ever, the apparent protein digestibility of 77.1% for PKPC compares favorably with those earlier reported for most conventional urorthodox Nigerian plant protein sources (Fetuga, 1972). It is envisaged that adequate heat treatment of the protein concentrate from PKM might inactivate any residual antinutritional factors present in the protein concentrate, improve the digestibility of its protein, and enhance a better utilization of the protein by monogastric animals. A comprehensive evaluation of the nutritive potential of the protein isolate from PKM is currently being investigated.

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Isolation of Yeast Protein with Reduced Nucleic Acid Level Using Reversible Acylating Reagents: Some Properties of the Isolated Protein

Jay K. Shetty and John E. Kinsella*

Yeast proteins isolated by isoelectric precipitation, by alcohol precipitation, or by ammonium sulfate precipitation contained high levels of contaminant nucleic acids (>22 g of nucleic acid/100 g of protein). Gel filtration of these yeast proteins on a Sepharose 6B column indicated that yeast proteins and nucleic acids were present as a nucleoprotein complex. Chemical modification of the ϵ-NH_2 group of lysine in the yeast proteins by maleic or citraconic anhydride destabilized the nucleoprotein complex and facilitated the separation of proteins and nucleic acids at pH 4.0–4.2. Subsequent incubation of the maleylated or citraconylated yeast proteins resulted in deacylation of the modifying groups. Some functional properties and the in vitro digestibility of the yeast proteins isolated by this method are reported.

In previous papers the problem of nucleic acids contamination in yeast proteins was reviewed, and the preparation of yeast protein isolate with a reduced nucleic acid level by succinylation of the ϵ-NH_2 group of lysine residues in the yeast protein was described (Kinsella and Shetty, 1979; Shetty and Kinsella, 1979a,b). This method offered some advantages over conventional methods for reducing nucleic acid levels in the yeast protein isolate. These include enhanced extraction of proteins, inhibition of proteolytic enzymes during the isolation, and improvement in the functional properties: i.e., solubility, emulsification, and foaming properties of the isolated proteins (Kinsella and Shetty, 1979). The disadvantages of the succinylation procedure is that the final product is succinylated protein. Because the succinyl group cannot be removed from the succinylated proteins under mild conditions (Means and Feeney, 1971), it is unlikely that succinylated yeast protein can be used as a practical source of dietary protein. However, we have demonstrated the feasibility of using a reversible modifying agent, citraconic anhydride, with β-lactoglobulin and soy protein and determined optimum conditions for deacylation while minimizing alteration of the protein (Brinegar and Kinsella, 1980, 1981). Palacian et al. (1981) used dimethylmaleic anhydride for dissociation of protein components from chromatin. In this paper, we report the successful use of reversible modifying reagents (maleic and citraconic anhydride) to isolate yeast proteins with low nucleic acid levels. Optimum conditions for removal of modifying groups were determined; some functional properties and the in vitro digestibility of the protein are described.

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EXPERIMENTAL SECTION

Materials. Brewer's yeast (*Saccharomyces carlsbergensis*) was obtained from Genesee Brewing Co., Rochester, NY, washed 3 times with distilled water, and freeze-dried. Bovine serum albumin (Cohn fraction V), pancreatin trichloroacetic acid, and orcinol were purchased from Sigma Chemical Co. (St. Louis, MO). Soy protein isolate was obtained from Ralston Purina Co. (St. Louis, MO), and 11S and 7S soy protein fractions were isolated according to the method described by Thanh and Shibasaki (1976). 2,4,6-Trinitrobenzenesulfonic acid (TNBS) and maleic and citraconic anhydride were purchased from Eastman Kodak Co. (Rochester, NY). Pepsin was obtained from Nutritional Biochemicals Corp. (Cleveland, OH). All other reagents were of reagent grade. Doubly distilled deionized water was used in the preparation of all solutions.

