Effects of Alkali Treatment and Heat Treatment in the Presence of Fructose on Digestibility of Food Proteins as Determined by an Immobilized Digestive Enzyme Assay (IDEA)

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An immobilized digestive enzyme assay (IDEA) system composed of two bioreactors, one containing pepsin and the other trypsin, chymotrypsin, and intestinal mucosal peptidases, was used to assess changes in protein digestibility resulting from alkali treatment or heat treatment in the presence of fructose. The degree of amino acid racemization and the amount of lysinoalanine (LAL) formed due to alkali treatment were determined. A linear correlation ($r = 0.84$) was obtained between loss of digestibility and the sum of the effects of both LAL formation and racemization. The effect of Maillard reactions on both digestibility, as measured with the IDEA system, and available lysine also was assessed. A linear correlation ($r = 0.87$) between loss of digestibility and loss in available lysine was obtained. These data indicate that the IDEA system is quite sensitive to protein modifications resulting from alkali treatment or Maillard reactions and may be a good predictor of in vivo digestibilities of processed proteins.

INTRODUCTION

Processing and storage of food proteins can result in damage to their nutritional quality. Such adverse effects are accentuated by alkali and/or heat treatments or by heating in the presence of sugars. Thus, exposure to alkali or high temperatures is known to cause cross-linking, e.g. by formation of lysinoalanine (for recent reviews see: Friedman, 1977; Mauron, 1982; Swaisgood and Catignani, 1982; Maga, 1984) and racemization (cf.: Friedman, 1977; Masters and Friedman, 1980; Swaisgood and Catignani, 1982; Maga, 1984). The Maillard reaction, which has been studied extensively (e.g. see: Adrian, 1982; Feeney and Whitaker, 1982), is initiated by a carbonyl–amine reaction between sugars and the ϵ-amino group in proteins and will proceed under relatively mild conditions such that increasing loss of bioavailable lysine will continue during storage.

Soluble proteolytic enzymes have been used in many studies to ascertain nutritional qualities of proteins (for recent reviews see: Stahmann and Woldegiorgis, 1975; Bodwell, 1977; Satterlee et al., 1977; Marable and Sanzone, 1981). Recently we developed a method using two bioreactors: one containing immobilized pepsin and the other immobilized pancreatic and intestinal mucosal proteinases and peptidases for determination of protein digestibility (Porter et al., 1984). Use of immobilized forms of proteinases has many advantages including (1) greatly increased catalytic stability chiefly due to elimination of autolysis, (2) conservation of enzyme permitting use of enzymes having limited availability, and (3) ease of separation of the products of the reaction from the catalyst.

In this study, the method was used to investigate the effects of amino acid racemization, formation of lysinoalanine, and Maillard reaction on protein digestibility. The immobilized digestive enzyme assay system (IDEA) was very sensitive to changes in protein structure resulting from exposure to alkali or heating in the presence of sugar.

EXPERIMENTAL SECTION

Materials. Controlled-pore glass beads (200-nm pore diameter, 80/120 mesh; 53-nm pore diameter, 120/200 mesh), used for enzyme immobilization, were obtained from Electro-Nucleonics. Porcine pepsin, trypsin, chymotrypsin, and intestinal mucosal peptidases were from Sigma Chemical Co. The intestinal peptidases were further purified by adsorption to and elution from DEAE-Sephacel (Porter et al., 1982).

Synthetic substrates used for assay of specific enzyme activities were benzoyl-L-tyrosine ethyl ester (BTEE), (*p*-tolylsulfonyl)-L-arginine methyl ester (TAME), and L-leucylglycine obtained from Sigma Chemical Co. and Gly-Gly-Phe-Phe ethyl ester purchased from Vega Biochemical Co. The protein substrates were α-lactalbumin and bovine serum albumin (BSA) obtained from Sigma Chemical Co., soy protein isolate (Supro 620) from Ralston Purina Co., and wheat protein isolate (#1200) from Manidra Protein Corp. β-Lactoglobulin was purified from milk by the method listed by McKenzie (1971), followed by elution from a DEAE-Sephacel column (1.0 cm × 55 cm) with a salt gradient of 0.01–0.05 M. The preparation was homogeneous for genetic variants A and B as determined by polyacrylamide gel electrophoresis.

Only reagent grade chemicals were used and included 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC) and fructose from Sigma Chemical Co., amino acid standards and *o*-phthalaldehyde (OPA) from Pierce Chemical Co., 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (CMC) and (3-aminopropyl)triethoxysilane from Aldrich Chemical Co., and lysinoalanine (LAL) from Miles Laboratories.

