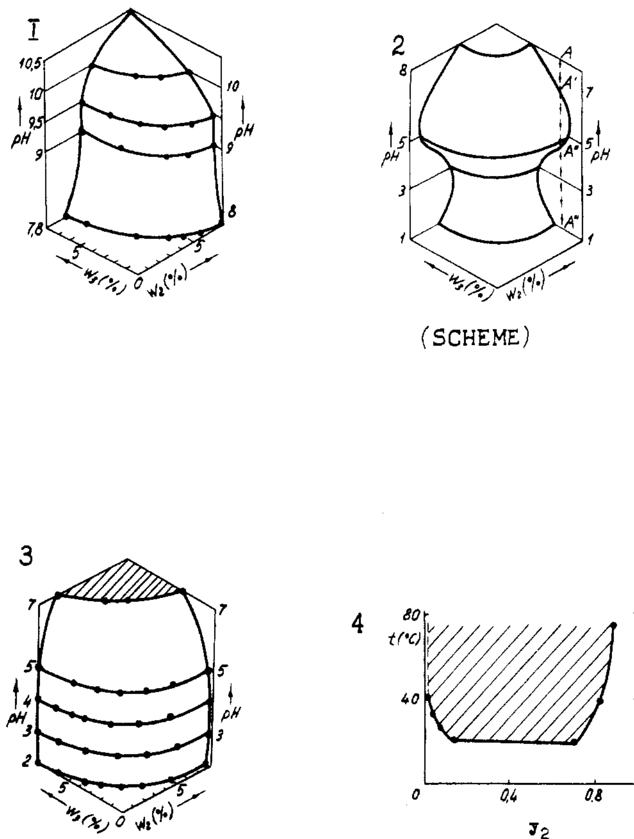

Figure 9. Effect of pH (1–3) and temperature (4) on the protein–neutral polysaccharide TDC. (1) Soybean globulins–dextran T-500–water, 20 °C, 0.25 M NaCl. (2) Casein–dextran T-2000–water, 20 °C, 0.5 M NaCl, 6 M urea. (4) Casein–dextran T-2000–water, 0.15 M NaCl. w_2 and w_3 = protein and polysaccharide concentrations. $y_2 = w_2/w_s$ = apparent weight fraction of protein at $w_s = w_2 + w_3$ = constant. Demixing gaps are shaded.

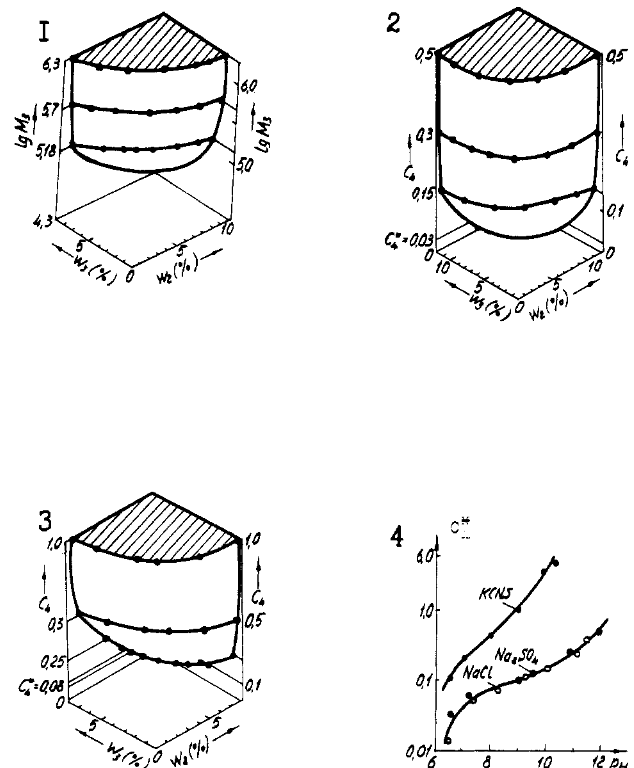

Figure 10. Effect of polysaccharide molecular mass (1), concentration (2, 3), and nature (4) of a low molecular weight salt on the protein–neutral polysaccharide TDC at 20 °C. (1) Casein–dextran–water, pH 6.5, 0.15 M NaCl. (2) Casein–dextran T-2000–water, pH 6.5. (3) Soybean globulins–dextran T-500–water, pH 9.0. (4) Casein–dextran T-2000–water. w_2 and w_3 = protein and polysaccharide concentrations. M_3 = polysaccharide molecular mass. C_4 = salt concentration, mol/L. C_4* = critical salt concentration above which a system is two-phased. Demixing gaps are shaded.

water, which is a poor solvent for the studied proteins. On the contrary, macrocomponents display a limited TDC in urea and potassium rhodanide solutions, which are good solvents for the two macrocomponents. This has no analogues in the thermodynamics of synthetic polymer solutions.

