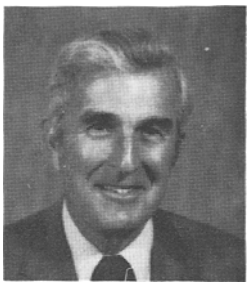

Chemistry and Characteristics of Enzyme-Modified Whipping Proteins

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ABSTRACT

Protein derivatives exhibiting aerating and whipping properties are produced by the action of a proteolytic enzyme on a suitable protein substrate, viz., wheat gluten, soy protein or casein. Various proteolytic enzymes can be utilized in the manufacture of the modified protein derivatives, but those enzymes displaying maximum proteolytic activity in the acid pH range produce whipping proteins with optimum aerating functionality and maximum stability characteristcs. Reaction parameters for the hydrolysis include: type of enzyme, enzyme concentration, temperature, pH and time of reaction. By careful control of these variables, modified proteins with unique and exceptional whipping properties can be produced. Enzyme-modified whipping proteins are bland, light cream-colored, spray-dried powders, soluble in hot or cold water, and functional over the entire pH range. Depending upon the source, protein and conditions of manufacture, products can be produced with a protein content ranging from as low as 50% to as high as 85%, and a whipping functionality equal to or twice that of egg albumen. Unlike egg albumen, these products do not coagulate to any appreciable degree when heated, and depending upon the specific end use of the product, this property may be either an advantage or disadvantage.

INTRODUCTION

Numerous articles have been written and many papers presented on the enzymatic hydrolysis of proteins as a convenient means of improving functional properties without diminishing nutritional values of proteins. The ultimate goal has been to produce bland, soluble, nutritious proteins with much improved functional properties, such as emulsion formation, fat absorption, film formation, and water absorption.

However, I shall confine my discussion solely to the manufacture, properties, and chemistry of enzyme-hydrolyzed proteins produced primarily for their whipping and foaming functionalities. Nutritional quality and flavor are, of course, desirable and important, but the primary objective of hydrolysis methods discussed here will be to produce proteins with optimum foam formation and foam stability.

Before proceeding further, it would be prudent to define precisely what we mean by an "enzyme-modified whipping protein." This is a class of protein products that have been modified, both in physical structure and chemical properties, by the action of a proteolytic enzyme and exhibit whipping and foaming properties.

MANUFACTURE

The early history of isolation of soy proteins records the investigations of Watts and Ulrich who, in 1939, prepared an active whipping substance from solvent-extracted soy flour. Despite the fact that the active principal in the whipping substance prepared by these investigators was probably a nonprotein, nitrogenous material, this early work is of scientific interest since it stimulated the develop-

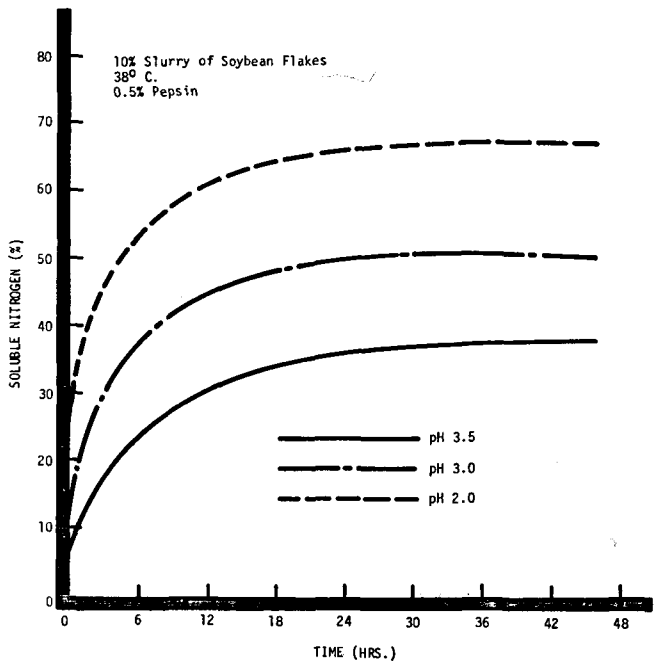

FIG. 1. Effect of papain concentration and temperature.

