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USE OF POLYSACCHARIDES TO REMOVE LIPIDS FROM THE PROTEIN GLOBULIN FRACTION OF BAKER'S YEAST

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ABSTRACT

The precipitation of lipid-protein complexes from the baker's yeast protein globulin fraction by polysaccharides (gum arabic and arabinogalactan) was investigated. Lipid-protein complexes were precipitated more readily with the polysaccharides under study than with other globulin fractions components. A method for the removal of lipids from the globulin fraction of baker's yeast by precipitation of the lipid-protein complexes with polysaccharides is suggested.

1. INTRODUCTION

Microorganisms are a promising source of food protein (Kinsella & Shetty, 1978; Pokrovsky, 1972). However, a considerable amount of lipid is extracted with the protein from yeast biomass. The presence of lipids in the yeast isolates is inadmissible for two reasons: firstly, yeast lipids contain non-edible lipids with an odd number of carbon atoms (Pokrovsky, 1972); secondly, the presence of lipids in the yeast isolates leads to an exceedingly rapid deterioration in the protein functional properties (Pokorny *et al.*, 1975; Van Deenen, 1978). Since, in yeast, lipids are present chiefly in the form of complexes with proteins, their direct extraction with organic solvents is not very effective. Sufficiently complete separation of lipids from proteins can be attained only by extraction using a chloroform-methanol mixture (Melton *et al.*, 1979; O'Leary, 1977). However, the use of this solvent mixture is undesirable if the protein is intended for food use. A general disadvantage of organic solvents, is that they cause denaturation of protein and reduce its functional properties.

Lipids coextracted with proteins can conveniently be regarded as either in the emulsified or solubilised form. Emulsified lipids are contained in the extract in the form of a fine, disperse lipid phase; their isolation by centrifugation or other physical

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methods is a simple task. This is not so in the case of solubilised lipids, i.e. lipids contained in soluble lipid-protein complexes. These lipids must be separated from the proteins to which they are bound rather strongly.

A systematic study of the thermodynamic compatibility of proteins and polysaccharides in water has shown that on mixing concentrated solutions of protein and polysaccharides a two-phase system can form. Maximum solubilities of different proteins in a particular polysaccharide solution, before a two-phase system is formed, can vary greatly. This property makes it possible to use polysaccharides to purify and fractionate the protein components (Antonov *et al.*, 1979).

Polson *et al.* (1964) described blood plasma globulin and fibrinogen precipitation with dextran, and also with several other water-soluble synthetic polymers, including polyethylene-glycol. Iverius (1968) reported precipitation of β-lipoproteins from a 1% solution with clinical dextran (molecular weight of 71 400 daltons) under physiological conditions (pH, ionic strength). Precipitation commenced at a dextran concentration of about 7%. Above this threshold the solubility of the β-lipoproteins decreased exponentially with increasing dextran concentration. Foster *et al.* (1973) described fractionation of the yeast enzymes of *Saccharomyces cereviseae* with polyethylene glycol. Zeppezaner & Brishammars (1965) demonstrated that it was possible to precipate lipoproteins and human blood plasma with water-soluble polymers.

It is therefore clear that protein mixtures can be purified by isolating certain protein fractions from protein mixtures using synthetic water-soluble polymers or polysaccharides. However, most investigations were carried out using polyethylene glycol which is non-edible and, hence, should not be used to separate proteins which are to be used in food.

In general lipid-protein complexes are more hydrophobic than other protein components and therefore have the greatest tendency to associate. Since the tendency to associate determines the thermodynamic compatibility of proteins and polysaccharides we may expect that that lipid-protein complexes will prove less compatible with polysaccharides than other protein components in the globulin fraction. If this is the case, then precipitation by polysaccharides, offers a method of separating solubilised lipids.

This paper considers the removal of lipid-protein complexes from the yeast, *Saccharomyces cereviseae*, using polysaccharides.

2. EXPERIMENTAL SECTION

2.1. *Materials*

Yeast protein globulins were removed from a pure culture of *Saccharomyces cereviseae* grown on molasses. The protein was extracted from the yeast when the latter was at the beginning of the stable growth stage. A 5% suspension of yeast cells was disintegrated mechanically using a ballistic disintegrator 'FUG-1' at 17°C. Protein was extracted from the homogenate at pH 8·8 for 1·5 h. The extract was separated by

centrifugation (3500 *g*, 60 min). Globulins were precipitated from the extract at pH 4·5 and the precipitate was separated by centrifugation (3500 *g*, 60 min) and dissolved at pH 8·0 using 0·1 M NaOH. This solution was centrifuged (20 000 *g*, 2 h) to separate insoluble components from emulsified lipids. Since no strong alkalis were used in protein extraction (pH did not exceed 9·0) the isolated fraction thus obtained was considered to consist of globulins.

Gum arabic (batch 7093912) was purchased from Merck Inc: $[\eta] = 16$ cm^3/g, $S_0 = 10{\cdot}6$ S, $M_{\eta s} = 190\,000$ daltons (0·25 M NaCl).