Methods. *Alkaline Extraction of Proteins from Disrupted Yeast Cells.* Yeast cells were disrupted according to the procedure described earlier (Shetty and Kinsella,

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1979a,b) using a Braun mechanical cell homogenizer (Melsung, West Germany). The pH of the disrupted yeast cell suspension (5% concentration, w/v) was increased to 9.5 by the addition of 1 N NaOH, and the suspension was stirred for 45 min and centrifuged at 28000*g* for 45 min at 5 °C. The clear supernatant (yeast extract) was recovered. The protein was then precipitated from this supernatant by three separate methods: i.e., by isoelectric precipitation at pH 4.5; by the addition of solid ammonium sulfate to full saturation; by precipitation with cold ethanol to a final concentration of 90% (ethanol–water, v/v). The precipitated protein was separated in each case by centrifugation, dissolved in a minimum amount of water (pH adjusted to 8.5), dialyzed extensively against deionized distilled water for 36 h at 5 °C, and then lyophilized (Shetty and Kinsella, 1979a,b).

Gel Filtration of Yeast Protein on a Sepharose 6B Column. Sepharose 6B (Pharmacia Chemical Co., Upsala, Sweden) was equilibrated with glycine–NaOH (0.01 M) buffer, pH 9.5, containing 0.1 M NaCl and packed into a column (110 × 3.2 cm). The column was then washed with the same buffer (3–4 times the bed volume). Protein was extracted from the mechanically disrupted yeast cell at pH 9.5 and separated from the cell debris by centrifugation. The clear supernatant was placed in a dialysis bag and dialyzed against glycine–NaOH buffer, pH 9.5 (0.01 M), containing 0.1 M NaCl for 24 h at 5 °C. The dialyzed protein was placed on the column and eluted with the same buffer (15 mL). Aliquots of the eluate were collected, and the absorbance of the fractions were measured at 260 and 280 nm. The gel filtration experiment was carried out at 5 °C.

Derivatization Using Maleic and Citraconic Anhydrides. The proteins, extracted from the yeast, at pH 9.5, were modified as described (Brinegar and Kinsella, 1980). Pure citraconic or maleic anhydride was added in small increments to a 1% yeast protein extract with continuous stirring over a period of 90 min. During the derivatization, the pH was maintained between 7.8 and 8.5 by the frequent addition of 3.5 N NaOH. After stabilization of the pH, i.e. following complete derivatization of the ϵ-NH_2 groups, HCl (3 N) was added dropwise to decrease the pH to 4.2. The precipitated protein was separated by centrifugation, dissolved in water (pH adjusted to 8.5), dialyzed against water (pH 8.5) for 26 h at 5 °C, and then lyophilized. Different degrees of derivatization of yeast protein was achieved by using varying concentrations of maleic anhydride and citraconic anhydride at 25 °C as described (Brinegar and Kinsella, 1980).

Removal of Modifying Groups from the Modified Protein. By use of conditions similar to those established for soy proteins (Brinegar and Kinsella, 1980), the modified proteins were deacylated. A 1% (w/v) suspension of maleylated or citraconylated yeast protein isolate (>90% ϵ-NH_2 groups modified) was dispersed in water. The pH was adjusted to 5.0, 4.0, and 3.0, and the mixture was then incubated at 30 °C for different intervals of time to determine the optimum pH for removal of the modifying group. After a specified time, the pH of the suspension was increased to 8.5, the mixture was dialyzed against water (pH adjusted to 8.5) for 36 h at 5 °C to remove the liberated acid, and the deacylated protein was then lyophilized for subsequent analyses.

In Vitro Digestibility Studies. (1) Pepsin. The peptic digestibility of the citraconylated and decitraconylated yeast proteins was determined. Soy proteins and egg albumin were also studied for comparison. In a typical experiment, protein (1% concentration) was dissolved in HCl–KCl buffer (0.01 M), pH 1.6, and pepsin was added at a ratio of 1:500 (w/w) enzyme to protein and the mixture was incubated at 37 °C. The rate of hydrolysis was then determined by withdrawing a known volume (5 mL) of the digested sample, and undigested protein was precipitated by the addition of 20% Cl_3AcOH (5 mL). Cl_3AcOH-soluble nitrogen was determined by the micro-Kjeldahl procedure (Association of Official Agricultural Chemists, 1975). The degree of hydrolysis was indicated by the quantity of Cl_3AcOH-soluble nitrogen released.

