The results from this study clearly show in many cases predetermined concentration of test compounds can improve the protein yields of processes to recycle organic wastes when the wastes are used as substrates to produce microbial protein. These or other agents may also enhance protein yields in on-going fermentation processes that utilize hydrocarbons as feed.

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Extraction of Protein, Low in Nucleic Acid, from *Saccharomyces fragilis* Grown Continuously on Crude Lactose

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Conditions for the maximum recovery of protein with a low nucleic acid content from yeast *Saccharomyces fragilis* using sodium hydroxide (0.4%), sodium chloride (4%), or water were established. Homogenization of a 5% cell suspension in NaOH (0.4%) in the presence of glass beads gave maximum extraction, *i.e.* 83% of Kjeldahl protein compared to 55% when water was used. Maximum yield of protein was obtained by acid precipitation (pH 4.3) of the alkaline extract, *i.e.* 88% *vs.* 86% from the aqueous extract. Total cell protein recoveries were 65 and 26% for alkaline and aqueous extracts, respectively. The yeast protein isolates contained >83% protein and less than 5% nucleic acids. Negligible amounts of nucleic acid occurred in yeast protein precipitated from a water extract at pH 6.0 and 80°.

The resistance of cell walls to rupture or digestion and the presence of nucleic acids are two major problems impeding the large scale use of single-cell protein (SCP) in food products. The indigestibility of the cell wall limits the availability of cell components thereby reducing the nutritional value of intact yeast cells (Tannenbaum *et al.,* 1966; Tannenbaum and Miller, 1967). The amino acid composition of cell wall is unbalanced, lacking sulfur and aromatic amino acids (Enebo, 1968; Ikawa and Snell, 1956; Salton, 1964). In order to improve nutritional value, the cytoplasmic protein must initially be extracted from microbial cells and then concentrated (Hedenskog and Ebbinghaus, 1972).

Several attempts were made for the direct extraction of protein from intact microbial cells (Mitsuda *et al.,* 1969; Mitsuda *et al.,* 1971; British Petroleum Co., 1970; Kyowa Hakko Kgyo, 1966). With intact cells an extensive chemical treatment or physical rupture is necessary to render the cell contents available to the extractant (Curran and Evans, 1942). When cells are first disintegrated, cell components can be extracted using mild chemicals, *i.e.,* water, dilute salts, and alkaline solutions.

Many have suggested the use of enzymes to digest the cell wall of yeast (Carenburg and Haden, 1970; Monreal and Reese, 1968; Sugimoto and Yokotsuka, 1968; Yamamoto *et al.,* 1972). However, these methods are limited because of extensive lysis and indiscriminate proteolysis. Very effective disintegration of yeast cell wall has been achieved using mechanical methods (Hedenskog *et al.,* 1969, 1970; Hedenskog and Ebbinghaus, 1972; Hedenskog and Mogren, 1973; Linnane and Vitols, 1962; Nossal, 1953; Novotny, 1964; Rehacek *et al.,* 1969; Wimpenny, 1967). Hedenskog *et al.* (1970) indicated that extraction of disintegrated yeast cells gave higher protein yields than non-disintegrated cells.

The nucleic acid content of microbial cells is regulated by microbial growth rate (Herbert, 1961; Kjelgaard and Kurland, 1963; Neidhardt and Fraenkel, 1961; Neidhardt and Magasanik, 1960). Nucleic acid content of yeast cells can be reduced by decreasing the growth rate (Vananuvat and Kinsella, 1975a,b), but this is not practical since rapid growth is obligatory in most processes for economic cell production. Thus, reduction of the nucleic acids present is necessary (Hedenskog and Ebbinghaus, 1972). Heat activation of endogenous yeast nucleases has potential (Maul *et al.,* 1970). Ohta *et al.* (1971) investigated the optimization of this process. A combination of a heat-shock process followed by dialysis or washing with phosphate solution, at an alkaline pH, was evaluated by Canepa *et al.* (1972). Other enzymatic methods in which exogenous ribonuclease was added to a suspension of yeast cells were described (Castro *et al.,* 1971; Dekloet *et al.,* 1961; Necas, 1958; Schlenk and Dainko, 1965). Chemical extraction of nucleic acids with acid, alkali, phenol, salts, and detergents has been used for analytical determination (Arnstein and Cox, 1966; Chargaff *et al.,* 1950). Although these methods are successful in reducing the nucleic acid content of protein extracts, they are complicated and expensive.

