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A Protein Isolate for Food by Phosphorylation of Yeast Homogenate

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ABSTRACT

A significant increase in protein extraction from Torula utilis *homogenate and substantial decrease in nucleic acid content (60%) in the resulting isolate was obtained by protein phosphorylation with sodium trimetaphosphate (STMP). Optimal conditions for this process were pH, 11·0; temperature, 38°C and concentration of STMP, 3%. This modification markedly improves such functional properties as solubility, emulsifying activity, foaming capacity and fat and water absorption capacity. The enzymatic digestibility is only lowered by about 10%.*

INTRODUCTION

Microorganisms, which are a potential source of valuable protein, have only been utilised in human nutrition on a small scale. To use them as food additives it is necessary to remove cell walls, separate the intracellular proteins in the form of an isolate, reduce the accompanying nucleic acids and obtain the required functional properties (Shetty & Kinsella, 1979).

In recent years a number of studies have been carried out to achieve that goal by chemical modification of microbial proteins (Lovland *et al.*, 1976;

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Schwenke & Rauschal, 1980; Shetty & Kinsella, 1982). According to some authors these techniques, despite their attractive character, demonstrate certain negative effects on the biological value of the protein (Proll & Schwenke, 1983; Shetty & Kinsella, 1982; Siu & Thompson, 1982).

According to Feeney & Whitaker (1977), among the known chemical modification techniques, acylation, alkylation, esterification and oxidation by various chemicals deserve special attention.

Shetty & Kinsella (1978) developed a modification procedure by treatment of disintegrated yeast cells with succinic anhydride and thiol compounds. Recently, Damodaran (1986) proposed a sulfitolysis technique by the application of sodium sulfite and sodium tetrathionate ($Na_2S_4O_6$). That technique seems to give interesting results since it improves protein properties but has no negative effect on the assimilation of the exogenous amino acids in the sulfonated proteins.

An interesting technique for modification and increasing protein extraction is the phosphorylation of yeast homogenates with $POCl_3$, reported by Damodaran & Kinsella (1982).

In this paper the phosphorylation procedure with sodium trimetaphosphate (STMP) was studied, which had been used earlier by Sung *et al.* (1983) in the modification of soy bean protein.

The modification, being carried out at high pH, may serve for the temporary protection of lysine ε-NH_2 groups against their participation in undesirable chemical reactions occurring in food (e.g. Maillard reactions). Because of reversibility of that reaction at pHs below 5 (precipitation of proteins takes place at pH = 4·2), it should cause no unfavourable changes in nutritional value.

The purpose of this paper was to study the various aspects of the modification procedure and to elaborate conditions necessary to obtain a finished product of optimum quality.

MATERIAL AND METHODS

Experiments were carried out on *Torula utilis* feed grade yeast (Fig. 1). The cyclic sodium trimetaphosphate (STMP) was used as a phosphorylation agent. It was obtained by roasting the monosodium orthophosphate (NaH_2PO_4), according to the Bell method (1950).

The degree of protein phosphorylation (DF) was expressed as the ratio of the quantity of pyrophosphate produced during the reaction to the quantity released theoretically on the basis of serine content in the protein, under the complete substitution of —OH groups. Pyrophosphate quantity was determined indirectly by complexometric titration of the bound Zn^{2+} ions