(2) Pancreatin. Pancreatin contains various proteolytic enzymes; the action of the proteases on citraconylated proteins was studied by using the procedure described by Lynch et al. (1977) using a Radiometer pH stat TTT-2 (London Co., Cleveland, OH). In a typical run, 4.9 mL of protein solution (containing 3.0 mg of protein/mL) was adjusted to pH 8.0 with 0.1 N NaOH. Pancreatin (0.12 mg) was added, and proteolysis was then allowed to proceed at 37 °C until no further uptake of base (0.125 N NaOH) was observed.

Determination of Functional Properties. (1) Nitrogen Solubility. Dispersions containing yeast proteins (obtained by isoelectric precipitation), citraconylated yeast proteins, and decitraconylated yeast proteins were made in water (1% by weight), and the pH of the suspensions was adjusted to pH 4, 5, 6, and 7 by the addition of 0.2 N HCl or NaOH. They were then stirred for 1 h at 25 °C. When the suspensions were stirred, the pH was maintained by adding the appropriate acid or alkali. The suspensions were centrifuged at 20000*g* for 30 min at 5 °C. The soluble nitrogen in the supernatant was determined by standard micro-Kjeldahl procedure.

(2) Emulsifying Activity. Emulsifying activity of decitraconylated yeast protein isolate and other food proteins was measured according to the procedure described by Waniska et al. (1981). Emulsions were prepared with 20 mL of protein solution (0.50%) in phosphate buffer, pH 7.0 (0.1 M), and 10 mL of peanut oil and homogenized with a Janke-Kunkel blender for 30 s at 15 °C. The resultant emulsion was then diluted 1000-fold with phosphate buffer, pH 7.0 (0.1 M), containing 0.05% sodium dodecyl sulfate to stabilize the dispersed oil droplets. The optical density of the diluted emulsions was measured at 550 nm with a Bausch & Lomb Spectronic 700 spectrophotometer.

(3) Foaming Capacity. The foaming capacity of decitraconylated yeast proteins was determined by using the method of Waniska and Kinsella (1979). The protein was dissolved in phosphate buffer, pH 7.0 (0.1 M), to give a final concentration of 0.1% (w/v). Then the protein solution (15 mL) was injected into the sparging chamber of the foaming apparatus. Nitrogen gas was sparged into the protein solution until the foam chamber was filled with foam (60 mL) while simultaneously maintaining the volume of the liquid in the sparging chamber by continuously injecting protein solution. The time taken to form 60 mL of foam was recorded in each case, and the foam strength was then determined (Waniska and Kinsella, 1979).

Analytical Methods. *Lysine Determination.* The extent of modification of yeast proteins by maleic and citraconic anhydride was measured by determining the free ϵ-NH_2 group of the lysine residues by using 2,4,6-trinitrobenzenesulfonic acid as described by Hall et al. (1973). A known amount of protein was dispersed in 0.8 mL of water (pH 9.2) and 1 mL of 4% $NaHCO_3$ was added. Then 0.2 mL of 2,4,6-trinitrobenzenesulfonic acid (1.25% in water) was added and the mixture incubated at 40 °C for 2 h. The reaction was stopped by adding 3.5 mL of concentrated HCl. The reaction tubes were stoppered, kept

Table I. Nucleic Acid Content in Yeast Proteins Isolated by Different Methods

treatment	nucleic acid, g/100 g of protein	amount of yeast protein precipitated, %
alkaline extract (pH 9.5) of mechanically disrupted yeast and dialyzed	25.50	
isoelectric precipitation (pH 4.5)	16.80	80.0
ammonium sulfate precipitation (full saturation)	17.0	88.9
alcohol precipitation (>90% concentration)	29.50	95.0
reversible modification of lysine using acid anhydride	2.20	84.0

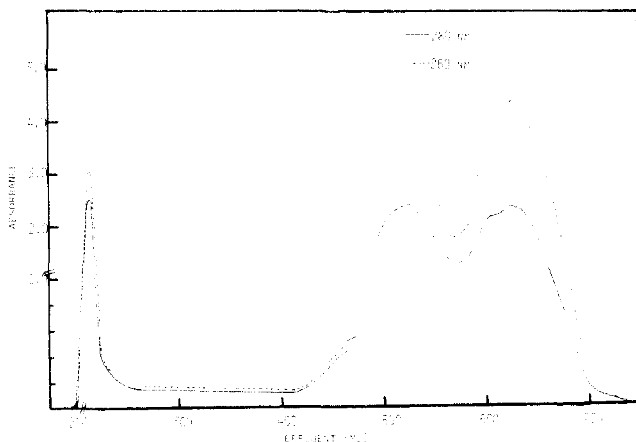


Figure 1. Gel filtration chromatography pattern on the Sepharose 6B column. Total proteins extracted at pH 9.5 from mechanically disrupted yeast cells.