Arabinogalactan (batch 19C-0101) was obtained from Sigma Inc: $[\eta] = 3{\cdot}2$ cm^3/g, $S_0 = 5{\cdot}2$ S, $M_{\eta s} = 29\,000$ daltons (0·25 M NaCl).

2.2. *Methods*

Intrinsic viscosity and sedimentation coefficient. The intrinsic viscosity, sedimentation coefficient and diffusion coefficient values of the polysaccharides were determined in 0·25 M NaCl using an Ubbelohde viscometer, and an ultracentrifuge 3170B (MOM Hungary), respectively.

Molecular weight. Molecular weights of gum arabic and arabinogalactan were calculated according to the following equation (Nefedov & Lavrenko, 1979):

$$M = \left[\frac{N_a \cdot S_0 [\eta]^{1/3}}{(1 - \bar{V}_2 \rho_0) \cdot \beta}\right]^{3/2}$$

where N_a is the Avogadro number, S_0 is the sedimentation constant, $[\eta]$ is the intrinsic viscosity, $\bar{V}_2 = 0{\cdot}6$ cm^3/*g* is the specific partial polysaccharide volume; ρ_0 is the solvent density; and $\beta = 2{\cdot}46 \times 10^6$.

Lipids. Lipids were determined using the spectrophotometric sulphophosphovanillin method (Zollner & Kirsch, 1962). The lipids were extracted using a chloroform-methanol mixture (0·8 parts of the protein solution; 1 part of chloroform: 2 parts of methanol). A 0·02% solution of calcium chloride was then added to the extract (1 part of the solution to 4 parts of the extract). The system separated into phases the lower phase consisting of chloroform with the lipids dissolved in it. An aliquot of this phase was taken and chloroform was evaporated using a rotary evaporator. Sulphuric acid (0·2 ml) was then added and the solution was boiled for 10 min in a hot water bath. After cooling to room temperature, 5 ml of the vanillin phosphate reagent consisting of 1 part of a 0·6% vanillin solution and 4 parts of phosphoric acid, were added to the test tube and a pink colour then developed. After 30 min, the optical density was measured at 530 nm against a water reference. Optical density, if the value does not exceed 0·9, is proportional to lipid concentration. The method was calibrated using the Folch solvent procedure (Folch *et al.*, 1957). To plot the curve, the control solutions were dried lyophilically, the lipids extracted using the Folch mixture, the chloroform phase separated then evaporated using a rotary evaporator, the retort with

the lipids brought up to a constant weight at $T = 100$-$105°C$ and the amount of lipids obtained determined gravimetrically (Folch *et al.*, 1957).

Protein. Proteins were precipitated from the test solution with hot perchloric acid. Kjeldahl's method was used to determine total nitrogen both in the initial solution and in the supernatant liquid. Protein nitrogen was assessed by a difference between the total nitrogen in the initial solution and the nitrogen of non-protein origin in the supernatant liquid. Protein concentration in the solution was determined by multiplying the protein nitrogen by the factor 6·25 (Spirin, 1958; Termikhatova & Shulga, 1974; Pilch, 1911).

Fractional precipitation of the globulin fraction by polysaccharides. Known amounts of concentrated polysaccharide solution were added to the globulin fraction solution. (The latter contained about 3-5% protein.) After each addition the system was mixed at room temperature for 10 min and its phase state analysed. Above a definite level of added polysaccharide the mixture separated into two phases. On centrifugation (20000g, 10 min) there appeared a gel-like protein-lipid residue, a supernatant containing polysaccharide, and the baker's yeast globulin fraction which was soluble at the appropriate polysaccharide concentration. The residue and the supernatant were analysed for lipid and protein.

3. RESULTS AND DISCUSSION

The solubility (s) of the globulin fraction components was calculated using the following relationship:

$$s = \frac{P_i}{P_0} \times 100\%$$

where P_i is the test component mass (lipid or protein) in the supernatant and P_0 is the test component mass (lipid or protein) in the initial solution.

Figure 1 shows a relationship between the solubility of lipid and protein components in the baker's yeast globulin fractions and concentration of gum arabic and arabinogalactan at 20°C and pH 8·0. In the presence of the polysaccharides both the protein and lipid solubility decreased, the latter decreasing more significantly. At the maximum polysaccharide concentration examined in the case of arabinogalactan the lipid solubility decreased more than two-fold, while the protein solubility fell by only 14%. In the case of gum arabic the relative lipid solubility fell almost 100 times as compared with a 10% reduction in the protein solubility.