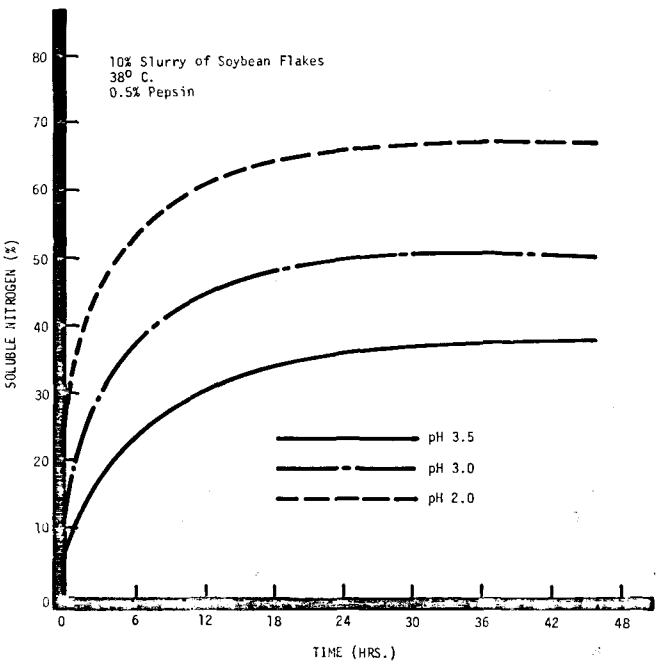

FIG. 2. Effect of reaction time and pH.

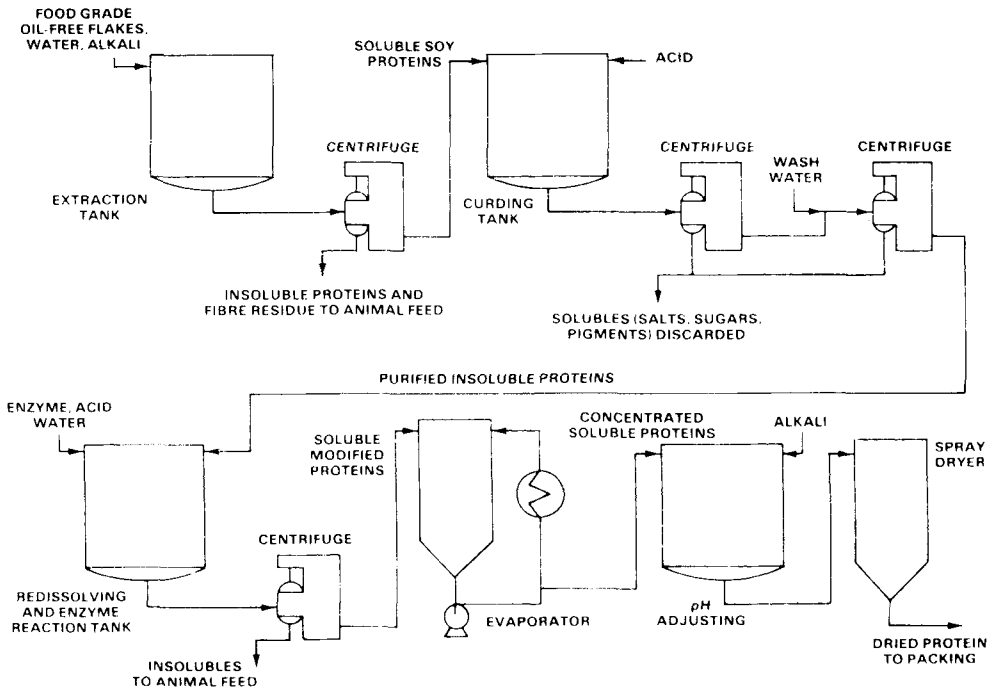

FIG. 3. Processing enzyme-modified soy protein - soy isolate process.

ment of processes and methods which led to the highly sophisticated whipping proteins and soy isolates which are available today. The severe shortage of egg albumen created by World War II gave further impetus to development and production of new type whipping proteins. Modified milk proteins produced by the alkaline hydrolysis of casein-and pepsin-modified soy protein whipping agents were commercially produced.

In the manufacture of these whipping agents, a number of proteins such as oil-free soy flakes or flour, soy protein isolate or concentrate, and wheat gluten may be utilized as the source material for the enzymatic hydrolysis. The particular substrate used will depend upon the product being produced and the specific process developed by the indivdual manufacturers.

Proteolytic enzymes commonly used are pepsin, papain, ficin, trypsin, and bacterial proteases. It should be pointed out that those enzymes possessing maximum activity in the acid pH range (pH 2.0 - 4.5) are more effective in producing proteins with maximum whipping functionality, whereas enzymes with maximum activity in the slightly acid to alkaline range (pH 5.0 - pH 8.0), although effective in producing proteins with some degree of foaming capacity, are inferior to the low pH-active enzymes. In many instances a combination of enzymes, generally used singly, one following the other, is often used to produce the desired whipping protein.