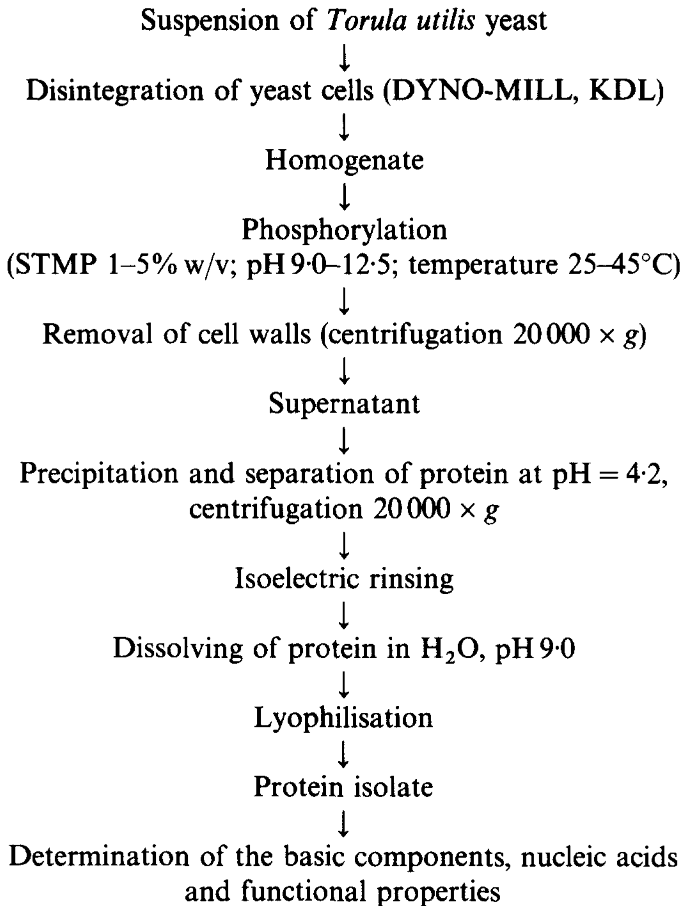

Fig. 1. Chemical flow diagram of yeast protein phosphorylation and isolation.

using 0·01 M EDTA against erichromate black as indicator (Flaschke *et al.*, 1969).

$$DF = \frac{X_p}{T_p} \times 100$$

in which DF is the degree of phosphorylation (%), X_p is the pyrophosphate quantity actually produced in the reaction mixture, and T_p is the theoretic pyrophosphate quantity calculated from serine content.

In the raw material and in the protein preparation after phosphorylation of the dry matter (temperature 105°C ± 2°C), crude protein (Kjeldahl method N × 6·25), nucleic acid content (colorimetric method of Herbert *et al.*, 1971) and threefold extraction with 0·5 N $HClO_4$ at 70°C (for 30 min) were

determined. The ribonucleic acid formed coloured complexes with orcine solution, while the desoxyribonucleic acid did so with diphenylamine.

The characteristics of the protein isolate were determined by functional properties such as:

—relative solubility (measurement of light absorption at 280 nm wavelength and pH from 1·0 to 12·0) (McElwain *et al.*, 1975),

—fat and water absorption capacity according to Sathe & Salunkhe (1981),

—emulsifying activity according to Schwenke (1981) by using the formula:

$$E_A = \frac{e_x 100}{t}$$

in which E_A is emulsifying activity (%), e_x is the volume of the emulsified layer (cm^3) and t is the total volume (cm^3),

—emulsion stability after heating at 80°C for 30 min. Results calculated with the above formula as E_S,

—foaming capacity and foam stability according to Puski (1975).

The enzymic digestibility *in vitro* was determined according to the classic Mauron method. After digestion with pepsin for 3 h and trypsin for 24 h the released amine nitrogen was determined colorimetrically by the ninhydrin method.

RESULTS AND DISCUSSION

Data in all figures and in the table are mean values from three replications.

In Fig. 2 the relationship between degree of yeast protein phosphorylation, STMP concentration and pH at two reaction temperatures (25° and 35°C) is presented.

As can be seen from Fig. 2, the degree of protein phosphorylation within the studied range increases with increase of pH and STMP concentration and for the same parameters is always higher at 35°C. High pH, which stimulates the phosphorylation reaction, might, however, diminish the nutritional properties of the isolate by stimulating the occurrence of antinutritional compounds (e.g. lysinoalanine). Similarly, the elevation of temperature not only increases the degree of phosphorylation (DF) but also contributes, together with the alkaline pH, to all the unfavourable changes in proteins such as: hydrolysis, depolymerisation, racemisation, etc.

The shape of the curves in Fig. 2 also indicates that at pH 9 to 10 a low degree of phosphorylation occurred, in the range 10 to 30%; by elevating pH

