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CONCENTRATION OF THE PROTEINS OF SKIMMED MILK BY MEMBRANELESS, ISOBARIC OSMOSIS

YU. A. ANTONOV, V. YA. GRINBERG, N. A. ZHURAVSKAYA & V. B. TOLSTOGUZOV

Institute of Elementoorganic Compounds of the USSR, Academy of Sciences, 117312 Moscow, B-212, Vavilova 28, USSR

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ABSTRACT

The concentration of skimmed milk proteins by polysaccharides such as gum arabic, arabinogalactan and apple pectin with a high content of methoxyl groups was studied. Investigation of the thermodynamic compatibility of skimmed milk proteins with these polysaccharides at different NaCl concentrations and pH has shown that above a certain polysaccharide concentration termed the 'threshold of complete incompatibility' the protein is almost completely excluded from the polysaccharide phase. Phase diagrams obtained for the systems: water-skimmed milk proteins-arabinogalactan, water-skimmed milk proteins-gum arabic and water-skimmed milk proteins-pectin, indicate that highly esterified apple pectin is superior to the other polysaccharides for concentrating skimmed milk proteins.

The proposed method of concentration which may be called 'membraneless isobaric osmosis' has a higher productivity and lower energy consumption than other methods of biopolymer concentration.

INTRODUCTION

Studies of thermodynamic compatibility of proteins and polysaccharides in aqueous medium have shown (Grinberg & Tolstoguzov, 1972; Antonov *et al.*, 1975*a*,*b*,*c*,*d*; Antonov *et al.*, 1976; Antonov *et al.*, 1977; Antonov *et al.*, 1979; Antonov *et al.*, 1980) that mixing of concentrated solutions of these biopolymers produces a two-phase system in which protein is contained mainly in one, 'protein' phase while polysaccharide is predominantly in the other phase. Protein concentration in the 'protein' phase may be very much higher than in the initial solution.

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The properties of water–protein–polysaccharide two-phase systems suggest their possible use for concentration of proteins by polysaccharides.

Concentration and fractionation of protein-containing systems by polysaccharides has been reported in the literature. Thus, Cohen (1942) described precipitation of plant viruses and haemocyanine in the crystalline state by such polysaccharides as heparine, hyaluronic acid, gum arabic and starch. The efficiency of polysaccharides as precipitating agents was essentially independent of pH and their charge density. The most effective precipitant was hyaluronic acid. However, hydrolysates of hyaluronic acid did not precipitate proteins. Starch precipitated proteins only in the presence of low molecular weight electrolytes. Polson *et al.* (1964) reported sedimentation of human globulin and fibrinogen by dextran and some other water-soluble synthetic polymers, including polyethylene glycol.

The present paper considers the possible use of gum arabic, arabinogalactan and highly esterified apple pectin for the concentration of skimmed milk proteins.

MATERIALS

Skimmed Milk

The milk used was obtained from one cow in one milking. The milk was skimmed by centrifugation in a K-70 (Janetzky, East Germany) centrifuge for 50 min at 3500 g and lyophilised in a Labor MIM (Hungary) apparatus at a shelf temperature of 20°C. The preparation thus obtained had the following characteristics: dry matter content, 93·6%; nitrogen, 6·7%; lipids, 0·05%; phosphorus, 1·1%; ash, 6·5% Solutions of dried skimmed milk had a pH of 6·4.

Polysaccharides

The polysaccharides used had the following characteristics:

Arabinogalactan, Sigma Chemical Co., batch no. 19C-0101, $S^{\circ}_{20} = 5{\cdot}2S$, $[\eta] = 3{\cdot}2 \times 10^{-2}$ dl . g^{-1} in 0·25 M NaCl, $M_{[\eta][S]} = 29$ kD.

Gum arabic, Merck, batch no. 7438976, $S^{\circ}_{20} = 11{\cdot}3S$, $[\eta] = 0{\cdot}142$ dl . g^{-1} in 0·15 M NaCl, $M_{[\eta][S]} = 219$ kD.

