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Comparison of Three Methods for Calculating Protein Content of Foods

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Comparison was made between three methods for calculating the protein content of 68 foods. The 3 methods of calculation were: (1) multiplication of Kjeldahl nitrogen by 6.25; (2) multiplication of Kjeldahl nitrogen by factors varying from 5.30 to 6.38 depending on food type; and (3) summation of amino acid content as determined by chemical analyses. New conversion factors were calculated based on amino acid analyses. Substantial differences (20 to 40%) were found in protein content for many foods depending on the calculation method. The protein content of six typical menus for mature American males was calculated using the three methods. The resulting protein content of menus did not vary substantially (less than 3%) as a function of calculation method since relatively large differences in various foods tended to cancel out. Conversion factors based on amino acid analyses are recommended whenever more accurate approximation of protein content of individual food is required.

The Skylab manned space flight program presented unique problems involving the food system. All food was required to be on board the Skylab laboratory at the time of its initial launch into orbit. The laboratory was subsequently manned by three separate crews of three astronauts each. These were the first men to subsist during weightless flight for extended periods on predetermined 6-day menu cycles. Sixty-eight different foods were used in these menus. The menus were nutritionally balanced and selected to contain foods highly typical of a diet of the vigorous American adult male. Mission lengths were approximately 28 and 56 days for the first and second Skylab crews, respectively. There was an interval of about 60 days between the first and second crew's visit to the orbiting laboratory. Hence, the food which was originally placed aboard Skylab had to have long-term storage stability.

In addition to the life support requirements of the food, it was required to be nutritionally characterized so as to support sophisticated life science experiments. The food was manufactured so as to be nutritionally homogeneous and stable after a long storage period (Heidelbaugh et al., 1973a). The food was accurately defined in regard to nutritional content (Stadler et al., 1973). The ingredients and manufacturing procedures for each of these Skylab foods have been described by Heidelbaugh et al. (1973b).

As part of the program to assure nutritional definition of the Skylab food, a detailed study was undertaken to accurately quantitate protein value. This was accomplished by analyzing each food for its content of nutritionally relevant amino acids. The results of these analyses were evaluated by comparing them to protein values obtained from calculations utilizing more conventional methods as recommended by Watt and Merrill (1963). The evaluation of such methods for determining protein value of foods is discussed in this paper.

MATERIALS AND METHODS

Skylab foods were packaged in sequentially serial numbered and individual portion sized containers. A table of random numbers was used to select three packages of each food by reference to the serial numbers. The selected samples were freeze dehydrated and ground for analysis. Total nitrogen content of each sample was determined by AOAC macro-Kjeldahl techniques. The nitrogen value used in this report was a mean value calculated from six separate Kjeldahl analyses for each item. Sample size for amino acid

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Table I. Comparison of Protein Content of Skylab-2 and -3 Menus[a]

Menu	Protein content calculated by the method indicated: A, conversion factor of 6.25, g	B, individual conversion factor,[b] g	C, sum of amino acids	% difference between A and B[c]	% difference between A and C[d]	% difference between B and C[e]
Commander, Skylab-2	98.56	97.28	95.66	+1.3	+2.9	+1.7
Science pilot, Skylab-2	105.63	103.98	102.98	+1.6	+2.5	+1.0
Pilot, Skylab-2	101.41	100.23	100.38	+1.2	+1.0	−0.2
Commander, Skylab-3	90.47	88.99	89.64	+1.6	+0.9	−0.7
Science pilot, Skylab-3	105.03	104.15	105.10	+0.8	−0.1	−0.9
Pilot, Skylab-3	157.96	158.26	154.69	−0.2	+2.1	+2.3

[a] Mean values for 6-day menu cycle selected as being representative of a nutritionally balanced diet recommended for a mature American male. [b] Conversion factors for meat, eggs, fruits, vegetables, and leguminous seeds, 6.25; milk and cheeses, 6.38; bakery products, 5.70; peanuts, 5.46; and pecans, 5.30 (from Jones, 1941). [c] Percent difference = 100(A − B)/A. [d] Percent difference = 100(A − C)/A. [e] Percent difference = 100(B − C)/B.