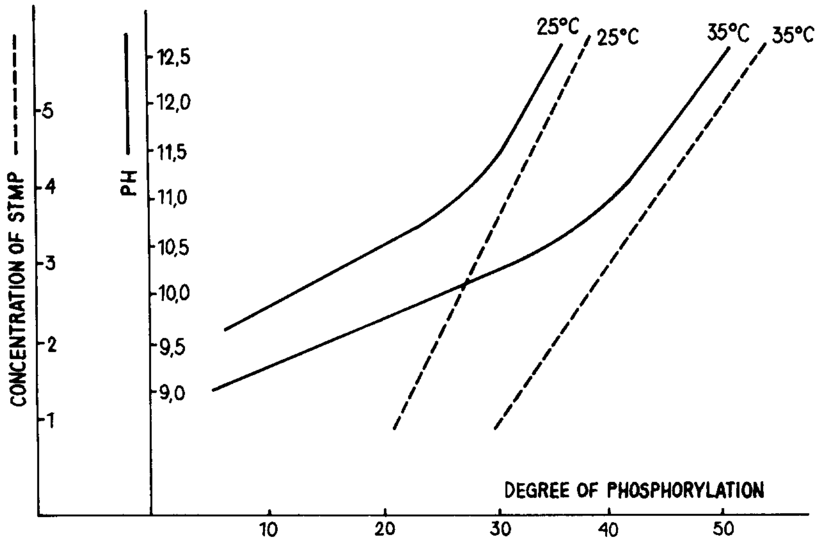

Fig. 2. Relationship between degree of phosphorylation (%), STMP concentration (%) and pH.

to 11·5 the DF increased to 45%. A further elevation had no effect on DF increase.

The effect of STMP concentration in the range 1 to 3% shows a nearly proportional relationship. Further increasing of STMP concentration to 5% is not desirable since the phosphorylating agent occurs in amounts excessive to the number of free OH and NH_2 groups.

The isolates obtained demonstrate quite high contents of crude protein, N × 6·25 (above 80% of dry matter) and of protein insoluble in 20% TCA (so called true protein); its content is above 75% of the dry matter. It can be seen that the phosphorylation procedure increases extractability and facilitates separation of proteins from nucleic acids. By increasing the degree of phosphorylation above 20% only an increase in the protein extractability has been observed.

The control sample protein isolates, obtained by alkaline extraction, has considerably lower crude protein and true protein contents and a higher content of nucleic acids. This resulted from the mild conditions of the alkaline extraction in which the hydrolysis of the nucleic acids is not complete.

Changes of the negative charge on the protein, resulting from phosphorylation or succinylation, make a considerable reduction of the nucleic acid content possible, as shown in Table 1.

The experimental findings indicate that the degree of nucleic acid reduction is proportional to the degree of protein phosphorylation.

The isoelectric point of the phosphorylated yeast protein isolate is at pH

TABLE 1
Effect of the Degree of Protein Phosphorylation on the Composition, Functional Properties and Enzymic Digestibility of the Preparation

Degree of phosphorylation	*Crude protein in % of dry matter (N × 6·25)*	*True protein in % of dry matter (N × 6·25)*	*Nucleic acids in % of dry matter (total)*	*Enzymic digestibility (%)*	*Functional properties*				
					Solubility at pH 7·0	*Emulsifying activity*	*Foaming capacity*	*Fat absorption capacity*	*Water absorption capacity*
Alkaline extraction									
0	80·2	66·6	4·22	54·9	0·5	72·0	55	4·93	4·71
35	81·16	75·6	2·29	51·1	0·6	80·4	70	4·94	4·72
40	81·12	76·5	1·77	50·5	0·65	82·5	75	4·99	4·94
45	82·10	77·2	1·70	50·5	0·72	75·5	85	4·94	4·99
50	81·37	74·7	2·08	50·5	0·82	80·0	72	4·90	4·94
55	82·95	77·9	1·64	49·4	0·80	83·5	90	5·11	5·0

3·5, whereas the greatest precipitation of the nucleic acids is observed at pH 6·4–4·5.

Introduction of the orthophosphoric radical to the protein molecule decreases the nucleophilic properties of the amino acid functional groups in the yeast proteins and in this way the destabilization of nucleoprotein complex is increased.

As shown earlier, the reduction of nucleic acid content in the yeast protein isolates by chemical phosphorylation is more advantageous when compared with other methods used to reduce the nucleic acid content (Fig. 3). One of the most significant factors affecting the application of modified yeast proteins in food is their susceptibility to enzymic digestion.

The degree of hydrolysis was expressed as the ratio of the quantity of α-amino nitrogen produced after enzymic hydrolysis to its quantity after acidic hydrolysis.

In principle, phosphorylation does not reduce the enzymic digestibility of protein when compared with that of protein extracted from the biomass under alkaline conditions. However, the reduction of digestibility becomes more evident with increasing degree of phosphorylation. Compared with the control sample, phosphorylation decreases enzymic digestibility of protein by 10% (Table 1).