Pectin, Fluka, batch no. 148860 102, $S^{\circ}_{20} = 1{\cdot}9S$, $[\eta] = 2{\cdot}95$ dl . g^{-1} in 0·15 M NaCl, $M_{[\eta][S]} = 69$ kD, methyl group content 62·7% polygalacturonic acid content 53·3%.

METHODS

Intrinsic Viscosity

Intrinsic viscosity was measured on a Ubbelohde capillary viscometer at 20°C.

Sedimentation

Sedimentation studies were performed in a 3170B ultra-centrifuge (MOM, Hungary) at 20°C.

Molecular Weight

The molecular weights of gum arabic and pectin were determined using the Flory-Mandelkern equation (Nefedov and Lavrenko, 1979):

$$N_A[S][\eta]^{1/3}M^{-2/3} = \beta$$

where N_A is the Avogadro number ($6{\cdot}022 \times 10^{23}$ mol^{-1}), $[\eta]$ is the intrinsic viscosity (dl . g^{-1}), β is a constant whose value was taken as $2{\cdot}46 \times 10^6$ and $[S]$ is the characteristic sedimentation constant which may be found from the following equation:

$$[S] = \frac{S_0\eta_0}{1-\bar{v}\rho_0}$$

where S_0 is the sedimentation constant, $\bar{v}$ is the partial specific volume (0·6 cm^3 g^{-1}) and η_0 and ρ_0 are the solvent viscosity and density respectively.

Degree of Esterification of Pectin

A sample of pectin (0·3–0·4 g) weighed to an accuracy of 10^{-4} g was dissolved in 100 g of distilled water. Six drops of Hinton indicator (0·4% aqueous solutions of bromothymol, cresol red and phenol red mixed in the ratio of 1:1:3) were then added, and the solution was titrated with 0·1 N NaOH until a crimson colour appeared which was stable for 20 s. Thereafter 20 ml of 0·1 N NaOH was added and incubated at room temperature for 2 h. After that 20 ml of 0·1 N HCl was added, and the carboxyl groups liberated were titrated with 0·1 N NaOH. The degree of esterification E was calculated from the formula:

$$E = \frac{b}{a+b}\,100\%$$

where a and b are the quantities of 0·1 N NaOH used in the first and second titrations, respectively.

The polygalacturonic acid content P was calculated from the equation:

$$P = \frac{a\,0{\cdot}0176 + b\,0{\cdot}01901}{d}$$

where d is the weight of the sample in grams.

Protein Content

The protein, both in the skimmed milk and the two-phase systems, was measured by the Kjeldahl technique for total nitrogen and non-protein nitrogen. The latter was determined after precipitation of proteins with 10% trichloroacetic acid.

Determination of Phase Diagrams

Phase diagrams for water-protein-polysaccharide systems were constructed at fixed values of pH, temperature and NaCl concentration. A sample of freeze-dried skimmed milk was dissolved in distilled water or NaCl solution by stirring at room temperature for 40 min. A polysaccharide solution was prepared separately under the same conditions. The solutions were then centrifuged for 30 min at 3500 g to separate undissolved particles, titrated with 1 N NaOH to the required pH, and assayed for dry matter content by drying triplicate aliquots to a constant weight. The solutions were mixed in different weight ratios with an accuracy of 10^{-4} g, and the mixture was stirred for 5 min. Phase separation due to thermodynamic incompatibility between the skimmed milk proteins and polysaccharides was determined as described earlier (Antonov *et al.*, 1975). The mixture was kept at room temperature for 1 h to attain equilibrium and then separated in a K-70 centrifuge for 60 min at 3500 g and 20°C. The compositions of the phases were determined by the phase volume ratio method (Polyakov *et al.*, 1980). Phase volumes were measured in glass centrifuge tubes on a KM-6 cathetometer (USSR).