A limited amount of information is available concerning

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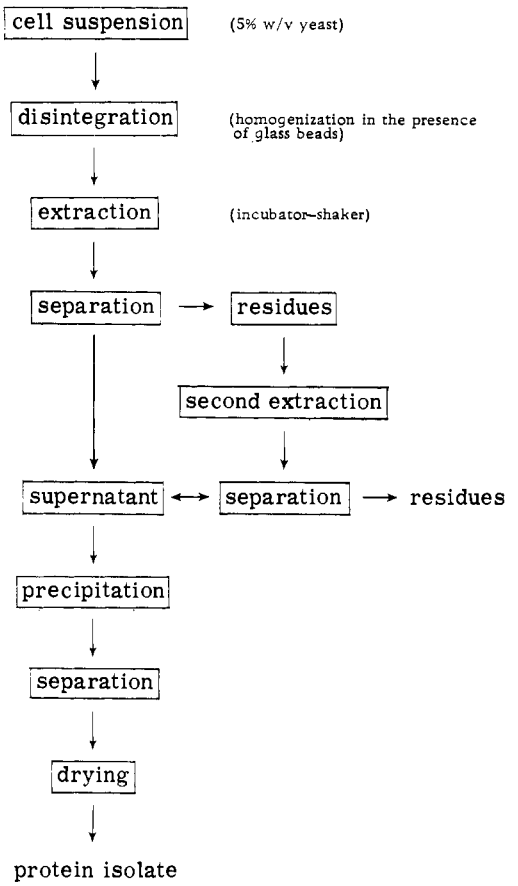

Figure 1. Outline of procedures used in extracting yeast protein from *S. fragilis*.

the preparation of SCP concentrate with reduced nucleic acid content using chemical extraction methods. Hedenskog and Ebbinghaus (1972) prepared protein concentrates from yeast by acid precipitation of the alkali-extracted protein after cell wall removal. Improved methods are required for the preparation of yeast protein concentrate with low nucleic acid content. Several practical procedures for preparation of protein from *Saccharomyces fragilis* were evaluated in the present study and the main objective was to maximize protein yield while minimizing nucleic acid contamination.

EXPERIMENTAL SECTION

Saccharomyces fragilis was grown by continuous culture in a medium containing 2% lactose at a dilution rate of 0.18 (Vananuvat and Kinsella, 1975b). The intact harvested *S. fragilis* cells contained about 50% Kjeldahl protein, 40% Lowry protein, and 10% nucleic acid. Extraction and precipitation of protein from *S. fragilis* were studied using freeze-dried yeast cells. The Kjeldahl nitrogen was determined by the AOAC method (1965). Kjeldahl (K) protein was N × 6.25. Lowry protein (L) of intact yeast cells and yeast protein isolates were determined according to the method described by Herbert *et al.* (1971) and Lowry *et al.* (1951). Bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) was used to prepare standard curves for both methods. Nucleic acid was directly determined by the method of Schmidt and Thannhauser (1945) and indirectly from the difference between K-protein and L-protein (Vananuvat and Kinsella, 1975a).

Initially the effects of homogenization, different extractants, temperature, time, and flotation ratio on protein extractability were studied using the scheme outlined in Figure 1. After each extraction step the suspension was centrifuged at 3000*g* for 20 min to remove cell debris. Protein concentration in the supernatant was measured for subsequent calculations.

Table I. Effect of Glass Beads during Homogenization upon the Release of Proteins from *S. fragilis* Cells into Water

	Protein released, %	
	K-Protein	L-Protein
No homogenization[a]	14.43	6.10
Homogenization without glass beads	27.98	15.04
Homogenization with glass beads[b]	38.78	29.87

[a] 5% yeast suspended in water and left standing for 1 hr. [b] Glass beads, 0.5 mm diameter, VWR Scientific, Rochester, N.Y. Homogenization was done in a Virtis homogenizer (Virtis-Research Equipment, Gardiner, N.Y.) at medium speed for 15 min. Protein released was calculated on the basis of K-protein or L-protein in the original yeast cells. Temperature was about 30° and the pH was 6.4 at the end of homogenization.