Thermodynamic Compatibility of Proteins. Based on nine protein–protein–water systems, it has been established that proteins of various classes (according to Osborne classification) exhibit a limited TDC under certain conditions (Polyakov et al., 1979, 1980, 1984). Figure 11 gives phase diagrams of the following systems: casein–ovalbumin–water, casein–soybean globulins–water, and soybean globulins–ovalbumin–water obtained by the phase volume ratio method (Polyakov et al., 1980, 1984). Compatibility of proteins is strongly affected by thermal treatment (V. I. Polyakov, unpublished results). Thus, in the native state, the BSA and ovalbumin are completely compatible in a water solution. Meanwhile, the thermotropic aggregates of ovalbumin are not compatible with BSA (Figure 11e) or native ovalbumin (Figure 11d).

Texturization of Proteins out of Two-Phase Systems. We have suggested a general scheme for spinneretless generation of anisotropic gels and fibers out of liquid two-phase systems (Tolstoguzov, 1978; Antonov et al., 1980). This scheme is given in Figure 12. Gelation of the dispersed phase during flow gives rise to fibers. However, if the dispersion medium rather than the dispersed phase undergoes gelation, we obtain gels filled with cylinders oriented downstream. Such gels have been termed "capillary-structure gels". And finally, when the two phases go over into a gel-like state, the result is gels filled with fibers oriented in the direction of deformation. The above trends of protein processing are of practical value in the production of new food forms imitating meat products.

The processing of two-phase liquid systems into food products requires that they should be brought to a gel-like state. Hence, it is essential to know the kinetics, formation conditions, and properties of gels. It should be borne in mind that the gel-like state is characteristic also of a final food product, not only of its intermediate (as is generally the case with processing through technical polymer solutions). In this connection, much thought has been given to gels of proteins and anionic polysaccharides.

Gelation of Proteins and Polysaccharides. We have studied the viscoelastic properties of thermotropic gels of soya bean globulins (Bikbov et al., 1979a,b, 1981, 1983), pectins (Plashchina et al., 1979), carrageenan (Plashchina et al., 1980b), furzellaran (Plashchina et al., 1980a), and maltodextrin (Braudo et al., 1979). It has been established that the rate of relaxation processes occurring in protein and polysaccharide gels is independent of the macromolecular concentration and is governed by the internal viscosity of structural elements. Relaxation processes are probably localized in macromolecular associates, which form a spatial gel network.

Using an example of carrageenan, which turns into a double-helix conformation during gelation, we have shown that the gelation process is characterized by different levels of cooperation, depending on whether the helical conformation is stable or it is in equilibrium with the coil conformation. In the former case, gelation involves a relatively