RESULTS

The thermodynamic compatibility of skimmed milk proteins with gum arabic, arabinogalactan and pectin have been studied. When polysaccharide and protein solutions of percentage concentrations W_3 and W_2 respectively have been mixed at specified values of pH and ionic strength, a two-phase system resulted in which one phase consisted of a concentrated solution or gel of milk proteins. The protein concentration in the other 'polysaccharide' phase rapidly decreased on increasing the polysaccharide concentration in the system. At a certain polysaccharide concentration, the protein was practically absent in the 'polysaccharide' phase. This concentration may be termed the 'threshold of complete incompatibility' with protein.

Figures 1–3 display phase diagrams for water-skimmed milk proteins-polysaccharide systems at pH 6·4 and [NaCl] = 0. The general shape of the diagrams are typical of water-protein-polysaccharide systems (Antonov *et al.*, 1979*c*).

Table 1 contains some parameters derived from these diagrams, including the 'threshold of complete incompatibility', degree of protein concentration (i.e. ratio of protein concentration in the protein phase to that in a skimmed milk), composition of the concentrate and its physical state. As can be seen from Table 1, the protein concentration in the water-skimmed milk proteins-arabinogalactan system is 1·5 times higher than the concentration in water-skimmed milk proteins-pectin and water-skimmed milk proteins-gum arabic systems. However, the concentration of polysaccharide in the water-skimmed milk proteins-pectin system at the 'threshold of complete incompatibility' is 26·6 times less than in the water-skimmed milk proteins-arabinogalactan system and 7·4 times less than in the water-skimmed milk proteins-

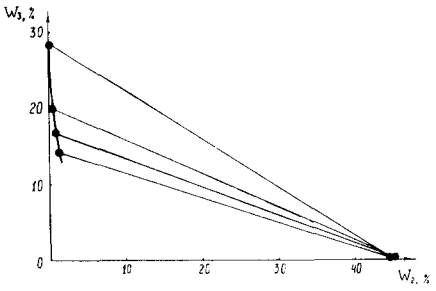

Fig. 1. Phase diagram of water-skimmed milk proteins–arabinogalactan system. $T = 20°C$, pH 6·4, [NaCl] = 0, —— binodal, —— tie lines.

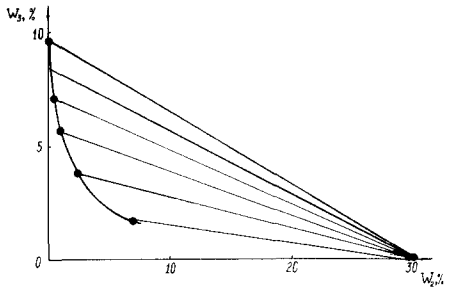

Fig. 2. Phase diagram of water–skimmed milk proteins–gum arabic system. $T = 20°C$, pH 6·4, [NaCl] = 0, —— binodal, —— tie lines.

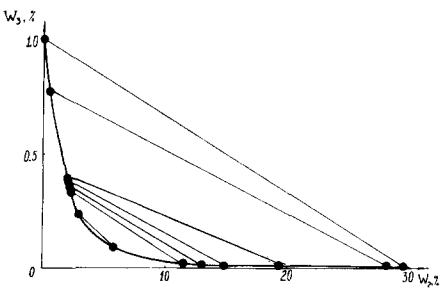

Fig. 3. Phase diagram of water–skimmed milk proteins–pectin system. $T = 20°C$, pH 6·4, [NaCl] = 0, —— binodal, —— tie lines.

gum arabic system. Therefore, of the three polysaccharides, pectin with a high degree of esterification is most effective at concentrating skimmed milk proteins.

We examined the thermodynamic compatibility of pectin with skimmed milk proteins over a range of pH (6·0, 6·4, 8·0, 9·5, 10·5) and at two NaCl concentrations (0 and 0·5 M). pH in the range 6·0–8·0, had little effect on the phase diagram. Figures 3–5 display typical phase diagrams determined in water at pH 6·4, 9·5 and 10·5, respectively. Figure 6 shows the phase diagram at pH 6·4 in 0·5 M NaCl. Several parameters derived from the phase diagrams are given in Table 2. It can be seen from this table that skimmed milk proteins can be best concentrated at the pH and salt level that naturally exist in skimmed milk.