Precipitation of protein from supernatant after each extraction step was achieved by pH adjustment with 1 *N* hydrochloric acid and the resultant suspensions were shaken at 25° on the Evapo-Mix shaking water bath (Buchler Instruments, Fort Lee, N.J.) for 30 min. The pH's of suspensions were readjusted, if necessary, and shaking continued for another 30 min. After centrifugation, 7700*g* for 20 min, the volumes of the supernatants were measured and aliquots from the supernatants were analyzed for K- and L-protein. The difference between the amount of protein in the supernatants before and after precipitation corresponded to the amount of protein precipitated.

The effect of heating on extent of protein precipitation at various pH values was studied. After adjusting to a specific pH value the suspension was heated with constant stirring in a boiling water bath until the temperature reached 80° in *ca.* 2 min. The suspension was rapidly cooled to room temperature and centrifuged to recover the protein.

RESULTS AND DISCUSSION

Factors Affecting Protein Extraction. *Effect of Glass Beads.* Both K- and L-proteins released during homogenization of *S. fragilis* in the presence of beads were higher than those obtained without beads and much higher than when no homogenization was used (Table I). These data indicated that glass beads aided rupture of cells and release of cell contents. The amount of protein released in this experiment was slightly lower than that obtained by Hedenskog *et al.* (1970) who obtained 50% K-protein from *Saccharomyces cerevisiae.* Though a similar ratio of yeast to beads was used in these two studies, other variables including disruption temperature, agitation speed, and weight of beads affected the extraction of protein (Currie *et al.*, 1972; Hedenskog *et al.*, 1970).

Effect of Temperature. Temperature variation, from 25 to 70°, had a pronounced effect on the extractability of yeast protein (Table II) with the highest value at 40°, *i.e.*, 52.5 and 50.76% for K- and L-protein, respectively. Above 50° the K-protein extracted decreased gradually and L-protein dropped sharply. This was probably due to coagulation of extractable proteins. This observation agrees well with the reports concerning protein extraction from *S. cerevisiae* (Hedenskog and Ebbinghaus, 1972), sunflower seed (Gheyasuddin *et al.*, 1970), and alfalfa meal (Lu and Kinsella, 1972). However, the optimum extraction temperature varied for each type of protein. Hedenskog *et al.* (1970) reported that an increase of extraction temperature gave a slight increase in the amount of nitrogen extracta-

Table II. Effect of Temperature upon the Quantity of Protein Extracted from *S. fragilis* Cells with Water

Extraction temp, °C	Protein extraction,[a] % of cell protein: Kjeldahl (N × 6.25)	Lowry
20	46.1	40.2
30	48.3	43.6
40	52.5	50.7
50	52.0	49.2
60	50.1	26.9
70	41.3	18.1

[a] Yeast suspension (5%) in water was homogenized in the presence of glass beads and extracted for 30 min in a temperature controlled water bath. The pH of the final extract was 6.2–6.3. Per cent protein extracted represents the amount of cell protein solubilized.

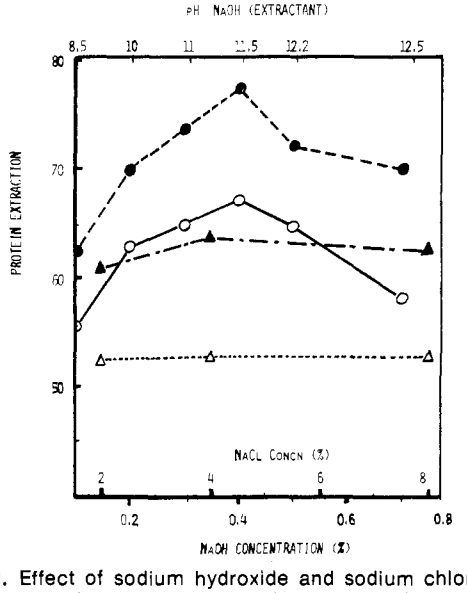

Figure 2. Effect of sodium hydroxide and sodium chloride concentrations on the amount of protein extracted from a suspension (5%) of *S. fragilis* cells that was homogenized in presence of glass beads and extracted for 30 min at 40°: (●,▲) Kjeldahl protein (N × 6.25); (○,△) Lowry protein extracted with NaOH and NaCl, respectively. NaCl extract was pH 6.3.