Hydrolysis parameters for the enzymatic reaction are pH, temperature, time, and concentrations of substrates and enzymes. It is important to recognize that modification behavior depends not only on the nature of the protein substrate and enzyme, but also on these hydrolysis parameters; all variables play a significant role in enzymatic hydrolysis. Figure 1 shows the effect of both papain concentration and temperature on the rate of hydrolysis as measured by soluble nitrogen at pH 4.5. It is indicated that a minimum enzyme concentration of 0.4 to 0.5% is required to produce a significant degree of nitrogen solubilization; higher concentrations solublize only a slightly larger amount of protein. Temperature of the reaction mix dramatically affects the hydrolysis, as evidenced by the fact that at 0.5% enzyme concentration, an 18 C increase in reaction temperature produced a 100% increase in soluble nitrogen from 30% to 60%.

Figure 2 demonstrates the effect of pH and reaction time in the digestion of soybean flakes with pepsin. In this digestion reaction, a critical stage of the hydrolysis is the first 3 to 12 hr during which time rapid hydrolysis occurs followed by a slow, linear digestion from ca. 12 to 48 hr. The effect of pH depends, of course, on the enzyme used, and, in this instance hydrolysis was not only more rapid but more complete at pH 2.0, as compared to the higher pHs of 3.0 and 3.5.

Enzyme-modified whipping proteins can be prepared by several methods from a number of different protein materials. Figure 3 shows a schematic drawing for the manufacturing process of a modified whipping protein using a soy isolate as the starting material. The isolate is prepared in the conventional manner by alkaline extraction of soybean flakes. The purified curded isolate is then subjected to enzyme hydrolysis for 12 to 24 hr under appropriate hydrolysis conditions to produce the functional protein. The hydrolysate is separated from the unreacted protein by centrifugation, concentrated and, following pH adjustment, is spray dried. Figure 4 represents the so called direct method of producing modified whipping proteins in which oil-free soybean flakes are first washed to remove a portion of the soluble salts and sugars, then treated directly with the enzyme without first isolating the soy protein. After enzyme digestion, the remaining process is essentially the same as in the preceeding process with separation of the soluble hydrolyzed fraction, concentration, pH adjustment and spray drying.

PROPERTIES

Typical chemical and physical properties of three different types of whipping agents are listed in Table I. As evidenced by the wide variation in properties of the three products, it is possible, by proper selection of substrate, enzyme and hydrolysis conditions, to "tailor-make" a whipping protein with just about any desired set of physical and chemical properties. Table II shows the essential amino acid analysis of the three whipping proteins. Generally, these products will have approximately the same

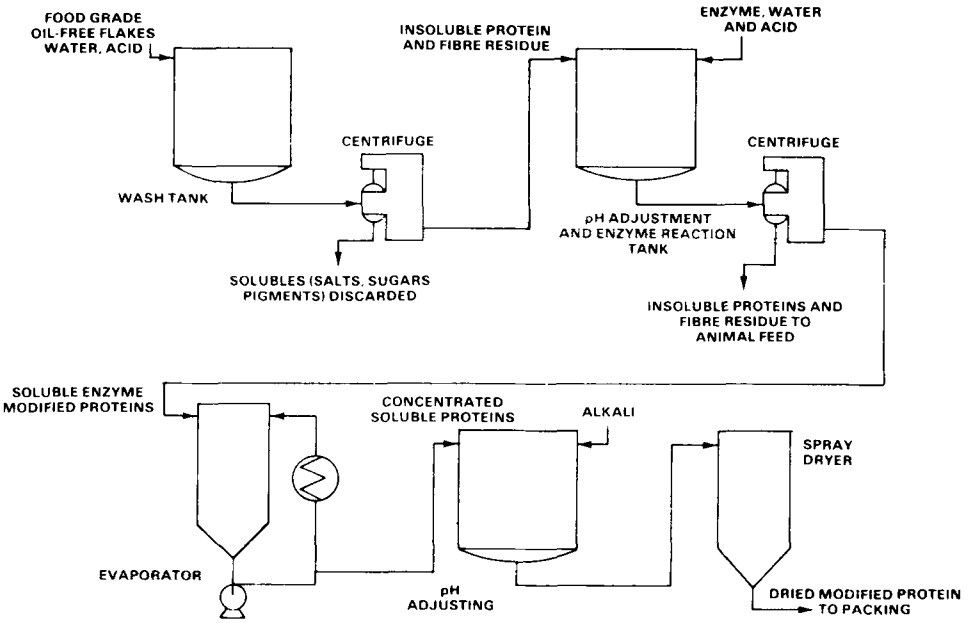

FIG. 4. Processing enzyme-modified soy protein - soybean flake process.