Enzyme Immobilization. Controlled-pore glass (CPG) beads were derivatized with (3-aminopropyl)triethoxysilane as previously described (Swaisgood et al., 1976; Janolino and Swaisgood, 1982). The aminopropyl–CPG was succinylated in acetone (Porter et al., 1984) to give succinamidopropyl–CPG. Pepsin was immobilized on aminopropyl–glass by activation of enzyme carboxyl groups with CMC as described by Porter et al. (1984). The immobilized trypsin, chymotrypsin, and intestinal peptidases were the same preparations previously described by Porter et al. (1984), which were covalently bound to succinamidopropyl–CPG by activation of the matrix carboxyl groups with EDC.

Enzyme Assays. Individual enzyme activities were assayed by using specific substrates and the microcirculation reactor system described by Taylor and Swaisgood (1980). Immobilized trypsin (150 μL of beads) was assayed at 25 °C by recirculation of 3.0 mL of 1.0 mM TAME in

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0.04 M Tris buffer, pH 7.5, containing 0.01 M $CaCl_2$, and the absorbance change was monitored at 247 nm. Immobilized chymotrypsin (150 μL of beads) was assayed similarly with 0.47 mM BTEE in 0.043 M Tris, pH 7.5, containing 0.05 M $CaCl_2$, and the absorbance change was monitored at 256 nm. A fluorometric OPA assay was used to measure immobilized pepsin and peptidase activity (Porter et al., 1982). Pepsin was assayed at 40 °C by recirculation of 3 mM Gly-Gly-Phe-Phe-OC_2H_5 in 0.04 M sodium citrate at pH 4.0. Peptidase activity was determined using 5 mM Leu-Gly as described by Porter et al. (1982) except the temperature was 37 °C.

Alkali Treatment of Proteins. With the exception of wheat protein isolate, 3 mg/mL of stock solutions of the proteins was prepared in 0.2 M NaOH, sealed in ampules, and heated for varying times at 40 °C. Following heating, the solutions (10 mL) were neutralized with 6 M HCl, 0.15 mL of 6% sodium azide was added, and the pH was adjusted to 2.0 with 1 M HCl and the volume to 15 mL. The solutions were filtered through 0.45-μm membranes or Whatman #1 filter paper (soy protein isolate). For the case of wheat protein isolate, 20 mg/mL solutions in 0.2 M NaOH were heated as above, followed by neutralization and adjustment to pH 2.0. These solutions were filtered through Whatman #1 filter paper and concentrated with an Amicon YM 10 membrane, and 0.15 mL 6% sodium azide was added to 15 mL of the solution.

The protein concentrations of the resulting solutions (2 mg/mL) were determined from their absorbancies at 280 nm using $E^{1mg/mL}$ values of 0.94 for α-lactalbumin (Swaisgood, 1982), and 0.67 for BSA (Goodno et. al., 1981). The concentrations of soy protein isolate and wheat protein isolate were determined by reaction with OPA using a fluorometric protein assay (Goodno et al., 1981).

Maillard Reaction with Fructose. Proteins were dissolved (3 mg/mL) in 0.5 M D-fructose solutions containing 0.01 M sodium phosphate, pH 7.0 (β-lactoglobulin and BSA), or 0.01 M sodium borate, pH 7.0 (α-lactalbumin), and 10 mL was sealed in ampules and heated for various times at 90 °C. Following heating, a 0.4 mL-portion was withdrawn for measurement of available lysine and the remaining 9.6 mL was prepared for pepsin digestion by dilution with distilled water, addition of 0.145 mL of 6% sodium azide, adjustment to pH 2.0 and a final volume of 14.5 mL, and filtration through a 0.45-μm membrane. To solubilize the soy protein isolate, it was first dissolved in 0.2 M NaOH at room temperature and then adjusted to pH 7.0 with 6 M HCl and filtered through Whatman #1 filter paper. Protein concentrations were determined as described above.

Determination of Digestibility. Digestibilities were measured with the IDEA system described by Porter et al. (1984). This system consists of two bioreactors, one containing 1.0 mL of immobilized pepsin and the other containing 0.5 mL each of a mixture of immobilized trypsin and chymotrypsin and 1.0 mL of immobilized intestinal peptidases (Porter et al., 1984; Swaisgood and Catignani, 1985).

Protein substrate solutions were first adjusted to pH 2.0 and digested with the pepsin bioreactor. The reactor was rapidly rinsed with 5 mL of the protein solution prior to assaying, and digestion was accomplished by recirculation of 9 mL at 37 °C for 20 h. Between assays the pepsin reactor was washed successively with 100 mL of 4 M urea, pH 2.0, and 100 mL of 0.01 M HCl at 4 °C and stored in the latter solution containing 0.06% sodium azide. The pepsin hydrolysate was adjusted to 50 mM sodium phosphate or 20 mM sodium borate (wheat protein isolate and α-lactalbumin), pH 7.5, before being subjected to hydrolysis in the trypsin–chymotrypsin–intestinal peptidase reactor. This reactor also was rapidly rinsed with a 5-mL portion of the hydrolysate prior to digestion of the remaining solution by recirculation at 37 °C for 20 h. Between assays the reactor was washed successively with 100 mL of 4 M urea in 10 mM sodium phosphate, pH 7.0, and 100 mL of the phosphate buffer at 4 °C and stored in the phosphate buffer containing 0.06% sodium azide.