ble from yeast, algae, and bacteria, while the amount of precipitable nitrogen was slightly decreased at higher temperatures (above 70°). The yield of protein and amino acids, especially lysine, decreased when the extraction temperature exceeded 70° (Hedenskog and Ebbinghaus, 1972; Hedenskog and Mogren, 1973).

Sodium Hydroxide. Protein extractability increased as the concentration of NaOH increased up to 0.4% w/v (Figure 2), with the maximum extraction of K- and L-protein being 77.21 and 67.09%, respectively. At concentrations above 0.4% NaOH, the amount of protein extracted dropped and L-protein decreased more rapidly than K-protein.

Sodium Chloride. The K-protein extracted with 4% NaCl was slightly higher than the values obtained using 2 and 8% NaCl (Figure 2). There were no differences in L-protein extracted at the three concentrations of NaCl. The average L-protein extracted was 52.59%.

Duration of Extraction. The effect of duration of extraction with water, 0.4% NaOH, or 2% NaCl was evaluated. Extractability of protein by water continually increased with time (Figure 3). There was a slight increase in the percentages of K- and L-protein extracted with NaOH from 0.5 to 2 hr. The amount of K-protein extracted with NaCl also increased, whereas L-protein was constant. Acceptable extraction, *i.e.*, 80% K-protein and 70% L-protein, was obtained from *S. fragilis* using 0.4% NaOH for 1 hr and only small increases occurred with longer time.

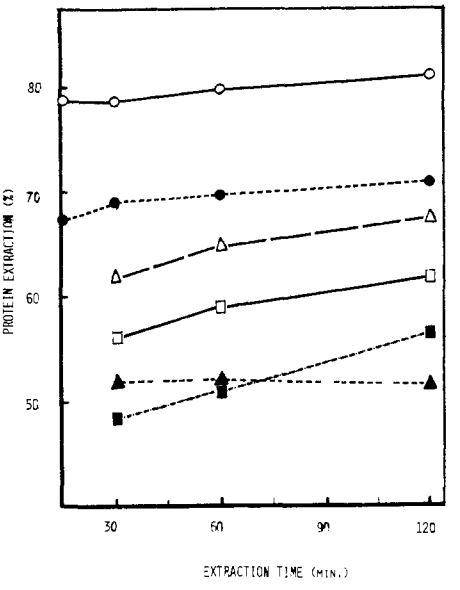

Figure 3. Effect of extraction time upon the quantity of protein extracted from *S. fragilis* cells with water, sodium hydroxide, and sodium chloride solutions. Extraction conditions: 5% yeast suspension was homogenized in the presence of glass beads and extracted for 30 min at 40° in a water bath with shaking: (○,△,□) Kjeldahl protein (N × 6.25); (●,▲,■) Lowry protein extracted with sodium hydroxide (0.4%), sodium chloride (2%), and water, respectively.

These yields are similar to the maximum yields of protein obtained from disintegrated cells of yeast and algae when extracted with NaOH (0.3–0.5%) for 1 hr (Hedenskog and Ebbinghaus, 1972). Lu and Kinsella (1972) found little effect of time on the extraction of alfalfa leaf protein where virtually all of the extractable proteins were in solution within 30 min of extraction. Intact cells of *Candida* species extracted with NaOH (2–5%) for 2–10 hr at 37° gave a 70–80% K-protein yield (Mitsuda *et al.*, 1971). Hedenskog *et al.* (1970) showed that an increase of the NaOH concentration above 0.5% gave no increase in the yield of soluble nitrogen when extracting disintegrated yeast cells. The extractability of both K- and L-protein from *S. fragilis* in the present study decreased when NaOH concentration exceeded 0.4%.

