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SOME FUNCTIONAL PROPERTIES OF COTTON PROTEIN HYDROLYSATES

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A comparative study of the solubility and emulsifying properties of cotton protein hydrolysates produced by the enzyme preparation Pektofoetidin has shown that the optimum functional properties are possessed by hydrolysates with a degree of proteolysis of 8-13%.

At the present time, the question of the search for new sources of food proteins and the expansion of the range of their application is becoming acute [1]. In addition to the biological value of protein substances, an important factor limiting the sphere of their use is undoubtedly their functional properties [2]. Among the considerable arsenal of known methods of regulating the functional properties of proteins, a definite place is occupied by proteolysis. The hydrolysis of proteins at the peptide bonds under the action of enzymes generally improves their solubility, particularly at the isoelectric point (IEP). At the same time, some other functional properties of the hydrolysates obtained (emulsifying, gel-forming, etc.) also change [3-7].

Protein isolates obtained by acid extraction from cottonseed meal possess a low solubility in the neutral pH range at low ionic strength of the solution [8]. In the present paper we give results of a study of the change in the solubility and emulsifying properties of cotton protein hydrolysates obtained on proteolysis of an isolate by the enzyme preparation Pektofoetidin P10x, which is used in industry [9].

Analysis of the products of the proteolysis of the cotton protein isolate by Pektofoetidin (Fig. 1) show that the enzyme exhibits considerable activity, and the rate of proteolysis depends on the enzyme:substrate ratio. As can be seen from Fig. 1, the maximum degree of proteolysis of the protein (α) amounted to 20.1% at a time of hydrolysis of 24 h.

Since proteins exist in aqueous solutions in the form of bipolar ions and the solubility of cotton protein at the IEP is low, it is possible to study their solubility with the aid of turbidimetric titration. The region of the pH for maximum precipitation of all the

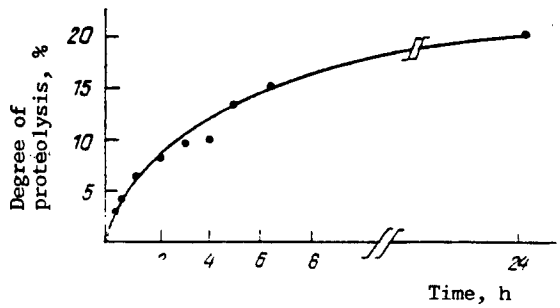

Fig. 1. Degree of proteolysis of hydrolysates of cotton protein by Pektofoetidin as a function of the time of the reaction.

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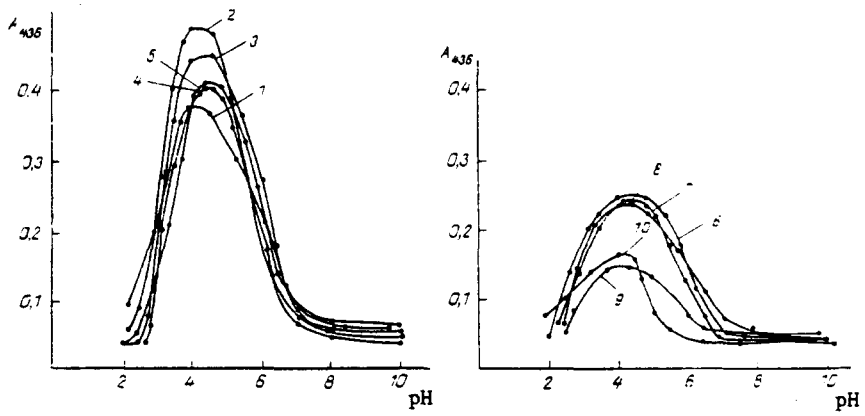

Fig. 2. Curves of the turbidimetric titration of cotton protein hydrolysates. Here and in Figs. 3-5: 1) unhydrolyzed isolates; 2) time of hydrolysis 15 min, α = 3.0%; 3) 30 min, α = 3.9%; 4) 1 h, α = 6.5%; 5) 2 h, α = 8.1%; 6) 3 h, α = 10.0%; 7) 4 h, α = 10.4%; 8) 5 h, α = 13.3%; 9) 6 h, α = 15.0%; 10) 24 h, α = 20.1%.

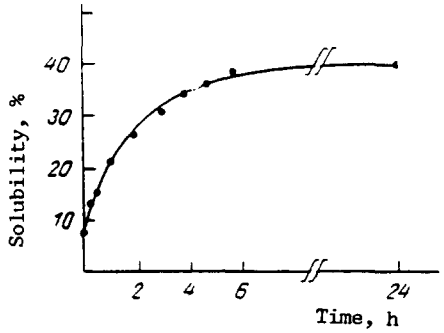

Fig. 3. Solubility of cotton protein hydrolysates in the region of the pH of minimum solubility.

hydrolysates investigated (Fig. 2) was close to the region of the maximum precipitation of the initial protein (pH 3.8-4.6). For samples with a degree of proteolysis below 10% (Fig. 2, curves 2-5) an increase in the turbidity of the solution was observed in the region of the pH of the minimum solubility of the hydrolysates, which can be explained by an increased aggregation of the peptides obtained under these conditions.