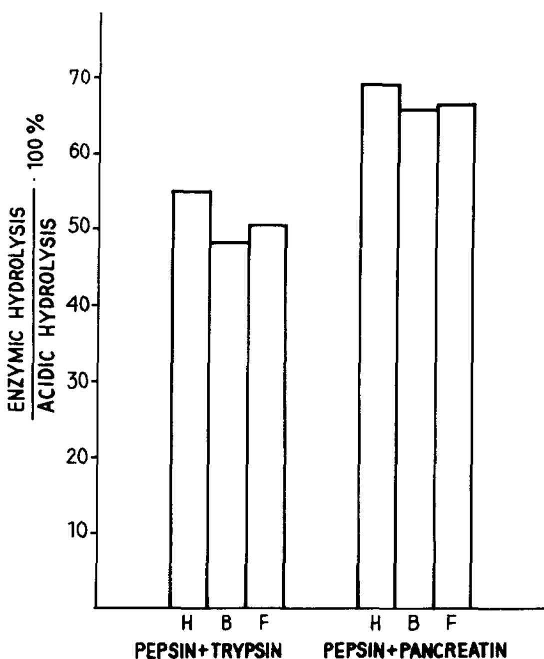

Fig. 3. Comparison of enzymic digestibility of yeast protein homogenates under the application of two enzyme systems.

Figure 3 presents the comparative enzymic digestibility *in vitro* of three yeast protein preparations; namely, the succinylated (B), the phosphorylated (F) and that obtained after alkaline extraction and used as the control sample (H).

The minor reduction of digestibility of the phosphorylated protein may be caused by various factors. First of all, the high pH decreases the nutritive value. Moreover, as has been found in the experiments not yet published, despite the reversibility of phosphorylation of the lysine ε-NH_2 groups at pH below 5, certain numbers of the orthophosphoric radicals remain in the protein, thus limiting the access of trypsin to lysine.

Reduction of the enzymic digestibility in the phosphorylated protein is difficult to explain. It may be associated with the changes discussed above, which occur in protein in the alkaline medium. Technological advantages of the chemically modified protein have to be checked taking into account its biological value. It can be best tested on animals, and such tests are to be carried out in the subsequent stages of this study.

The chemical modification which results in minor structural changes in the protein molecule can improve certain functional properties which are of importance to the food additives. This is not the case in the yeast protein isolates obtained by the conventional method, i.e. by isoelectric or alcoholic precipitation.

Phosphorylation decreases, in the protein molecule, the number of positive charges, thus increasing the number of negative ones.

Phosphoric group addition alters the electrostatic forces between polypeptide chains and results in structural changes which may alter functional properties in the modified proteins.

As can be seen in Table 1, protein phosphorylation had no apparent effect on fat absorption capacity. However, a significant improvement was observed in the other functional properties examined, i.e. the emulsifying and foaming capacities. That improvement was proportional to the number of phosphorylated radicals in serine and lysine.

CONCLUSIONS

The occurrence of typical phosphoproteids containing mainly orthophosphoserine radicals is known in nature, e.g. milk casein, egg white ovalbumin, egg yolk phosvitine (Taborsky, 1974).

Enzymic phosphorylation of protein with phosphoprotein kinase or transferase has also been reported. However, those methods have not been used on a large scale due to high costs of the ATP and enzymes (Bingham, 1968).

The usage of STMP as phosphorylation agent is economically justified, and the yeast protein converted into phosphoproteids may be used as a protein additive to food products. STMP has been considered as the least toxic and least hazardous to the human organism, compared with the other polyphosphates (Ellinger, 1972).

In this study a phosphorylation procedure of yeast protein was developed and modification of that type demonstrates the following features:

(1) Introduction of phosphoric acid radicals into the protein molecule to replace the —OH groups of serine and partially the ε-NH_2 groups of lysine reduces the number of cation radicals and thus destabilizes the nucleoproteid complexes. In this way the protein yield can be increased and the content of the nucleic acids in the isolate markedly reduced.

(2) The degree of yeast protein phosphorylation is dependent on temperature, pH and STMP concentration. The optimum parameters of the procedure are as follows:

temperature	~35°C
pH	11·0
STMP concentration	3·0% w/v

(3) Phosphorylation of homogenates at the level of 50% decreases the nucleic acid content in the protein isolated from 4·22% to 1·64%, markedly improves the functional properties of protein, and slightly decreases its enzymic digestibility.

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