Mixture of Sodium Hydroxide and Sodium Chloride. Studies were made to determine the effect of a mixture of 0.4% NaOH plus 2% NaCl and 0.4% NaOH plus 4% NaCl at various extraction times upon the extractability of protein from *S. fragilis.* The amount of protein extracted with the former was much higher over the entire extraction period (Figure 4). In both systems, the amount of protein extracted decreased as extraction time increased. The highest yields, 75.09 and 63.08% for K- and L-protein, respectively, were obtained with NaOH (0.4%) plus NaCl (2%). Thus, mixtures of NaOH and NaCl did not improve the extractability of protein from *S. fragilis* over NaOH (0.4%) alone.

Gheyasuddin *et al.* (1970) and Mattil (1971) studied the effect of several concentrations of inorganic salts, *i.e.*, NaCl, $CaCl_2$, Na_2SO_3, and Na_3PO_4 on the extractability and solubility of various types of protein and found that extractability varied with type of protein and salt. The extractability of protein from coconut meal was rather constant with 0.1 to 1.0 *M* Na_2SO_3, whereas that of sunflower seed meal decreased as the concentration of Na_2SO_3 rose. Extractability of protein from *S. fragilis* remained constant as the concentration of NaCl was increased from 2 to 8%.

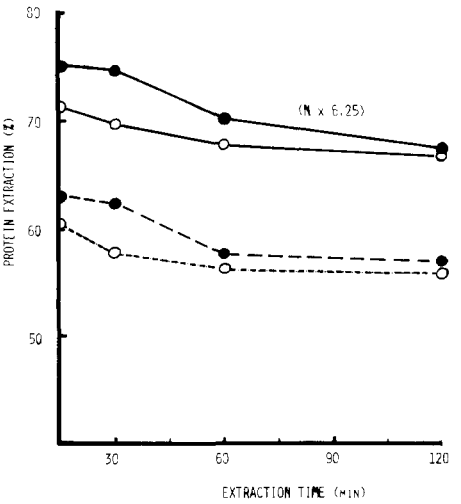

Figure 4. Effect of mixture of sodium hydroxide and sodium chloride on the quantity of protein extracted from *S. fragilis* cells. Extraction conditions: 5% yeast suspension was homogenized in the presence of glass beads and extracted at 40° in a water bath with shaking. The final pH values of solutions extracted with 0.4% sodium hydroxide and 2% sodium chloride and 0.4% sodium hydroxide and 4% sodium chloride were 11.5 and 11.2, respectively: (●–●, O–O) Kjeldahl protein (N × 6.25); (● - - - ●, O - - - O) Lowry protein extracted with sodium hydroxide (0.4%) plus sodium chloride (2%) and sodium hydroxide (0.4%) plus sodium chloride (4%), respectively.

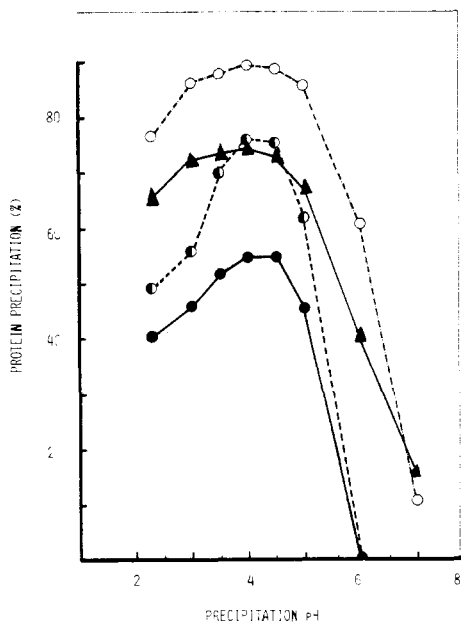

Figure 5. Effect of pH upon the quantity of protein precipitated from *S. fragilis* extracted with water and sodium hydroxide solutions. Extraction conditions: 5% yeast suspension was homogenized in the presence of glass beads, and extracted with either water or 0.4% sodium hydroxide at 40° for 1 hr. After water extraction, the final pH of the suspension was 6.0, whereas after sodium hydroxide extraction the final pH was 11.5. Per cent protein precipitated was calculated on the basis of K- or L-protein in extractant supernatant before precipitation: (▲,●) amount of Kjeldahl protein (N × 6.25); (O,◐) amount of Lowry protein precipitated from sodium hydroxide (0.4%) and aqueous extracts, respectively.