TABLE I

Typical Properties of Enzyme-modified Whipping Protein

	Soy Albumen	D-100WA	Hyfoama 66
Protein, %	65.0	81.0	77.7
Ash, %	15.0	17.0	7.8
Moisture, %	4.0	4.5	3.7
% Nitrogen-free-extract	16.0	1.5	10.8
Color	light cream	cream	cream
Flavor	slightly bitter	bland	bland
Density (lbs./cu. ft.)	25	25	24
Granulation	thru 80 m.	thru 80 m.	thru 80 m.
pH (5% solution)	4.8	5.2	6.6

TABLE II

Essential Amino Acid Analyses[a]

	Soy Albumen	D-100WA	Hyfoama 66
Valine	1.87	2.56	2.14
Leucine	4.37	5.14	4.38
Isoleucine	1.73	2.63	1.74
Phenylalanine	2.82	3.38	3.52
Tryptophane	0.64	0.60	0.31
Threonine	2.52	2.62	1.78
Methionine	0.98	0.81	0.92
Lysine	3.94	4.25	0.92

[a]G amino acid/16 g nitrogen.

amino acid profile as the base starting proteins, and it will be noted that the two products derived from the soybean, Soy Albumen, and D-100WA have the approximate same composition, with the exception of lysine content, as Hyfoama-66, a wheat-derived product.

There are numerous ways to test and measure whipping functionality of the enzyme-modified proteins. Since their primary function is whipping (or foaming), it is most important that a test method be used which fairly represents the conditions under which the product will eventually be used. Unfortunately, there is no single test which will evaluate the whippability of a product under all possible conditions of use. Many factors affect functionality of whipping proteins: the type of beater - completely different results are obtained in a horizontal type beater as compared to a vertical beater - presence of fats and oils - lipids inhibit the whipping function of all protein-whipping agents, and depending upon the particular system under study, even trace amounts of a fat or oil can seriously affect the whippability of a mix. Also, nearly all type emulsifiers have varying inhibitory effects on whippability of protein-whipping agents, and care must be exercised when the products are used in the presence of these materials.

Figures 5,6 and 7 show the comparative whip rates of several enzyme-modified proteins when compared with egg albumen and soy isolate in three different type whip tests. Figure 5 shows the comparative rate of whip in the first stage whip of a conventional corn syrup - sucrose confectionery frappe. The enzyme-modified product, Soy Albumen, not only whips faster and to a greater volume than egg albumen, but also does not exhibit the so called "beat down," which is characteristic of egg albumen in systems of this type. Figure 6 shows the comparative results of a test designed to measure whipping efficiency in a fat-

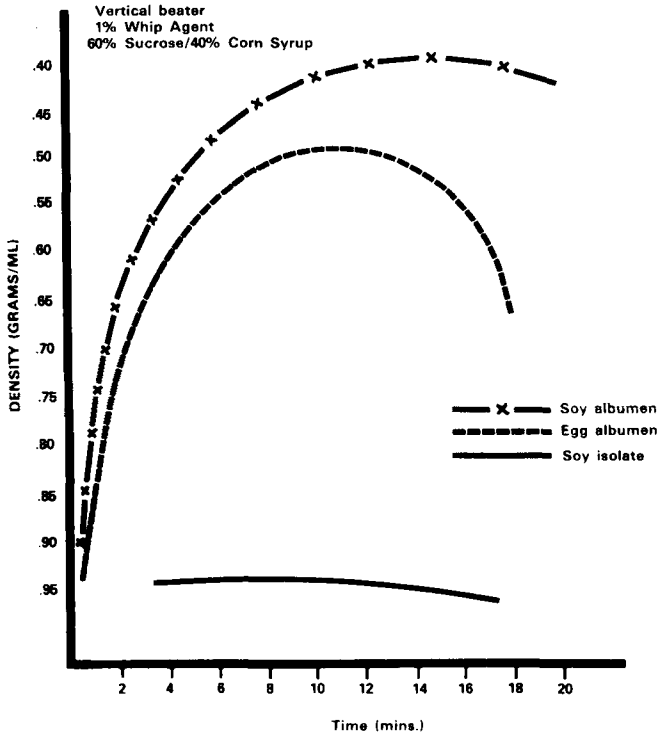

FIG. 5. Comparative whip rates in confectionery frappe.