in an oven maintained at 110 °C for 4 h, and cooled to 10 °C, the volume of the solution was made up to 10 mL with distilled water, and the mixture was then extracted twice with anhydrous diethyl ether (15 mL). Residual ether was evaporated by keeping the tubes in a water bath maintained at 50 °C for 10–15 min. The absorbance of the aqueous solution was measured at 415 nm. Appropriate blanks and standards were concurrently run.

Nucleic Acid. The Orcinol method described by Herbert et al. (1971) was used to determine the amount of nucleic acid associated with the yeast protein precipitated at pH 4.2 following different levels of modification with maleic or citraconic anhydrides. Protein (10–50 mg) was suspended in 0.5 M HCl (10 mL) and incubated at 50 °C for 2 h with constant stirring. The solution was then filtered. An aliquot (0.5 mL) was taken for the determination of ribose. The percent nucleic acid was calculated from the standard graph obtained by using purified yeast RNA (Herberts et al., 1971).

RESULTS AND DISCUSSION

The yeast proteins prepared from alkaline (pH 9.5) extracts of mechanically disrupted yeast cell by isoelectric precipitation at pH 4.5, by the addition of ethanol, or by ammonium sulfate yielded proteins with a high content of contaminating nucleic acids (Table I). The elution pattern of alkaline extracted yeast proteins from the Sepharose 6B column showed greater absorbance at 260 nm compared to that at 280 nm in all the fractions, indicating that nucleic acids and proteins were present as nucleoprotein complexes in the disrupted yeast (Figure 1).

The complexation of the protein and nucleic acid may have occurred during disruption of cell wall and/or the extraction procedure. Nucleoprotein complexes are stabilized by weak noncovalent forces such as electrostatic interactions, hydrophobic interactions, and hydrogen bonding (Spitnik-Elson and Elson, 1976). Ionic linkages occur between the anionic phosphate groups of the nucleic acids and the cationic groups of the basic amino acids of the proteins, and hydrophobic associations occur between hydrophobic regions of the protein and bases of the nucleic acids. Because of the large number of such weak associations, the complex is quite stable under the mild conditions used to isolate the proteins. Thus, purification of proteins and/or enzymes from yeast is complicated because of the interference from and contamination by the nucleic acids. However, modification of the ϵ-NH_2 groups of the lysine residues in the yeast proteins using succinic anhydride destabilizes the nucleoprotein complex and facilitates the separation of nucleic acids from yeast proteins at pH 4.2 (Shetty and Kinsella, 1979a,b). Succinylation of yeast proteins during extraction reduced the number of cationic residues and greatly enhanced electrostatic repulsion between the added carboxylic groups and the anionic phosphate groups of the nucleic acids. This probably also caused disruption of the hydrophobic associations between the protein and nucleic acids and resulted in dissociation of nucleic acid from the proteins. However, the succinyl groups cannot be easily removed from the succinulated protein under mild conditions (Means and Feeney, 1971); hence, the bioavailability of lysine from the succinylated protein is greatly reduced (Bjarnason and Carpenter, 1969; Groninger and Miller, 1979). Therefore, removal of modifying groups from the proteins after separating the nucleic acids is essential in proteins intended for food uses. Therefore, we explored the possibility of using reversible acylating agents.

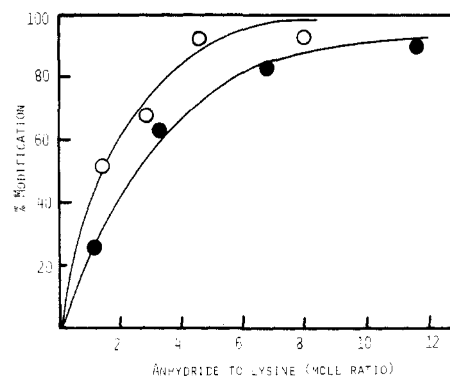


Figure 2. Effect of cyclic acid anhydride concentration on the modification of ϵ-NH_2 groups in the yeast proteins: (○) maleic anhydride; (●) citraconic anhydride.