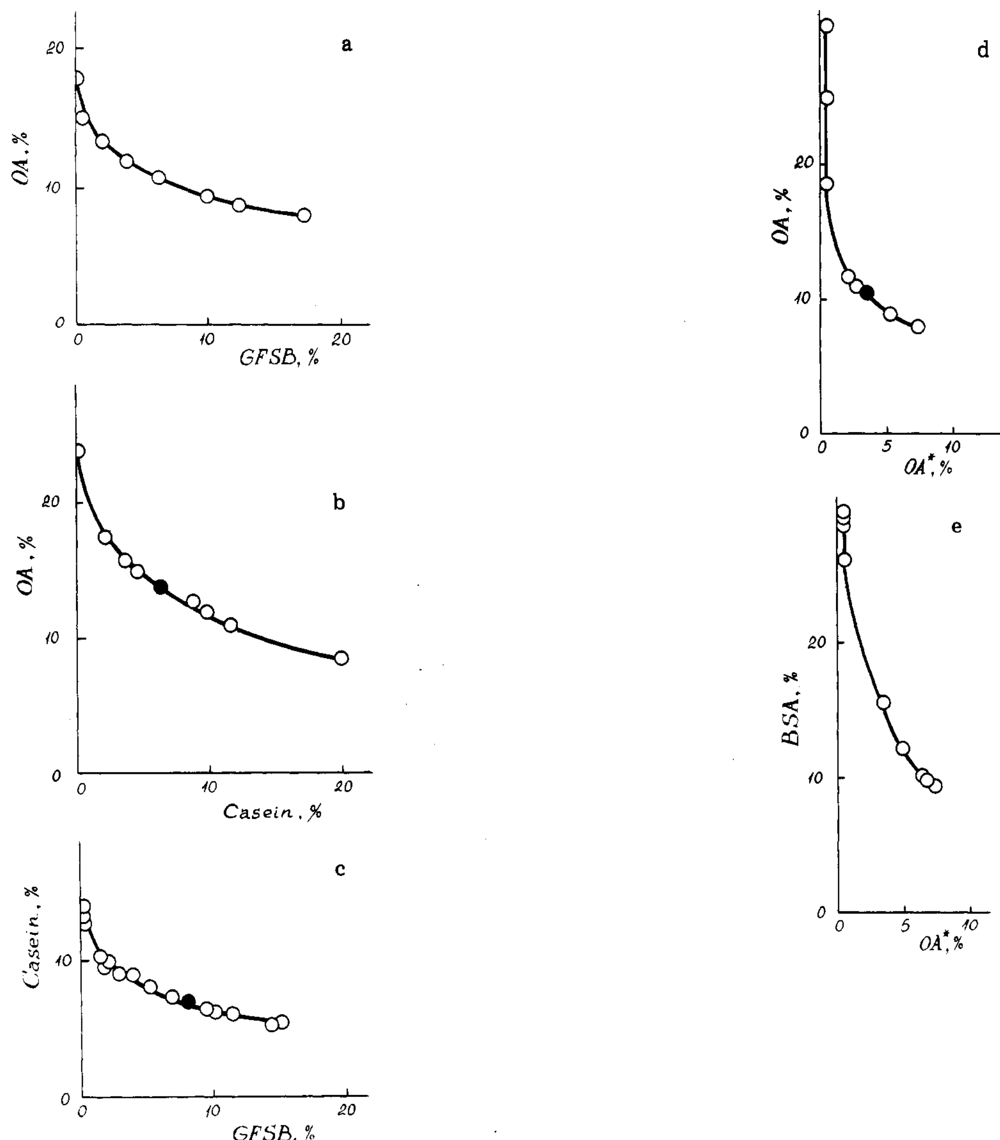

Figure 11. Phase diagrams for some protein A-protein B-water systems at 20 °C: (a) ovalbumin (OA)-globulin fraction of soybeans (GFSB)-water, pH 6.6; (b) ovalbumin-casein-water, pH 6.6; (c) casein-GFSB-water, pH 6.9; (d) ovalbumin-thermally modified ovalbumin (OA*)-water, pH 6.6; (e) bovine serum albumin (BSA)-OA*-water, pH 6.6. (O) Binodal; (●) critical point. The systems are two-phased above and to the right of the binodals.

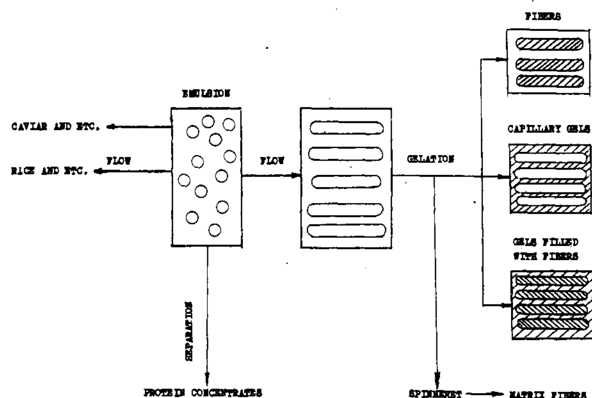

Figure 12. General scheme for processing two-phase protein systems.

small reduction of entropy (−165 J/deg mol of junction), whereas the change in enthalpy is an order of magnitude higher than the energy of thermal motion (−30 kJ/mol of junction). This mechanism of gelation is typical of polymers with rigid or semirigid chains, say of pectins and maltodextrin. As the temperature rises, causing the helix to melt (according to the optical rotation data), the gelation entropy and enthalpy are reduced abruptly. The entropy drops to −795 J/deg mol of junction and the enthalpy drops to −235 kJ/mol of junction. Here, intermolecular interactions play a decisive role in the stabilization of helical conformation and the formation of a helix is necessary for the formation of gel network junctions. In this case, helix formation is related to considerable loss of the conformational entropy. The latter is compensated by increasing enthalpy due to the intermolecular hydrogen bonds. Thanks to the compensation of the two effects, the free energy of formation of gel network junctions remains at a constant level.