analysis was determined by the percentage of protein in each food item. Sample size ranged from 100 to 300 mg, depending upon the protein content. Amino acid analysis, except for tryptophan, was performed utilizing an automated amino acid analyzer (Beckman Model 121-C Amino Acid Analyzer) following the methods of Spackman et al. (1958). Beckman's Custom Research Resins were used for separation. For analysis of acidic and neutral amino acids, type UR-30 with spherical particles having a mean diameter of 22 ± 6 μ was used. For analysis of basic amino acids, type PA-35 with spherical particles having a mean diameter of 15 ± 6 μ was utilized. For analysis of acidic and neutral amino acids, pH 3.25 ± 0.01 (0.20 *N*) and pH 4.25 ± 0.02 (0.20 *N*) sodium citrate buffers were utilized. For analysis of basic amino acids, a pH 5.25 ± 0.02 (0.35 *N*) sodium citrate buffer was used. Buffer flow rates were 68 ml/hr. Ninhydrin color reagent flow rate was 34 ml/hr. For cystine analyses, the sample was oxidized with performic acid following the method suggested by Hirs (1967). Cystine was then quantitated by ion exchange chromatography utilizing the automated amino acid analyzer. Tryptophan was determined by microbiological assay (Greene and Black, 1944). Amino acid analyses were performed in triplicate for each food item and a mean value was used for all calculations subsequently reported.

Three different methods to calculate protein content of these foods were compared. These three methods were: (1) multiplication of Kjeldahl nitrogen by the conversion factor 6.25; (2) multiplication of Kjeldahl nitrogen by the conversion factors selected by food type, i.e. meat, eggs, fruits, vegetables, and leguminous seeds, 6.25; milk and eggs, 6.38; bakery products, 5.70; peanuts, 5.46; pecans, 5.30 (Jones, 1941); (3) summation of the amino acid content of each food.

An overall evaluation of the effect of these three methods for protein content calculation was made by applying the various conversion factors to the Skylab menus. These menus had been carefully selected to be nutritionally balanced and recommended for mature American males working under stress.

RESULTS AND DISCUSSION

The grams of amino acids per 100 g of the edible portion of Skylab foods were calculated (see paragraph at end of paper regarding supplementary material). Kjeldahl nitrogen was compared with the total nitrogen contained in the amino acids. These data indicate that a substantial quantity of Kjeldahl nitrogen is not derived from amino acids.

The protein content of 68 individual foods calculated by the three different methods was compared and analyzed. Variations in percent differences as a function of method of calculation ranged as high as 20 to 40% in many foods. To correct for this variance, new factors for conversion of Kjeldahl nitrogen to protein content were devised. Use of these factors is recommended when the protein values of individual foods comparable to those studied here are required. Generally the differences were higher in foods which had a lower nitrogen content. The analytical error would be expected to be higher in these foods. However, the analytical error was minimized by the six Kjeldahl nitrogen analyses and the triplicate amino acid analyses which were performed on each food item.

Comparison of percent differences indicates a larger difference when the protein content from traditional factors is compared to amino acid analysis. Some of the differences may be attributed to composition and the appropriate conversion factor not being used. Bread, for example, is composed of flour, nonfat dry milk, yeast, and several other ingredients and poses some problem in factor selection.

Whenever an accurate estimate of protein content of a food is required, and that food is dissimilar to the type reported here, consideration should be given to developing a conversion factor patterned after the way such factors were developed in this study.

The overall evaluation of the impact of the selection of different conversion factors on the evaluation of typical American nutritionally balanced menus is presented in Table I. These data indicate that variations in calculated protein content of individual foods resulting from the methods of calculation tend to be modulated when a balanced menu is examined. This finding suggests that the errors resulting from use of the "traditional" Kjeldahl nitrogen conversion factors tend to be randomly distributed among any variety of foods. Errors in amino acid analysis would also be randomly distributed. Thus, the use of these conversion factors with nutrient balanced menus made up of a variety of foods typical to the diet of the mature American male would be expected to be as accurate as using more rigorous values derived from analysis of the food for each individual amino acid. On the other hand, the use of such "traditional" conversion factors for individual foods for the purpose of individual evaluation or "nutritional labeling" may be questioned. The best estimate of the protein content of a food is the summation of the amino acid content. Conversion factors based on amino acid analyses are recommended whenever more accurate approximation of the protein content of individual foods is required.

Supplementary Material Available. Supplementary data containing information on the amino acid composition and protein content of 68 individual foods will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× re-

duction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.50 for photocopy or $2.50 for microfiche, referring to code number JAFC-75-611.