Reversible Modification of Yeast Proteins Using Citraconic and Maleic Anhydride. The modification of ϵ-NH_2 group of lysine residues in the yeast proteins with maleic and citraconic anhydride increased with increasing concentration of anhydrides (Figure 2). However, the extent of lysine modification was greater with maleic anhydride compared to that of citraconic anhydride for the same molar ratio of anhydride to lysine. No comparative data are available concerning the relative rates of acylation by different anhydrides. Conceivably, the methylation of the maleic anhydride decreased the reaction rate due to steric hindrance. The methyl group is an electron-donating substituent; hence, its contribution to the electron density at the carbonyl carbon may have decreased the electrophilicity of the carbonyl carbons in citraconic anhydride.

The nucleic acid content of the protein precipitated at pH 4.2 following modification of the proteins to varying degrees by these anhydrides was progressively decreased

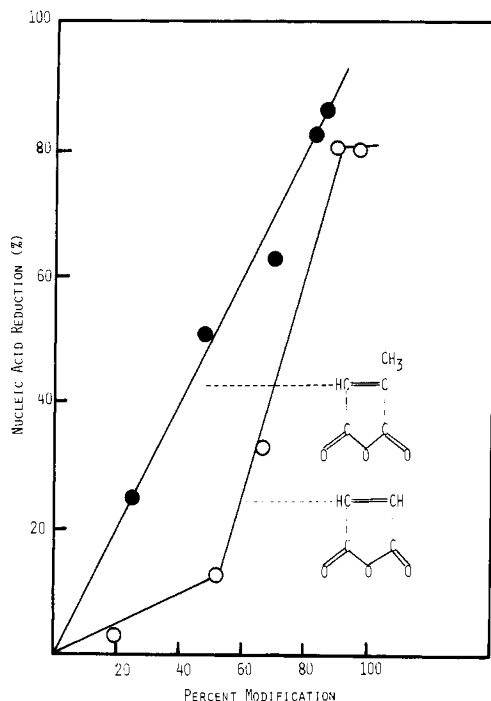


Figure 3. Effect of ϵ-NH_2 group modification on the reduction of nucleic acid content in the yeast protein precipitated at pH 4.2: (○) maleic anhydride; (●) citraconic anhydride.

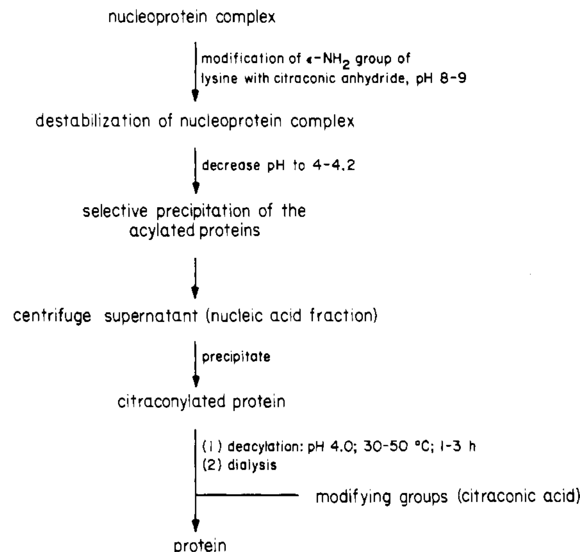
Table II. Conditions for Optimal Deacylation of Citraconylated Yeast Proteins at 30 °C

treatment time, h	% of ϵ-NH_2 groups regenerated at 3.0	4.0	5.0
0.5	48.0	50.0	
1.0	68.0	60.0	60
2.0	95.5	96.0	78
4.0	100	98	90
6.0	100	100	98

(Figure 3). The extent of nucleic acid reduction in the yeast proteins precipitated at pH 4.2 was proportional to the number of lysine residues modified with citraconic anhydride. The nucleic acids were more effectively separated from the citraconylated yeast proteins compared to the maleylated yeast proteins. At 50% protein modification, maleylation and citraconylation resulted in 12% and 50% reduction in the nucleic acid, respectively. Thus, while the introduction of a methyl group into maleic anhydride to give citraconic anhydride decreased the electrophilicity of the anhydride, it enhanced the destabilization of the nucleoprotein complex and facilitated the separation of nucleic acid, making it a preferable acylating agent.