Solvation Ratio. Using water, NaOH (0.4%), and a mixture of NaOH (0.4%) and NaCl (2%) the extractabilities of protein using solvation ratios of 20:1 and 10:1 were studied. The extractability of protein with NaOH (0.4%) was affected most at the higher concentration of yeast (Table III). Both K- and L-protein extracted from 10% yeast suspension with NaOH were 20% lower than from a 5% yeast suspension, whereas with water it was only 7–8% lower. The effect of extraction time was negligible which agrees with the report of Hedenskog *et al.* (1970). In general, for the extraction of protein from seeds, leaves, and meal a solvation ratio of 20:1 is used (Gheyasuddin *et al.*, 1970; Betschart and Kinsella, 1973; Lu and Kinsella, 1972).

Table III. Effect of Ratio of Extractant to Yeast upon the Quantity of Protein Extracted from *S. fragilis* Cells with Water, Sodium Hydroxide, and a Mixture of Sodium Hydroxide and Sodium Chloride Solutions for Various Extraction Times[a]

		Protein extracted, %			
		10% yeast suspension		5% yeast suspension	
Extractant	Time, hr	K-Prot.	L-Prot.	K-Prot.	L-Prot.
H_2O	0.5	51.95	43.54	56.05	48.49
H_2O	1.0	51.66	42.87	58.91	50.05
H_2O	2.0	53.06	43.57	61.75	65.39
0.4% NaOH	0.5	59.52	48.55	78.60	68.79
0.4% NaOH	1.0	59.84	48.76	79.70	69.54
0.4% NaOH	2.0	59.60	50.03	80.90	70.88
0.4% NaOH + 2% NaCl	0.5	56.59	42.81	74.73	62.28
0.4% NaOH + 2% NaCl	1.0	57.48	42.90	70.25	57.82
0.4% NaOH + 2% NaCl	2.0	54.87	41.18	67.91	57.22

[a] Extraction conditions: 5 and 10% yeast suspensions were homogenized in the presence of glass beads and extracted at 40° in a water bath with shaking. Values represent two extractions.

Table IV. Comparison of the Effects of pH and Heating upon the Quantity of Protein Precipitated from Water and Sodium Hydroxide Extracts of *S. fragilis*[a]

	Protein precipitated, %			
	NaOH extract		Water extract	
Precipitation conditions	K-Prot.	L-Prot.	K-Prot.	L-Prot.
pH 11.9 + heat	0	0		
pH 9.0 + heat	6.56	12.53		
pH 7.0 + heat	44.60	68.84		
pH 7.0	10.67	15.76		
pH 6.0 + heat	49.50	76.13	57.65	88.84
pH 6.0	40.25	60.59		
pH 4.0 + heat	72.89	88.45	57.54	86.17
pH 4.0	71.02	87.72	54.32	75.80

[a] Extraction conditions: 5% yeast suspension was homogenized in the presence of glass beads and extracted with 0.4% sodium hydroxide or water at 40° for 1 hr in a water bath with shaking. The pH obtained from extraction of *S. fragilis* with water was about 6.0 without any adjustment.

Precipitation of Protein. *Effect of pH.* The precipitation of yeast protein following extraction was studied in order to maximize protein yield and reduce nucleic acid contamination of the protein isolate.

