Analysis of Liquid Protein Products for Amino Acids, Carbohyrates, and Peptides

Twenty-one samples of commercially available liquid protein products, partial hydrolysates derived from collagen or gelatin, were analyzed for amino acids, carbohydrates, and peptides. For each of the parameters measured, there were large variations from product to product in analytical results compared to label declaration and in product composition. One product contained 0% of the label declaration of tyrosine and another 424% of the label declaration of methionine. Glycerol and sorbitol also were detected in the products, which purported to contain no calories from carbohydrates or fats. Xylitol was not found in any of the samples. The composition of the liquid protein products and the deaths associated with the ingestion of these products cannot be correlated on the basis of the data now available.

Obesity, a major nutritional health problem, has been implicated as a contributory factor in cardiovascular disease, diabetes, and numerous other disease conditions (Bray, 1976). A number of techniques for the management of obesity have been proposed, including diets low in carbohydrate and fat (Stillman and Baker, 1967), exercise (National Institutes of Health, 1976), drug treatment (Gershberg, 1972), diets high in fat and protein (Atkins, 1972), surgery (Bray, 1976; Maine et al., 1977), behavior modification (Stunkard, 1975), and diets high in protein with supplementary vitamins and minerals (Bistrian et al., 1976, 1977a,b). The latter is one of the most popular diets used to date. The diet, which is based on the use of liquid proteins, is commonly called the protein-sparing modified fast diet and was popularized by Linn and Stuart (1976) as the "last chance diet." These products, most of which are hydrolysates made from either collagen or gelatin, were promoted as aids to help obese individuals lose weight. A number of human deaths have been attributed to the use of liquid protein products (Center for Disease Control, 1977). Liquid protein products were analyzed for heavy metals, pesticides, chlorodioxins, and bacterial contamination, as well as amino acids, carbohydrates, and peptides, to determine whether they contained any component that could be linked to the deaths. The results of the analysis for amino acids, carbohydrates, and peptides are reported here.

MATERIALS AND METHODS

Materials. Twenty-one liquid protein products were collected from manufacturers throughout the United States. All products were commercially available partially hydrolyzed protein products derived from collagen and gelatin.

Amino Acids. Amino acids were determined on a Beckman Model 121-HP automatic amino acid analyzer. Sodium citrate buffers, pH 5.10 (0.35 N), 3.15 (0.20 N), and 4.20 (0.38 N), were used to elute the amino acids by using a two-column system. Ninhydrin (E. Merck, Elmsford, NY) was used to produce the color reaction. Amino acid standards were purchased from Pierce Chemical Co., Rockford, IL. Samples were hydrolyzed for 48 h according to the procedure outlined in the Beckman Model 121 instruction manual.

Carbohydrates. Carbohydrates were determined by using reverse-phase high-pressure liquid chromatography according to the method of Conrad and Palmer (1976). A Waters ALC 100 liquid chromatograph with a μBondapak/carbohydrate column was used. The mobile phase was 80% acetonitrile (Burdick and Jackson Laboratories, Inc., Muskegon, MI)–20% water at a flow rate of 2 mL/min. The effluent was monitored with a Waters R401 refractive index detector. Sorbitol and xylitol standards were purchased from Supelco, Inc., Bellefonte, PA. Glycerol was obtained from Fisher Scientific, Silver Spring, MD.

Table I. Amino Acid Composition of Liquid Protein Products

amino acid	range of concn, mg/30 mL	range of label claim, %
L-alanine	907–2728	70–194
L-arginine	453–2332	38–194
L-aspartic acid	533–1483	59–162
L-glutamic acid	876–2908	58–173
glycine	2346–11 682	63–373
L-histidine	39–236	35–215
L-hydroxylysine	60–323	40–215
L-isoleucine	139–450	70–212
L-leucine	265–830	64–180
L-lysine	221–1163	34–179
L-methionine	84–465	82–424
L-phenylalanine	165–525	53–166
L-proline	667–4330	29–153
L-serine	247–927	25–148
L-threonine	165–520	55–165
L-tyrosine	0–192	0–210
L-valine	207–642	59–183

Peptides. Peptide fragments for molecular weight estimation were separated by gel filtration chromatography. A 2.2 cm diameter column was filled to a height of 45 cm with Sephadex G-25 coarse gel (Pharmacia Chemicals, Piscataway, NJ). The column was eluted with 0.1 M $CaCl_2$ at a flow rate of 25 mL/h. The effluent was monitored at 280 nm on a Gilford Model 250 spectrophotometer.

A Pharmacia No. JE-01 calibration kit containing chymotrypsinogen (M_r 25 000) and ribonuclease (M_r 13 700) was used for molecular weight standards. Bacitracin (M_r 1411), obtained from the Division of Drug Biology, was also used for molecular weight determinations.