Figure 7 outlines the process of milk protein concentration with pectin and indicates some potential uses for the product. Initially the pectin is dissolved in water. The pectin solution is then mixed with skimmed milk to obtain a two-phase system. This concentration stage lasts several minutes. The pectin and protein phases are then

TABLE 1
Some characteristics of water-skimmed milk proteins-polysaccharide systems at 20°C, pH 6·4, [NaCl] = 0

Parameters	*Polysaccharides*		
	Arabino galactan	*Gum arabic*	*Apple pectin*
Threshold of complete incompatibility (%)[a]	24	6·7	0·9
Degree of protein concentration[b]	15	10	10
Concentration of concentrate (%)[b]			
protein	45	30	30
polysaccharide	0·01	0·01	0·01
water	47	58	–
State of the concentrate[b]	Gel	Gel	Liquid

[a] This represents the total polysaccharide concentration in the whole system after mixing.
[b] Determined at the threshold of complete incompatibility.

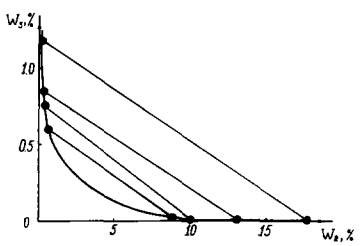

Fig. 4. Phase diagram of water-skimmed milk proteins–pectin system. $T = 20°C$, pH 9·5, [NaCl] = 0, —— binodal, —— tie lines.

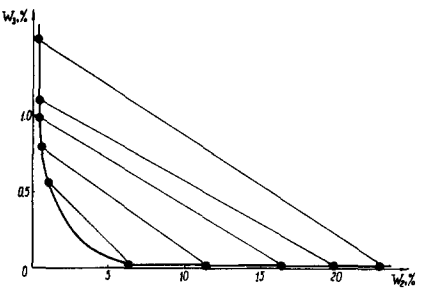

Fig. 5. Phase diagram of water-skimmed milk proteins–pectin, system. $T = 20°C$, pH 10·5, [NaCl] = 0, —— binodal, —— tie lines.

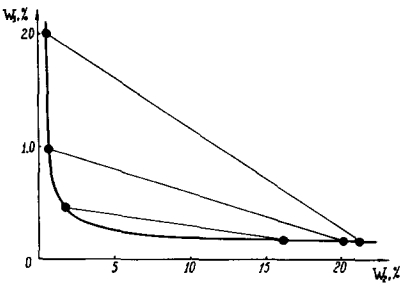

Fig. 6. Phase diagram of water-skimmed milk proteins–pectin system. $T = 20°C$, pH 6·4, [NaCl] = 0·5 M, —— binodal, —— tie lines.

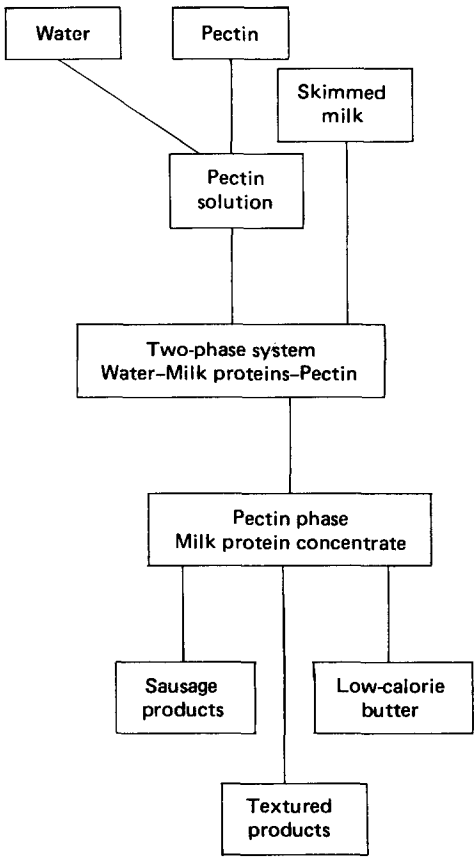

Fig. 7. Scheme for protein concentration by the method of membraneless isobaric osmosis.

separated. When this is done on a centrifuge at 3500 g the separation takes 30 min. We have also separated the protein phase on a LAPX-202 Alfa-Laval laboratory separator (at 38–40°C, plate rotation rate 10 000 rpm, number of plates 32, flow rate 18 litres h^{-1}).