Removal of Modifying Groups from the Modified Yeast Proteins. The isopeptide bond formed between ϵ-NH_2 and acid anhydrides containing a cis double bond is acid labile (Singhal and Atassi, 1971; Butler et al., 1967; Dixon and Perham, 1968; Kirby et al., 1974; Aldersley et al., 1974). Thus, the maleylated and citraconylated yeast proteins (>90% modified) were incubated at different acidic pHs for different intervals of time at 30 °C. Complete regeneration of ϵ-NH_2 groups from maleylated yeast proteins was not obtained (Shetty and Kinsella, 1980). However, in case of citraconylated yeast proteins almost complete deacylation occurred within 4 h at pH 3.0 and 4.0 at 30 °C (Table II). The rate of deacylation of citraconylated yeast proteins increased with increasing temperatures (Shetty and Kinsella, 1980). At 50 °C complete deacylation occurred within 30 min of incubation at pH 4.0. Prolonged incubation was necessary for removal of citraconyl groups at lower temperatures.

Scheme I. Scheme Showing the Separation of Proteins and Nucleic Acids from a Nucleoprotein Complex Using Reversible Modifying Reagents of Free Amino Groups in the Proteins



Several mechanisms have been proposed for reversible deacylation (Means and Feeney, 1971; Kirby et al., 1974; Aldersley et al., 1974). Deacylation may possibly be enhanced by weakening of the amide bond resulting from the decreased positive charge at the amide carbonyl carbon induced by the electron-rich double bond (Butler et al., 1967; Butler and Hartley, 1972; Brinegar and Kinsella, 1981). Means and Feeney (1971) suggested a mechanism by which deacylation is initiated by electron withdrawal from the carbonyl group by hydrogen bonding with the free proton. The decreased partial positive charge at the carbonyl carbon would then facilitate nucleophilic attack by the ionized carboxyl oxygen. Although the electronic and geometric effects of the cis double bond of the various derivatives allows the deacylation process to occur, the degree of methylation at the unsaturated carbons has a profound effect on the rate of deacylation (Aldersley et al., 1974). This was further confirmed in the present study by the increased rate of deacylation of citraconylated yeast proteins compared to that of maleylated yeast proteins.

During the deacylation of the citraconylated yeast proteins we consistently observed a decrease in the pH of the system in the pH range between pHs 3.0 and 4.5, whereas above pH 5.0 no significant change in the pH was observed. During deacylation the pH was maintained by the addition of alkali. the maximum amount of alkali was consumed at pH 4.0. The pH was not altered when acetate buffer of pH 4.0 was used during decitraconylation. On the basis of the experimental data an integrated procedure for separation of proteins and nucleic acids from yeast is shown (Scheme I).

Functional Properties. The solubility of isoelectrically precipitated yeast nucleoprotein, citraconylated and decitraconylated yeast protein isolate at pHs 4, 5, 6, and 7, was determined. Citraconylation significantly improved the solubility of yeast protein above pH 5.0 (Figure 4). The presumably was due to the increased hydration of the additional carboxylic acid groups and the loosened structure of the derivatized protein. Removal of the citraconic acid residues resulted in decreased solubility between pH 5.0 and pH 7.0. This obviously reflected the loss of the charged citraconic acid residues and probably some denaturation caused by the acidic conditions. However, the