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Some Functional Properties of Protein Isolates from Yeast, *Saccharomyces fragilis*

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Functional properties of protein isolates prepared from the yeast *Saccharomyces fragilis*, by alkaline and water extraction and precipitated by acid (pH 4.0) and by heating (80°) at pH 6.0, respectively, were examined. Typical solubility curves were obtained with maxima occurring above pH 7.5. Minimum solubility points occurred at pH 4 to 4.5 and 4.5 to 5 for alkali and water extracts, respectively. Heat precipitation reduced protein solubility by approximately 5% compared to acid precipitation (pH 4 at 26°). The foaming capacity of yeast proteins, with the exception of the water extract precipitated at pH 4, was inferior (about 50%) to that obtained with soy isolate. Foam stability of all yeast proteins was poor. The emulsifying activity of all yeast proteins, except the sample prepared by heat precipitation of alkaline extract at pH 6.0, had higher emulsifying activity than the soy isolate. Protein prepared from water extract and precipitated at pH 4.0 showed the highest value. Yeast protein isolates exhibited lower surface tension than water.

In the last decade intensive research has been carried out to find cheaper sources of protein to alleviate protein malnutrition and new sources of functional proteins for food industry. Proteins from fish, oilseed, leaves, and microbes have been intensively investigated and some new proteins are available commercially for human consumption. Higher production rates and protein yields, ease of production control, and possible food production without the use of limited land makes single-cell protein (protein derived from cells of yeast, mold, bacteria, and algae) more attractive as a protein source compared to conventional plant and animal sources (Mateles and Tannenbaum, 1968). However, a number of problems associated with single-cell protein (SCP) have to be solved to render it a safe and cheap source of protein for human use. Proteins from microbial cells should be low in cell wall fragments to improve nutritional value, i.e. bioavailability of protein. Nucleic acid content should be reduced to minimize the intake of nucleic acid to less than 2 g per day (Edozien et al., 1970) and the protein should have acceptable color, flavor, and texture (McCormick, 1973). These criteria are fundamental and necessitate the isolation of protein from yeast cells prior to its use in foods. Initially the utility and marketability of isolated yeast protein will depend to a large degree on their functional properties, cost notwithstanding. Information on the functional properties of proteins prepared from microbial cells is limited (Labuza et al., 1972). Rheological properties were investigated by Huang and Rha (1971). Fiber formation was studied by Huang and Rha (1972) and Mitsuda et al. (1971).

In this paper some functional properties of proteins isolated from *Saccharomyces fragilis* were determined.

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EXPERIMENTAL SECTION

Saccharomyces fragilis was grown in continuous culture on crude lactose as reported (Vananuvat and Kinsella, 1975a). Final cell concentration was about 11 g/l. Recovery of yeast and extraction of protein were performed as described (Vananuvat and Kinsella, 1975c). Protein was extracted from the broken cells with 0.4% sodium hydroxide or water. Protein was precipitated from these respective extracts by acidification with 1 *N* HCl to pH 4.0 and by heating the extracts to 80° for 30 sec following adjustment of the extract to pH 6.0. The precipitated protein was recovered by centrifugation (10,000*g* for 15 min) and freeze dried. Thus, four types of yeast protein isolates were tested for functionality. Protein samples 1 and 2 were prepared by extracting the broken cells with NaOH and precipitating the protein with acid at pH 4.0 (26°) and by heat (80°) at pH 6.0, respectively; samples 3 and 4 were prepared by extracting with water and precipitating the protein under identical conditions.

Solubility. Solubility of yeast protein isolates (1% concentration) was determined according to Lu and Kinsella (1972). Kjeldahl nitrogen was determined by the method of AOAC (1965) and protein was calculated using a factor of 6.25. Protein was also determined according to the method of Lowry et al. (1951) for comparison. Bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) was used to prepare the standard curve.

Whippability. Whipping capacity was determined according to the method of Yasumatsu et al. (1972) where foam expansion and foam stability were taken as indices of whippability. Fifty milliliters of protein suspension (0.1 g/ml) in a 100-ml stoppered cylinder was shaken horizontally for 1 min. The resulting foam volume (milliliters) was defined as foam expansion. The residual foam volume, measured after 30 min, was used as an index of foam stability.