After separation, the pectin phase is used to prepare a pectin solution by bringing it to the nominal pectin concentration by the addition of solid pectin. This stage provides for recycling of pectin.

The milk protein concentrate is a viscous liquid with a protein concentration of 20–30%. It is a good emulsifier and may therefore be used as an extender in the production of sausage and low-calorie butter. The liquid state of the concentrate and its ability to form an ionotropic gel enables it to be used to form textured products by the spineretteless technique (Antonov *et al.*, 1980).

TABLE 2
Some characteristics of water-skimmed milk proteins–pectin systems at different pH and NaCl concentrations

pH	*[NaCl] (mole litre^{-1})*	*Threshold of complete incompatibility (%)*	*Factor of concentration*	*Concentrate composition (%)*	
				Protein	*Polysaccharide*
6·4	0·0	0·9	10[a]	30	0·01
9·5	0·0	–	5·7[a]	17	0·01
10·5	0·0	–	9·0[a]	27	0·02
6·4	0·5	–	6·0[a]	20	0·18

[a] Determined at a pectin concentration of 0·9%.

DISCUSSION

The method of concentration of skimmed milk proteins described in this paper illustrates a new principle of protein concentration which is based on a general phenomenon of thermodynamic incompatibility between proteins and polysaccharides. This principle has much in common with concentration by osmosis but does not require a membrane or pressure difference between the system and environment. This concentration principle may be termed membraneless, isobaric osmosis.

The essence of the principle is explained in Fig. 8 which shows a typical phase diagram of a water-protein-polysaccharide system. The bold line here is termed a binodal and separates a two-phase system (above) from a single-phase system (below). Point A represents a protein solution and point B represents a polysaccharide solution. After mixing in a certain ratio, these solutions produce a water-protein-polysaccharide mixture whose initial composition is represented by point C. Since point C is above the binodal, the mixture separates into phases D and E which are polysaccharide and

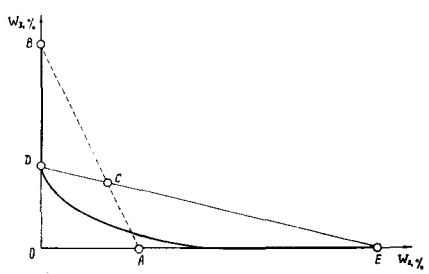

Fig. 8. Principle of the process of skimmed milk proteins concentration with pectin.

protein solutions, respectively. The protein concentration in phase E is higher than in the initial solution A.

Concentration of protein occurs as a result of transfer of water, i.e. osmosis, from the protein solution into the polysaccharide solution. This transfer is accounted for by two factors:

1. Separation of the water-protein-polysaccharide system into liquid phases each of which is composed either mainly of protein or mainly of polysaccharide.
2. A stronger dependence of water activity upon concentration in the case of the polysaccharide. This is explained by the fact that water is a very good solvent for polysaccharide and a poor solvent for protein.

Figure 9 shows water activity (a_w) as a function of percentage concentration (W) in a protein solution (curve 1) and in a polysaccharide solution (curve 2). Points A and B are initial solutions of protein and polysaccharide, respectively. Mixing of these two will result in the transfer of water from the former into the latter until the water activities in them become equal (points D and E). This will naturally mean concentration of the protein solution and dilution of the polysaccharide solution.

Two-phase systems composed of water, protein and polysaccharide have a very low interfacial tension of about 0·01 dyne cm^{-1} (Gulov, 1973). Therefore, a very high degree of dispersion can be obtained by such simple means as a conventional laboratory stirrer. Particles of 1–5 μm in size can be obtained without difficulty. Owing to the small particle size water-protein-polysaccharide systems have a large interfacial area. For instance, a 100 ml system whose dispersed phase occupies 0·2% of the volume may have an interfacial area of several dozen square metres. The high rate of protein concentration in these systems can be explained by the large interfacial area available for the transfer of water.