For a quantitative evaluation of the solubility of the hydrolysates we used the static method (Fig. 3), determining the amount of protein that had passed into solution in a region close to the IEP (pH 4.5). It can be seen from Fig. 3 that with a rise in the degree of proteolysis the solubility of the hydrolysates increased. For a sample with the maximum degree of proteolysis (α = 20.1%) the solubility under these conditions was four times higher than for the unhydrolyzed protein.

For their successful application as additives to food products, the emulsifying properties of hydrolysates are important. Curves of the dependence of the amount of decane passing into an emulsion on the amount of protein (hydrolysate) in the aqueous phase were plotted. The tangent of the angle of slope of the titration curves showed the characteristic emulsifying activity of the protein:

Sample	Degree of proteolysis, %	Intrinsic emulsifying activity
1	0	0,125
2	3,0	0,375
3	3,9	0,410
4	6,5	0,453
5	8,1	0,620
6	10,0	0,680
7	10,4	0,800
8	13,3	0,800
9	15,0	0,760
10	20,1	0,550

As can be seen from the figures given above, the emulsifying activities of the hydrolysates reached a maximum, and the further proteolysis of the protein led to a deterioration of the emulsifying properties.

The dependence of emulsion stability on the concentration of protein (hydrolysates) is shown in Fig. 4. A system stabilized by a hydrolysate with a degree of proteolysis $\alpha = 8.1\%$ (Fig. 4, curve 5) was characterized by the highest stability. Hydrolysates with higher degrees of proteolysis stabilized emulsions less well.

Thus, among the samples obtained it is possible to single out two groups with respect to functional properties: I) samples 1-5; and II) samples 6-10. The main differences of these groups consist in the nature of the turbidimetric titration and emulsion stability curves. The latter is due above all to a change in the qualitative composition of the hydrolysates on proteolysis.

Under the conditions of disk electrophoresis in PAG, the unhydrolyzed cotton protein gave eight protein bands of different intensities (Fig. 5). With a rise in the degree of proteolysis the number of bands corresponding to protein subunits decreased, while the intensity of the fast-migrating band of low-molecular-mass peptides increased. For the hydrolysate with a degree of proteolysis $\alpha = 8.1\%$, we observed a disappearance of the subunits and an intense band corresponding to the low-molecular-mass components. For a sample with a degree of proteolysis $\alpha = 20.1\%$ peptides precipitable by TCA were practically absent from the electrophoretogram.

The results obtained show that the proteolysis of a cotton protein isolate can be used successfully for regulating its functional properties. The change in these properties has a complex nature, since the hydrolysates obtained consist of mixtures of high- and low-molecular-mass proteins and peptides. For the given conditions (ratio of enzyme to substrate,

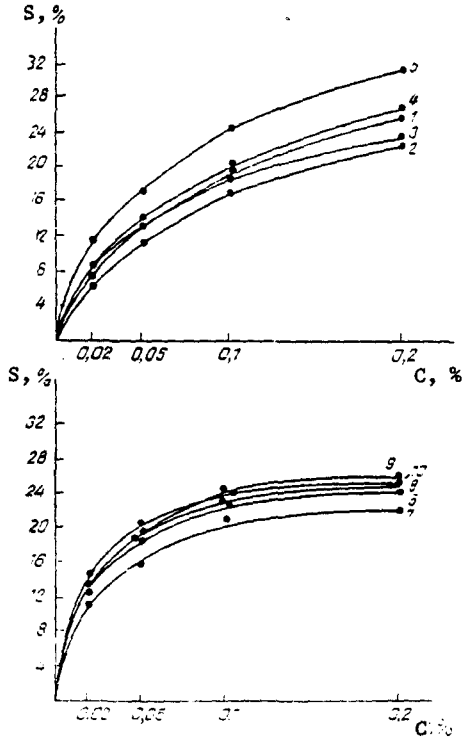

Fig. 4. Curves of the S/C relationship for cotton protein hydrolysates (where C is the concentration of the hydrolysate in the solution, %, and S is the area corresponding to the emulsion on diagrams of emulsion stability, %).

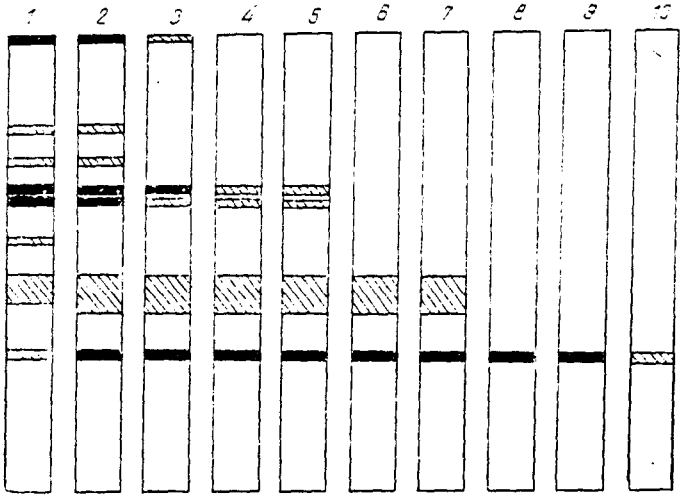

Fig. 5. Electrophoretograms of cotton protein hydrolysates in 15% PAG in the presence of 2% Na—DDS.

concentration of substrate in the solution, and time of proteolysis), hydrolysates with a degree of proteolysis α = 8-13 possessed the optimum functional properties.