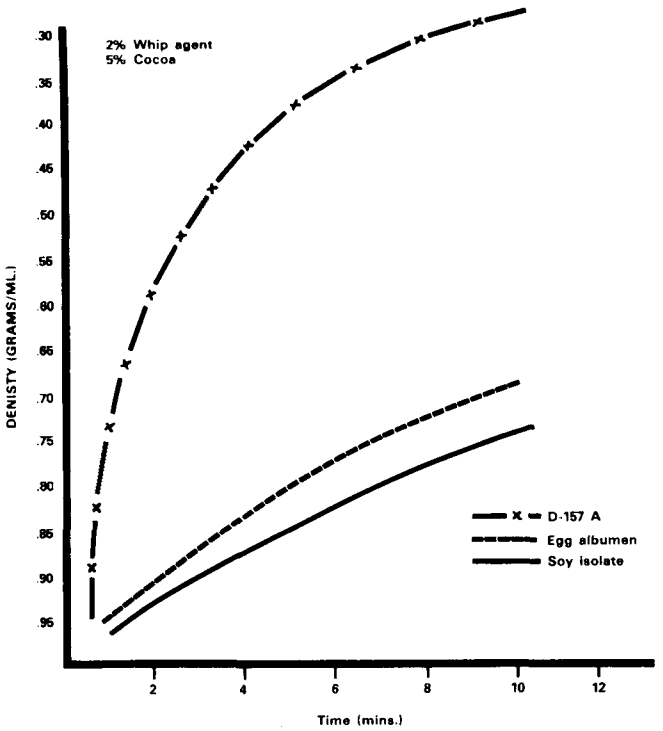

FIG. 6. Comparative whip rates in fat-inhibited chocolate chiffon.

inhibited chocolate chiffon dessert. Because of its instant solubility and greater fat tolerance, the modified vegetable protein, D-157A, very significantly outperforms egg albumen and the soy isolate. In Figure 7 an enzyme-modified, wheat protein product is compared with soy isolate and egg albumen in an emulsifier-inhibited system. As in the previous two tests, the enzyme-digested product whips much faster and to greater volume than does egg albumen. It will be noted that in all three tests, the

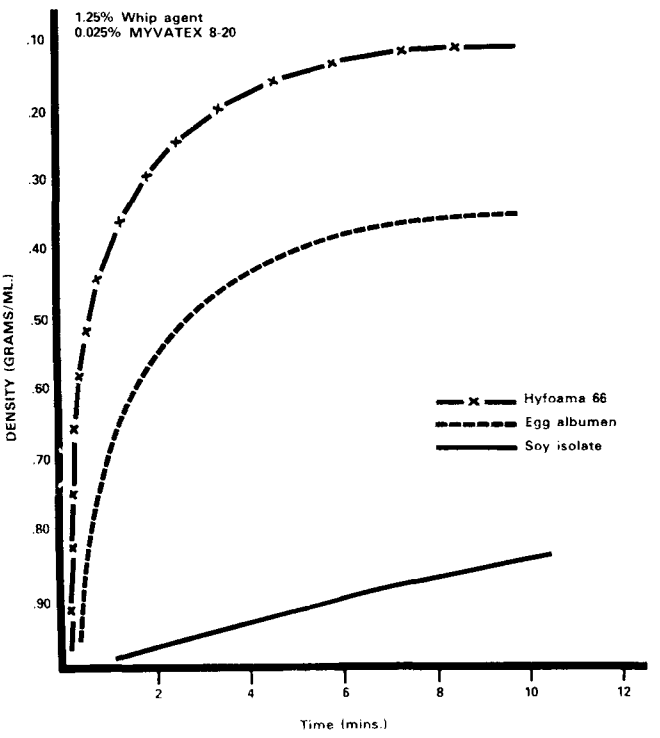

FIG. 7. Comparative whip rates in emulsifier-inhibited sucrose mix.

modified soy isolate exhibited very poor whipping and aerating properties.