In conclusion Table 3 compares the membraneless, isobaric osmosis with the two most common methods of concentrating protein, vacuum evaporation and ultrafiltration, in terms of productivity and energy consumption. It can be seen that

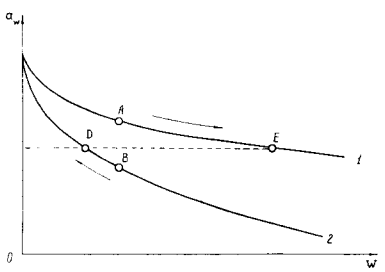

Fig. 9. Schematic representation of water activity as a function of protein (curve 1) and polysaccharide (curve 2) concentrations in solutions.

TABLE 3
Technical and economical characteristics of different methods used to produce a 30% concentrate of skimmed milk proteins

Concentration method	*Energy consumption (kJ kg^{-1} of protein concentrate)*	*Productivity (kg of protein concentrate h^{-1})*
Vacuum evaporation[a]	9 700	440
Ultrafiltration[b]	3 830	190
Membraneless, isobaric osmosis	90[c]	500

[a] Data for a typical installation with thermocompression by used steam.
[b] Data for a typical installation with a membrane area of 342 m^2.
[c] Calculated for a typical continuous flow separator; this does not include energy consumption for stirring and pumping the solutions.

membraneless, isobaric osmosis is preferable on both criteria. Another advantage of this method is that it requires no complex equipment, only stirrers, a separator, and pumps for liquids.

REFERENCES

Antonov, Yu. A. (1978). Doctoral Thesis, Moscow, INEOS, USSR Academy of Sciences.

Antonov, Yu. A., Grinberg, V. Ya. & Tolstoguzov, V. B. (1975). *Starke* **27**, 424.

Antonov, Yu. A., Grinberg, V. Ya. & Tolstoguzov, V. B. (1976). *Visokomol. Soid.* **B18**, 566.

Antonov, Yu. A., Grinberg, V. Ya. & Tolstoguzov, V. B. (1977). *Coll. and Polym. Sci.* **255**, 937.

Antonov, Yu. A., Lozinskaya, N. V., Grinberg, V. Ya., Dianova, V. T. & Tolstoguzov, V. B. (1979*a*). *Coll. and Polym. Sci.* **257**, 1159.

Antonov, Yu. A., Grinberg, V. Ya. & Tolstoguzov, V. B. (1979*b*). *Nahrung* **23**, 207.

Antonov, Yu. A., Grinberg, V. Ya. & Tolstoguzov, V. B. (1979*c*). *Nahrung* **23**, 597.

Antonov, Yu. A., Grinberg, V. Ya. & Tolstoguzov, V. B. (1979*d*). *Nahrung* **23**, 847.

Antonov, Yu. A., Grinberg, V. Ya., Zhuravskaya, N. A. & Tolstoguzov, V. B. (1980) *J. Texture Studies* **II**, 199.

Grinberg, V. Ya. & Tolstoguzov, V. B. (1972). *Carbohydr. Res.* **25**, 31.

Gulov, V. Ya. (1973). Doctoral Thesis, Moscow, INEOS, USSR Academy of Sciences.

Cohen, S. S. (1942). *J. Biol. Chem.* **144**, 353.

Nefedov, P. P. & Lavrenko, P. N. (1979). *Transport Methods in the Analytical Chemistry of Polymers*, Khimiya, Leningrad.

Polson, A., Portgieter, G. M., Largier, J. F., Mears, G. E. F. & Joubert, F. J. (1964). *Biochim. Biophys. Acta* **82**, 463.

Polyakov, V. I., Grinberg, V. Ya. & Tolstoguzov, V. B. (1980). *Polymer Bull.* **2**, 757.