The effect of pH on the precipitation of protein from the supernatant after the extraction of *S. fragilis* with 0.4% NaOH and water was studied (Figure 5). The pH of precipitation significantly affected the yield of yeast protein obtained. The highest yield was obtained at pH between 4

Table V. Quantity of Protein Extracted and Recovered from *S. fragilis* Cells Grown in Continuous Culture on 2% Lactose[a]

Extractants	Protein extracted,[b] %		Precipitation method	Protein pptd,[c] %		Protein recovd,[d] %	
	Kjeldahl	Lowry		Kjeldahl	Lowry	Kjeldahl	Lowry
0.4% sodium hydroxide	82.87	76.25	pH 4.0	83.76	88.63	67.13	65.16
			pH 6.0 + heat	55.57	72.60	44.53	54.20
Water	55.32	51.50	pH 4.0	48.01	60.92	20.55	25.82
			pH 6.0 + heat	60.94	78.14	26.08	33.11

[a] Vananuvat and Kinsella (1975b). Extraction conditions: 5% yeast suspension was homogenized in the presence of glass beads and extracted at 40° for 1 hr in a water bath with shaking. Kjeldahl corresponds to N × 6.25. Heat treatment involved heating protein solution up to 80° in a water bath (100°) and immediate cooling of sample. [b] % protein extracted = (amount of protein in supernatant)/(amount of protein in yeast cells) × 100. [c] % protein precipitated = (amount of protein precipitated)/(amount of protein in supernatant before precipitation) × 100. [d] % protein recovered = (amount of protein precipitated)/(amount of protein in yeast cells) × 100.

Table VI. Protein and Nucleic Acid in the Protein Prepared from Alkaline and Aqueous Extracts of *S. fragilis*[a]

Extractants	Precipitation method	Total protein, %	Nucleic acid, %
0.4% sodium hydroxide	pH 4.0	83.22	5.65
	pH 6.0 + heat	83.50	1.40
Water	pH 4.0	87.70	1.90
	pH 6.0 + heat	94.50	n.d.

[a] Extraction conditions, see Table IV; n.d. = none detectable.

and 4.5 from both water and alkaline extracts. The maximum values, *i.e.*, 89.11% L-protein and 74.40% K-protein, were obtained from alkaline extract while 75.80% L-protein and 54.32% K-protein were recovered from the water extract. Above pH 5, protein precipitation decreased sharply and the difference between K- and L-protein became smaller. In contrast, at a pH below 4 the decrease was gradual. It is noteworthy that the yield of L-protein was higher than K-protein at each pH value indicating that a larger amount of protein nitrogen was precipitated. The maximum K-protein precipitated from *S. cerevisiae* yeast was 70% between pH 3 and 5 (Hedenskog *et al.*, 1970).

Effect of Heat. The effect of heating the protein solution to 80° and immediately cooling following pH adjustment was studied. With NaOH as extractant, heating following precipitation at pH 4 caused no increase in yield of either K- or L-protein (Table IV). At higher pH values, heating caused an increase in quantity of protein precipitated. At pH 11.9 no precipitation of protein occurred even though heat was applied. In the case of the aqueous extract, heating at pH 6 caused an increase in the amount of protein precipitated (Table IV).

The K-protein recovery was around 67% when *S. fragilis* was extracted with alkali and subsequently adjusted to the isoelectric point of pH 4.0 (Table V). This was much higher than the protein recovered from *S. cerevisiae*, algae, and bacteria under comparable conditions (Hedenskog and Ebbinghaus, 1972; Hedenskog *et al.*, 1970). About 40% of protein was recovered from coconut meal under similar extraction conditions (Samsons *et al.*, 1971).

Protein recovery from the aqueous extracts was about half that obtained with alkali. Significantly higher amounts were recovered by heat precipitation at pH 6.0 (Table V). The extraction of disintegrated yeast and algae with water or weak salt solution and precipitation at isoelectric point gave very low protein recovery (Hedenskog *et al.*, 1970). Cococnut meal extracted with water and precipitated at isoelectric point gave only 10% protein recovery (Samsons *et al.*, 1971).

The protein content of yeast protein isolates from *S. fragilis* was high, *i.e.* over 85% protein (Table VI). The crude protein contents of lyophilized yeasts, *S. cerevisiae* and *S. carlbergenesis*, green algae, bacteria, and hydrocarbon-assimilating yeast, *Candida* species, were in the range of 70–80% K-protein (Hedenskog *et al.*, 1970; Mitsuda *et al.*, 1971).