Spinneretless Spinning. In a spinneretless processing of two-phase liquid systems, significant considerations are the phase viscosity ratio, the size of particles of the dispersed phase, interfacial tension, and the gelation rate of one or both phases of a system in flow (Antonov et al., 1980). Optimal conditions are satisfied over a certain range of compositions of processed systems. They can be established by analyzing phase diagrams of such systems under the specified conditions. Figure 13 represents phase diagrams for several protein-polysaccharide-water systems, whose range of separation falls into four subranges. The first subrange is most favorable for processing into

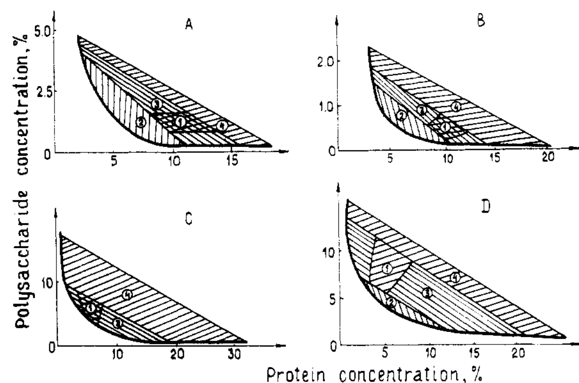

Figure 13. Phase diagrams for some protein–polysaccharide–water systems used in spinnertless spinning (for an explanation, see the text). (A) Casein–sodium alginate–water. (B) Casein–gum arabic–water. (C) Casein–dextran–water. Demixing gaps are shaded.

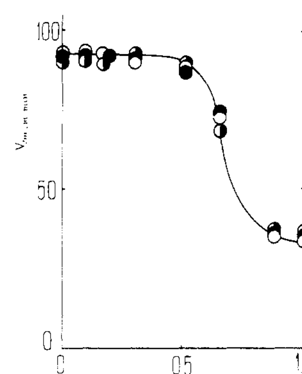

Figure 14. Spinnability of two-phase casein–sodium alginate–water system vs. volume fraction (ϕ) of the casein phase. Emulsions 1 (○), 2 (◑), and 3 (●) differ in the compositions of the coexisting phases. V_{2m} = the maximal uptake velocity of fiber.

anisotropic materials. Over subranges 2–3, processing is impracticable for a number of causes: In subrange 2, the form of deformed dispersed particles is rapidly relaxed in a coagulating bath. In subrange 3, processing is not feasible because of the high-volume fraction of the polysaccharide phase. In subrange 4, the viscosity of the dispersed phase is more than 10-fold that of the dispersion medium so that the drop cannot be deformed any longer.

The anisotropic structure of the capillary gels of systems C and D and of the fiber-filled gels of systems A and B is responsible for an appreciable anisotropy of their mechanical properties. The splitting of these gels into fibers contributes to the heterogeneous nature of the food product, which resembles, in chewing, a meat product.

An interesting property of anisotropic two-phase gels is their ability to fibrillate, i.e., to split into fibers in the course of deformation.

Of definite interest is also the feasibility of spinneretless production of infinitely long fibers 1–4 μm in diameter. This is achievable at a certain size of the dispersed phase particles and a given volume fraction of this phase due to the orientation and coalescence of deformed particles in flow.

Capillary gels are notable for a very interesting phenomenon. With a large volume fraction of the dispersed phase, the continuous phase of the gel represents noncylindrical fibers. These are produced not only by fibrillation of anisotropic gels but also by the processing of foams and suspensions. In the latter case, protein–polysaccharide–water systems are frozen and thawed, thereby leading to lyotropic porous gels of a fibrous structure. Such gels may be useful in the manufacture of combined meat products. We should like also to note that allowing for a limited TDC of some proteins with others we can produce relatively coarse protein suspensions, whose particles become plastic on swelling, when the system composition and temperature are changed. For example, by extruding moderately heated powder-like mixtures of soya bean and sunflower globulins, we obtain fibrous protein texturate utilized in the manufacture of meat products.