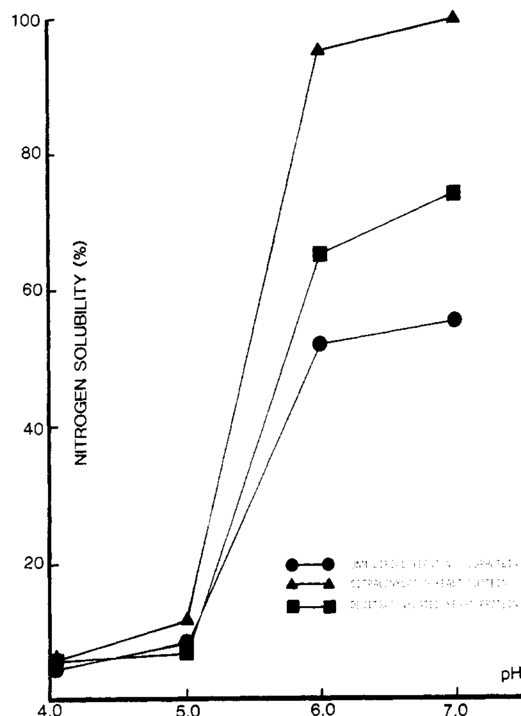


Figure 4. Solubiltiy of unmodified, citraconylated, and decitraconylated yeast proteins at various pHs (see Methods for details).

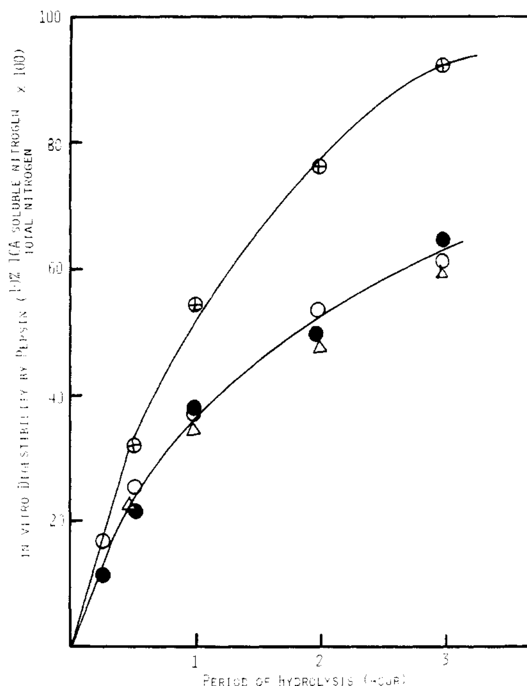


Figure 5. Rate of hydrolysis of citraconylated and decitraconylated yeast proteins by pepsin: (⊕) egg albumin; (○) soy proteins; (●) decitraconylated yeast proteins; (△) citraconylated yeast proteins.

Table III. Comparison of the Emulsifying Activities and Foaming Properties of Decitraconylated Yeast Protein Isolate with Other Food-Grade Proteins

protein	emulsifying act. (optical density at 550 nm)	foamability and foam strength: foam time, s	foam strength, cm/s
bovine serum albumin	0.89	409 ± 4	0.046 ± 0.003
egg albumin	0.62	399 ± 40	0.050 ± 0.001
soy 11S fraction	0.42	475 ± 21	0.064 ± 0.001
soy 7S fraction	0.54	438 ± 28	0.045 ± 0.005
decitraconylated yeast protein isolate	0.37	415 ± 18	0.107 ± 0.005

decitraconylated protein was significantly more soluble than the unmodified nucleoprotein complex, suggesting that the contaminating nucleic acid reduced hydration and solubility, particularly at pH 6.0 and above.

The emulsifying and foaming activities of decitraconylated yeast protein isolates were measured and compared with those of other food proteins (Table III). The emulsifying activity of decitraconylated yeast protein was much lower than that of bovine serum albumin and the other proteins tested under identical conditions. The data suggest that the decitraconylated yeast protein did not easily unfold at the oil–water interface when compared to the bovine serum albumin or egg albumin (Kinsella, 1982). The soy 11 S, a compact, extensively disulfide cross-linked protein, also showed limited emulsifying capacity in this study.

The increased time required to form 60 mL of foam probably reflects the slower rates at which the soy 11 S and decitraconylated yeast protein unfolded to form a cohesive film around the air bubbles and probably indicates the more stable tertiary structure of these proteins, especially compared to egg albumin (Kinsella, 1981; Phillips, 1981; Waniska and Kinsella, 1979). This was consistent with the greater strength of the foam formed from these proteins, probably reflecting the formation of a thicker and stronger protein film by decitraconylated yeast and soy 11 S protein.