The yeast protein isolates prepared in this study were devoid of their original characteristic yeasty flavor and they were a light cream in color similar to *Torula* yeast protein isolates as reported by Mitsuda *et al.* (1969, 1970).

Intact *S. fragilis* cells contained about 10% nucleic acid. The protein isolate prepared from alkaline extract precipitated at pH 4.0 possessed about 5% nucleic acid compared to 1% in the protein precipitated at pH 6.0 with heating (Table VI). This corresponded to 50 and 90% reductions in nucleic acid, respectively. The protein isolate prepared from the aqueous extract and precipitated at pH 4.0 contained only 2% nucleic acid, and less than 1% nucleic acid was found in the protein isolate precipitated at pH 6.0 with heat. Hence extraction of protein from *S. fragilis* with water resulted in significant reduction in nucleic acid. However, protein recovery from yeast was only one-third to one-half of that obtained by alkaline extraction.

The yeast protein isolate obtained in the present study was better than the protein concentrate obtained from *S. cerevisiae* where the lowest nucleic acid content was 1.1% (Hedenskog and Ebbinghaus, 1972). Castro *et al.* (1971) used a heat-shock process followed by pancreatic ribonuclease to reduce acid content in *Candida utilis*. The resulting cell pellet contained 40–50% protein and 1–2% nucleic acid. This gave a protein to nucleic acid ratio of less than 50 which was lower than our yeast protein isolate which was 90. Daily intake of nucleic acid from yeast should not exceed 2 g because higher quantities have been reported to increase the uric acid content of blood plasma to an abnormal level (Edozien *et al.*, 1970; Waslien *et al.*, 1968, 1970). This intake of nucleic acid would only be attained with intakes of 50–100 g of *S. fragilis* protein depending on preparation methods. It has been reported that only 20–30 g of whole yeast can be consumed in order not to exceed the nucleic acid limit (Edozien *et al.*, 1970; Waslien *et al.*, 1968). Therefore, the methods of preparation of yeast protein isolates low in nucleic acids, described in the present study, may be of significance in eventually producing a high quality protein for human consumption.

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Determination of Four- and Five-Ring Condensed Hydrocarbons. I. Analysis of Polynuclear Aromatic Hydrocarbons in Yeast Produced by Growth on Both *n*-Hydrocarbon and Dextrose Feeds

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A method has been developed and tested for the analysis of polynuclear aromatic hydrocarbons in the biomass produced by growth of a pure *Candida* strain on both *n*-hydrocarbon and dextrose feeds. The method involves digestion of the yeast and the polynuclear aromatic material is concentrated by extraction, chromatography on alumina, partition, and chromatography on Florisil. Polynuclear aromatic compounds are separated and isolated by two successive thin-layer chromatographic plates, and measured by ultraviolet spectrophotometric procedures. Recovery studies of four components, benzo[*a*]pyrene, benzo[*e*]pyrene, benz[*a*]anthracene, and benzo[*g,h,i*]perylene each at the 5-ppb level averaged 63 to 80% for both *n*-hydrocarbon and dextrose grown yeast. Recovery of benzo[*a*]pyrene added to yeast from the 3- to 250-ppb level ranged from 84 to 88%. Recovery of polynuclear aromatics added individually to yeast was generally greater than that for a multicomponent system. Analysis of dextrose-grown yeast did not yield any of these compounds, whereas analysis of *n*-hydrocarbon-grown yeast showed some of these compounds in a range of 1–11 ppb. Other polynuclear aromatic compounds were detected in each of these yeast materials.

This work describes the first part of a two-part series dealing with the analysis of polynuclear aromatic hydrocarbons in a petroleum fermentation process. Polynuclear aromatic determinations of the biomass product will be considered initially.

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Pryor (1969) considers synthetic foods to be a possible solution to the population–food problem as a means of meeting the demand for new sources of protein. The culture of microorganisms, especially *Candida* yeast strains which grow actively on hydrocarbon substrates, appears feasible for the production of protein. Although there are a number of producers (Bennett *et al.*, 1969; Decerle *et al.*, 1969) employing various organisms and hydrocarbon feeds for production of this "petroprotein" outside the