EXPERIMENTAL

The protein isolate was obtained as described in [8]. A flask was charged with 10 g of cotton protein isolate, and 160 ml of 0.01 M acetate buffer, pH 4.5, was added; the mixture was thermostated at 50°C for 1 h, after which 0.2 g of Pektofoetidin was added and the solution was frozen and was lyophilized; sample 1 was the control unhydrolyzed protein. Hydrolysates with the following times of proteolysis were obtained similarly: 15 min, sample 2; 30 min, 3; 1 h, 4; 2 h, 5; 3 h, 6; 4 h, 7; 5 h, 8; 6 h, 9; and 24 h, 10.

The degree of proteolysis was determined as described in [10], where the degree of proteolysis is the ratio of the number of hydrolyzed bonds to the total number of peptide bonds, expressed as a percentage.

The turbidimetric titration of solutions of the proteins (C_p = 0.3%) was carried out as described in [11].

Determination of the Solubility of the Hydrolysates at the IEP. Weighed samples of the cotton protein hydrolysates were suspended in distilled water and each suspension was stirred for 1 h (pH 4.5), after which the mixture was centrifuged at 6000 rpm for 10 min and the amount of protein in the supernatant liquid was determined by microbiuret method [12].

The emulsifying properties of solutions of the hydrolysates were investigated in a neutral medium (pH 7.2) by the method proposed by Gurov et al. [13].

The disk electrophoresis of the hydrolysates was performed in 15% PAG in Tris-glycine buffer (pH 8.3) on a Reanal instrument (Hungary) in the presence of a 2% solution of Na—DDA. The amount of hydrolysate deposited in one tube was 200 μg [14].

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ISOLATION, PURIFICATION, AND SOME PHYSICOCHEMICAL PROPERTIES OF GLUCOSE ISOMERASE FROM Streptomyces atratus

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A highly purified glucose isomerase with a specific activity 23.5 times greater than that of a homogenate of the mycelium has been obtained from Streptomyces atratus by methods of homogenization from the freeze-dried biomass, heat treatment (70°C, 10 min), ion-exchange chromatography, and gel filtration. The yield of enzyme on the initial biomass was 51.8%. The molecular mass of the enzyme has been determined by various methods as 160 kDa but in the presence of sodium dodecyl sulfate in thin-layer PAG it amounts to 40 kDa.

Glucose isomerase [xylose isomerase; D-xylose ketol-isomerase (EC 5.3.1.5)] – an enzyme catalyzing the isomerization of certain aldosugars and ketosugars, is attacting the attention of many researchers by the possibility of its use for the industrial production of glucose-fructose syrups and of crystalline fructose [1-3]. Among the numerous microorganisms of various taxonomic groups described in the literature as producers of glucose isomerases with a high activity and productivity, the streptomycetes stand out [4]. Glucose isomerases have been obtained in a highly purified state from various species of streptomycetes by very simple methods of purification [5-7]. The aim of the present work was to obtain a homogeneous glucose isomerase from a culture of Str. atratus, Uz GIT-1, and to study its physicochemical properties.

The glucose isomerase from Str. atratus is an intracellular enzyme localized in the periplasmic space and in the cell wall of the producing organism [8] and various approaches have therefore been used for its isolation (both with and without the disruption of the cells). The most effective method of isolating the enzyme proved to be homogenization from the freeze-dried biomass. We used this method in subsequent work. A homogenate of the mycelium was centrifuged at 8000g for 30 min, and then the precipitate was removed. The cell-free buffer extract obtained in this way did not contain a large amount of ballast proteins. Since glucose isomerase possesses thermal stability, the following stage of purification was heat treatment at 70°C for 10 min in the presence of Mg^{2+} and Co^{2+} ions. The product was freed from denatured proteins by centrifugation at 6000g for 10 min. After this, the enzyme solution was deposited on a column containing DEAE-cellulose that had previously been equilibrated with 0.05 M phosphate buffer, pH 7.8. The glucose isomerase was eluted at a concentration of 0.4 M NaCl in 0.1 M phosphate buffer at the rate of 60 ml/h (Fig. 1). This concentration of sodium chloride was established after the preliminary performance of stepwise and linear gradient elution. The fractions with enzymatic activity were collected and were concentrated in a dialysis bag against polyethyleneglycol at 4°C for 5 h. The concen-

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