RESULTS AND DISCUSSION

Aliquots of the liquid protein products were subjected directly to amino acid analysis and yielded a complex chromatographic pattern, indicting that the manufacturing process only partially hydrolyzed the starting material. The resultant products are therefore mixtures of peptide fragments with no free amino acids except for tryptophan, which had been added after hydrolysis.

The peptides in the products were then characterized. The samples were passed through a Sephadex G-25 resin, which yielded the best species separation in the range of interest and also quantitatively recovered the eluted samples. A plot of the log of the molecular weight vs. the ratio of the elution to void volumes resulted in a straight line from which the molecular weight distribution of the peptides could be calculated. The results showed great variation among the products. Two representative patterns are shown in Figure 1. Product 1 contained 30% of the peptides with a molecular weight of 2000, 40% with a

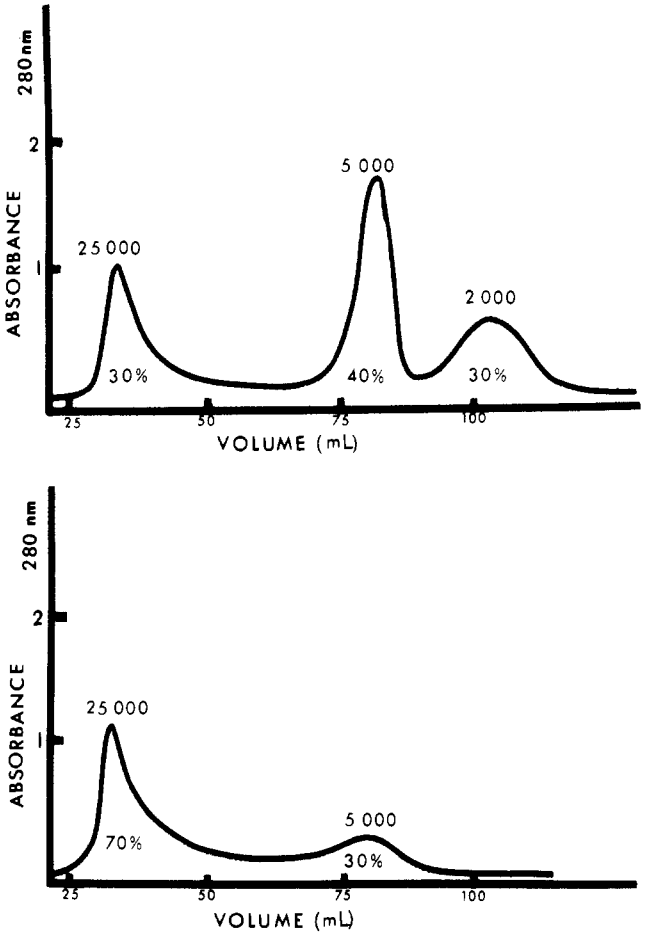

Figure 1. Sephadex G-25 filtration of two liquid protein products.

molecular weight of 5000, and 30% with a molecular weight of 25 000 or more. The second product contained 30% of the peptides with a molecular weight of 5000 and 70% with a molecular weight of 25 000 or more. Some of these high molecular weight fractions were collected and subjected to further gel filtration. The results indicated that the polypeptides were as large as 100 000. The variability in peptide length probably reflects differences in the degree of hydrolysis achieved by the various manufacturing processes.

Amino acid profiles of the completely hydrolyzed liquid protein products are shown in Table I. The amounts of amino acids found in the samples differed considerably from the amounts declared on the label. For the 17 products that had label declarations, analyses showed from 0% of the label declaration for tyrosine to 424% of the label declaration for methionine.

The quantitative label declaration on the liquid protein products stated that the only source of calories in the products was derived from protein, in contrast to the ingredient statement which indicated that the product contained glycerol or sorbitol or both. Analytical results showed that 6 of 17 liquid protein products contained from 0.1 to 5.9 g of glycerol/30 mL of product. The latter would add 23.6 cal/serving. Nine of the liquid protein products contained sorbitol. The lowest level was 0.3 g/30 mL; the highest level was 7.5 g/30 mL of sample, which would contribute an additional 30 cal/serving. Three products contained neither glycerol nor sorbitol. None of the products contained xylitol.

The data available at this time do not provide any evidence of a relationship between the composition of liquid protein products and the deaths of persons ingesting them.

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Mass and Nuclear Magnetic Resonance Spectra of Some Alkylpyrazines

The mass spectra and NMR spectra of 11 alkylpyrazines were measured. The alkylpyrazines were synthesized from dihydropyrazine and the corresponding aldehyde or ketone. The major mass spectral fragmentation of the alkylpyrazines underwent McLafferty rearrangement. The compounds which showed similar mass fragmentation patterns were easily distinguished by NMR spectra.

Since the development of the direct combination of capillary gas chromatography and mass spectrometry (GC–MS), the comprehensive analysis of flavor extracts from certain foods has become practical. Mass spectral

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