The relationship between the solubility of non-polar compounds, such as gases, carbohydrates and proteins, and salt concentration can be described by the Sechenov

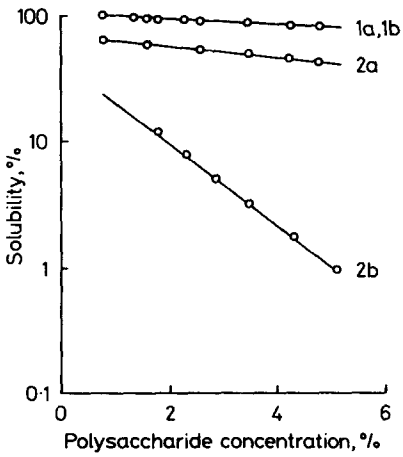

Fig. 1. Relationship between the solubility of the (1) protein and (2) lipid components of the baker's yeast globulin fraction and varying concentrations of (a) arabinogalactan and (b) gum arabic, at 20°C and pH 8. The polysaccharide concentration is expressed as (mass of polysaccharide)/(total mass of system after addition of polysaccharide solution) × 100.

equation (von Hippel & Schleich, 1979):

$$\ln Y = K_1 - K_2 C \tag{1}$$

where Y is solubility of the non-polar compound, K_1 and K_2 are empirical constants and C is the salt concentration.

The results of Laurent (1963) who studied the relationship between protein solubility in salt solutions and polysaccharide concentration can be described by a similar equation:

$$\ln Y = K_1 - K_2 C_{ps} \tag{2}$$

where Y is the protein solubility, K_1 and K_2 are empirical constants and C_{ps} is the polysaccharide concentration.

Figure 1 shows that solubility of the lipids and, hence, of the lipid-protein complexes (since the lipids are part of these complexes) depends on polysaccharide concentration, and that this relationship can also be described by eqn (2). We have also found that the coefficient K_2 depends on the polysaccharide molecular weight as follows:

$$K_2 = (3{\cdot}3 \pm 0{\cdot}4) \times 10^{-6} M \tag{3}$$

where M is the polysaccharide molecular weight.

The different effects of arabinogalactan and gum arabic on lipid solubility can thus be explained by molecular weight differences between these polysaccharides. A higher

molecular weight fraction of arabinogalactan would be expected to have a greater effect on lipid solubility.

Figure 2 shows the relationship between the relative protein concentration (P) in the precipitate, defined as the following ratio:

$$P = \frac{\text{(protein concentration)}}{\text{(protein concentration)} + \text{(lipid concentration)}} \times 100\%$$

and the gum arabic concentration. It indicates that as the polysaccharide concentration increases from 1·73 to 5·2% the protein content in the precipitated fraction increases from 44 to 94%. These findings show that at the initial stage, polysaccharides precipitate mainly lipid-protein complexes.

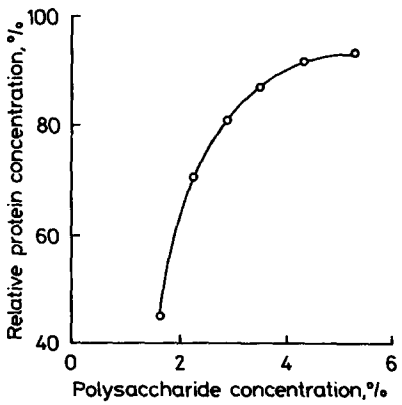

Fig. 2. Relationship between the relative protein concentration in the precipitate and the gum arabic concentration at 20°C and pH 8.

Figure 3 shows relationships between the relative lipid concentration in the globulin fraction after precipitation and the polysaccharide concentration. Relative lipid concentration (L) is defined as follows:

$$L = \frac{\text{(lipid concentration)}}{\text{(protein concentration)} + \text{(lipid concentration)}} \times 100\%$$

These relationships show that as the polysaccharide concentration increases, the relative lipid concentration in the globulin fraction decreases considerably. The effect is especially great in the case of gum arabic when L falls to 0·1%. None of the known methods of lipid separation, with the exception of extraction using a Folch mixture (methanol + chloroform), can remove lipids from these systems so effectively.

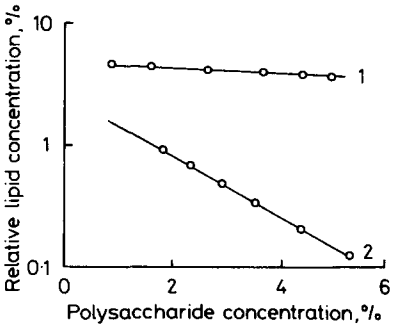

Fig. 3. Relationship between the relative lipid concentration in the baker's yeast globulin fraction (after precipitation of the solubilised lipids) and the concentrations of (1) arabinogalactan and (2) gum arabic, at 20°C and pH 8.

4. CONCLUSION

Lipid-protein complexes are far less compatible with polysaccharides than other proteins in the baker's yeast globulin fraction. This makes it possible to separate solubilised lipids from the main protein components in the globulin fraction by fractional precipitation using polysaccharides. In the case of gum arabic the efficiency of this procedure is comparable with extraction using the Folch solvent mixture. A considerable advantage of the fractional precipitation technique compared with solvent extraction is that proteins are not contamined with toxic materials.

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