Peptic Digestion. Because acidic conditions caused the removal of citraconyl groups from the citraconylated yeast proteins it is conceivable that the conditions of the stomach, i.e, low pH and temperature, may cause deacylation following consumption of citraconylated proteins. This would then make lysine nutritionally available. Therefore, hydrolysis of citraconylated and decitraconylated yeast proteins by pepsin was determined. Soy proteins and egg albumin were studied for comparison. The rate of hydrolysis of egg albumin by pepsin exceeded that of soy proteins and decitraconylated yeast proteins (Figure 5). More than 90% egg albumin was hydrolyzed within 3 h of incubation, whereas only 60% of the soy proteins and decitraconylated yeast proteins were hydrolyzed under the same conditions. To check whether free citraconic acid has any inhibitory effect on pepsin activity, we measured the rate of hydrolysis of egg albumin by pepsin in the presence of added citraconic acid (0.01 and 0.5 M). Addition of citraconic acid had no effect on the pepsin activity. No differences were observed either in the rate or in the extent of hydrolysis of yeast protein compared to that of decitraconylated yeast proteins.

Pancreatic Digestibility. Since pancreatin contains various proteolytic enzymes, the action of these on citraconylated and decitraconylated yeast proteins was measured. In figure 6A, the rate of hydrolysis of yeast proteins by pancreatin is shown. Citraconylated yeast proteins and yeast proteins obtained by isoelectric precipitation (control) were hydrolyzed at identical rates and to the same extent. Decitraconylated yeast protein that had been subsequently extensively dialyzed against water to remove free citraconic acid was hydrolyzed at a slightly lower rate. Citraconylated yeast protein that was subjected to deacylation without subsequent dialysis to remove liberated citraconic acid was hydrolyzed to a lower extent. This indicated that free citraconic acid inhibited one or more

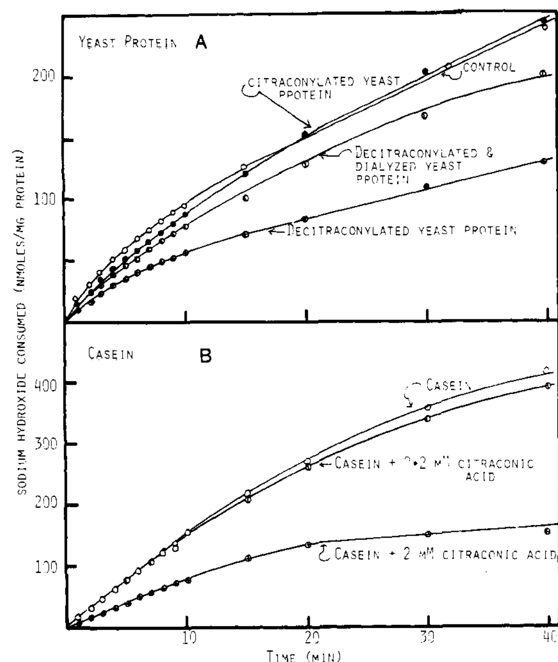


Figure 6. (A) Relative rates of hydrolysis of various yeast protein preparations by pancreatin. (B) Inhibitory effect of citraconic acid on hydrolysis of casein by pancreatin.

proteolytic enzymes present in the pancreatin. This was confirmed by showing that citraconic acid significantly reduced the hydrolysis of casein (Figure 6B) under identical conditions.

The increased pancreatic hydrolysis of citraconylated yeast proteins compared to that of the dialyzed decitraconylated yeast proteins might be due to the increased charge repulsion on the citraconylated yeast proteins. The additional negative charges on the citraconylated yeast proteins caused a greater dispersion of the protein that resulted in a "looser structure", which increased the susceptibility of the protein to pancreatic enzymes.

In conclusion, reversible modification of lysine residues using citraconic anhydride facilitated the separation of nucleic acids from yeast proteins, and the isolated protein exhibited good functional properties. However, more thorough nutritional studies and safety evaluation of the isolated protein need to be conducted before decitraconylated yeast proteins are used in foods. More recent in vivo studies have shown that decitraconylated yeast protein is a good biological source of nitrogen for fish (Kinsella et al., unpublished data, 1982).

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