Another application of TDC is the production of granular protein texturates, say, cavier, berries, and groats. Protein–water solutions can be shaped in aqueous media, which simplifies the process considerably. The shape of granules is determined by the condition of shear deformation and gelation of the protein solution drops. Gelation may be thermotropic, ionotropic, or lyotropic.

The findings regarding the scientific basis for spinneretless spinning offered the opportunity for initiating the development of this technological process. Several variants of the spinneretless spinning process have been suggested. They differ in the methods of generating a flow, compositions and types of systems, methods of fixing the structure of emulsions, and design features of equipment.

Spinneret Matrix Spinning. Spinnability of binary solutions of casein (Dmitrienko et al., 1978a) and sodium alginate has been studied as a function of the composition of a coagulating bath (Dmitrienko et al., 1978b). Later on, spinnability of a three-component two-phase casein–sodium alginate–water systems was examined vs. phase composition and volume ratio. The spinnability criterion was provided by a maximum rate of fiber takeup. Although the optimal compositions of the coagulating bath ($CaCl_2$, acid) are different for casein and sodium alginate solutions, nevertheless, an optimal composition of the bath can be specified to provide for a simultaneous gelation of the two phases of a casein–sodium alginate–water system.

The spinnability studies of emulsions based on two-phase casein–sodium alginate–water systems (Suchkov et al., 1980) have demonstrated that spinnability is very dependent on the phase composition and volume ratio (Figure 14). Away from the phase inversion point, emulsion spinnability is governed by the spinnability of the dispersion medium, i.e., by the continuous matrix phase, whereas in the phase inversion region, the spinnability switches from one constant value to another. The effect of the volume fraction of the dispersed phase on emulsion spinnability is equivalent to the change in the effective cross section of the matrix phase, except for the phase inversion region.

It has been found out that matrix casein–alginate fibers are much stronger and hydrothermally more stable than casein fibers (Antonov et al., 1980, 1982b).

Matrix spinning has very bright prospects, for it ensures processing of nonspinnable protein solutions and permits generation of protein fibers in soft conditions, compared to the conventional wet spinning, without deterioration of the biological value of proteins.

Concentration of Proteins. Investigations of phase equilibria in two-phase liquid systems have opened up promising prospects of their application, say, for purifying and concentrating proteins.

It has been established that proteins capable of solubilizing lipids are the first to precipitate due to polysaccharides; i.e., they precipitate before the basic protein components. This effect was demonstrated by using polysaccharides to purify the globulin fraction of baker's yeast (Bogracheva et al., 1982, 1983). As a result, the lipid

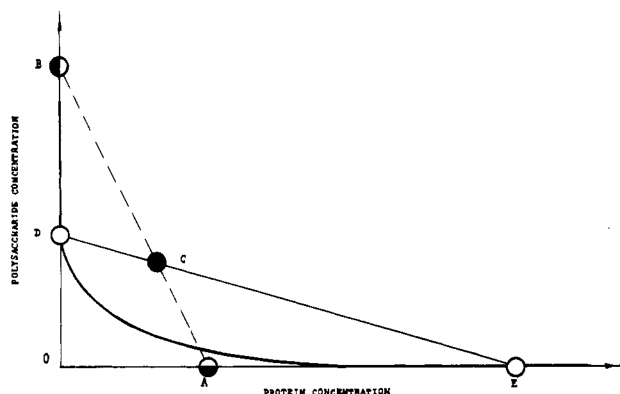

Figure 15. Scheme of membraneless osmosis. (A) Protein solution. (B) Polysaccharide solution. (C) Mixture of solutions A and B. (D) Polysaccharide phase. (E) Protein phase. Thick line = binodal; thin line = tie line.

content was reduced to 0.1%.

A new method is particularly promising for concentrating proteins. It has been called "membraneless osmosis" (Antonov et al., 1982a). Preliminary estimates indicate that this method of protein concentrating is much more efficient and less power intensive than other processes used to concentrate biopolymers. It underlies the process for concentrating skimmed milk proteins.

The principle of membraneless osmosis is illustrated by the diagram of Figure 15. This is a phase diagram for a protein-polysaccharide-water system. Point A represents a protein solution, point B a polysaccharide solution, and point C a mixture of the two solutions. This mixture separates spontaneously into phases D and E. Phase E is a protein solution, where phase D is a polysaccharide solution. It is important that the protein concentration in phase E is higher than that in the initial solution. When apple pectin is used, the milk proteins are separated completely at a 1% concentration of a polysaccharide.

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