CHEMISTRY

The progress of the enzymatic digestion of proteins can be monitored by a variety of techniques. One of the most common methods is removal of unmodified proteins, large polypeptides and enzyme by precipitation with trichloroacetic acid (TCA), followed by a suitable analysis of the TCA soluble polypeptides, peptides, and amino acids. The net result of digestion and analysis shows a progression from TCA insoluble proteins to TCA soluble peptides and amino acids.

Methods of analysis of the TCA soluble materials include the following: (a) spectrophotometric absorption at 280 nM, which measures the aromatic amino acids tyrosine, tryptophan and phenylalanine; (b) spectrophotometric absorption at 220 nM, which is representative of the peptide bonds; (c) trinitrobenzenesulfonic acid (TNBS) procedure, which measures free amino groups (1); (d) ninhydrin color reaction, which also identifies free amino groups (2); (e) total soluble nitrogen as assayed by the Kjeldahl technique.

A convenient method for direct measurement of peptide bond cleavage without prior protein precipitation is the pH-stat method (3). In this method, if the digestion is kept at a constant pH in the neutral or alkaline range by the addition of alkali, the alkali consumed is a direct measure of the peptide bonds hydrolyzed. This method is not feasible if the digestion is carried out in the acidic range or where the digestion pH varies.

The trinitrobenzenesulfonic acid procedure can also be applied as a direct measure of the primary amino groups formed during peptide bond cleavage (4). Some difficulties may be encountered with this determination where heterogeneous reaction mixtures are employed.

Using a standard set of reaction conditions, the hydrolysis parameter measured can be related to the total protein or nitrogen solubility in such a way that the rate and degree of digestion can be carefully controlled. Employing the

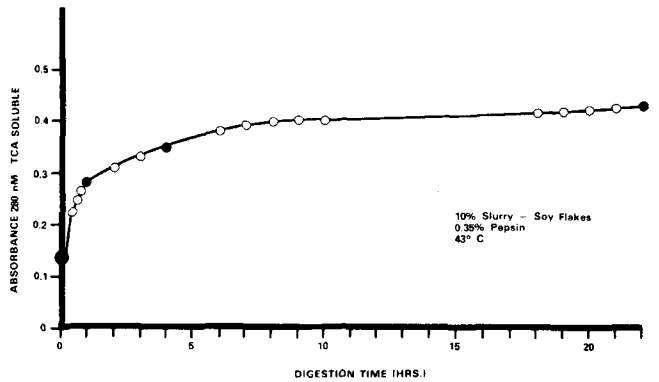

FIG. 8. Enzymatic digestion progress during soy albumen preparation.

pH-stat technique for digestion monitoring, Alder-Nissen (3) describes the degree of hydrolysis (DH) as the number of peptide bonds cleaved divided by the total number of peptide bonds, times 100. A molecular weight distribution of the products of enzymatic digestion can be assessed by gel filtration chromatography (1) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (5).

Figure 8 shows the digestion progression in the preparation of Soy Albumen. A 10% slurry of defatted soybean flakes was digested with 0.35% pepsin (1:10,000 activity). The initial pH was 2.3 and a temperature of 43 C was maintained. Samples were removed at various time intervals, precipitated with 2.5% TCA, and the absorbance at 280 nM measured on the supernatant after appropriate dilution. The absorbance at 280 nM is plotted vs. the digestion time in hours. The digestion is characterized by a rapid initial increase in absorbance between 0 and 5 hr, followed by a slower, almost linear increase from 5 to 22 hr. The closed circles in Figure 8 represent points at which samples of the digestion mixture were taken for molecular weight profiles.

Molecular weight spectra were obtained at 0, 1, 4 and 22 hr digestion times. The pHs of the samples for molecular weight characterization were adjusted to 7.3 and the samples then applied to a 2 X 95 cm column containing Sephadex G-50. The elution buffer contained 0.05 M sodium phosphate, 0.05 M sodium chloride, 0.02% sodium azide at pH 7.3. The absorbance at 280 mM was plotted vs. elution volume in ml. Sephadex G-50 has an effective protein fractionation range of 1,500 to 30,000 molecular weight, with those molecules of molecular weight greater than 30,000 essentially excluded. The column was calibrated with standard proteins of known molecular weight.

Prior to enzyme addition (0 hr), the mixture is characterized by species predominantly in the greater than 30,000 molecular weight region. With increasing digestion time, the quantity of these components is rapidly reduced with a corresponding increase in progressively smaller molecules. Thus, after 22 hr of digestion, 6% remain in the greater than 30,000 range, 2% in the less than 30,000 to 14,000 range, and 92% in the less than 14,000 molecular weight range.

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