Proteolysis was quantitated by direct measurement of the released α-amino groups by using a spectrophotometric OPA assay (Porter et al., 1984; Church et al., 1983, 1985). Digestibility is defined as the fraction of the total peptide bonds hydrolyzed and was calculated from the following relationships (Porter et al., 1984)

$$\text{digestibility} = n/n_0 = M_r\Delta A_{340}/d\epsilon n_0[\text{P}] = M_r\Delta A_{340}/115 n_0[\text{P}] \quad (1)$$

where M_r, d, ϵ, and [P] are the protein molecular weight, dilution factor, molar absorptivity, and protein concentration (mg/mL), respectively. For mixtures of proteins, the following relationship can be used for the dilution factor employed (1:51):

$$\text{digestibility} = n/n_0 = m_{0,w}\Delta A_{340}/d\epsilon[\text{P}] = \Delta A_{340}/[\text{P}] \quad (2)$$

Determination of Racemization. The degree of racemization of phenylalanine was measured with the immobilized D-amino acid oxidase–catalase reactor system previously described (Chung et al., 1985). Alkali-treated proteins were acid hydrolyzed, an aliquot was treated with the reactor system to completely oxidize the D-amino acids, and both samples were analyzed with an amino acid analyzer. The difference in values for sample pairs represents the amount of D form present.

Determination of Available Lysine. An OPA fluorometric method described by Goodno et al. (1981) was used to measure the available lysine. The relative fluorescence, RF, is related to the total amount of α- and ε-amino groups that reacts with OPA by the equation

$$\text{RF} = n_\alpha I_\alpha^\circ + n_\epsilon I_\epsilon^\circ \quad (3)$$

where n_α and n_ϵ are the moles of α- and ε-amino groups in the sample and I_α° and I°_ϵ are the relative fluorescences per mole. Thus, the change in available lysine is related to the change in relative fluorescence by

$$n_\epsilon - n_\epsilon' = \Delta n_\epsilon = \text{RF}/I_\epsilon^\circ = \text{RF}/2.41 \times 10^8 \text{ mol}^{-1} \quad (4)$$

where n_ϵ' is the moles of available lysine in the treated protein. The value for I_ϵ° is that given by Goodno et al. (1981). The fluorescence of a 10-μL sample reacted with 3 mL of OPA reagent was measured relative to 0.1 g/mL of quinine sulfate in 0.1 N H_2SO_4.

Determination of Lysinoalanine. The amount of lysinoalanine in treated proteins was measured by amino acid analysis of acid hydrolysates. An LAL standard, 100 nmol/mL, was used to calibrate the analyzer.

RESULTS

Characteristics of IDEA Bioreactors. The kinetic characteristics of immobilized pepsin were very similar to those of the soluble form. Michaelis constants determined for the substrate Gly-Gly-Phe-Phe-OC_2H_5 at pH 4.0 and 40 °C were 2.5 and 1.8 mM for soluble and immobilized enzymes, respectively. These values are in excellent agreement with that for the soluble enzyme reported by Hollands et al. (1969) for this substrate under these conditions (1.6 mM). The immobilized pepsin also was found to be very stable, with observed activities being unchanged

Table I. Initial Activities and Activities at the Conclusion of This Study for the Immobilized Enzymes Used in the IDEA Reactor System

enzyme	substrate	activity,[a] U/mL init	activity,[a] U/mL final	no. of assays
trypsin	TAME	19.3	17.0	35
chymotrypsin	BTEE	1.5	1.2	35
peptidases	Leu-Gly	0.13	0.11	35
pepsin	Gly-Gly-Phe-Phe-OEt	6.6[b]	4.6[b]	35

[a] One unit of activity is defined as 1 μmol/min of substrate hydrolyzed. [b] One unit of activity is defined as 1 ΔRF/min resulting from substrate hydrolysis and reaction of the α-amino group with OPA.

by use at temperatures of 55 °C or in 4 M urea solutions. Temperature studies up to 55 °C yielded linear Arrhenius plots, giving an activation energy of 12.9 kcal/mol.

Previous studies had suggested that immobilized trypsin, chymotrypsin, and intestinal peptidase activities also are quite stable (Porter et al., 1984). The data in Table I indicate that all of the immobilized enzyme activities were very stable during all of the assays performed in this study.

Effects of Alkali Treatment of Food Proteins. The digestibilities as measured with the IDEA system were quite sensitive to changes caused by alkali treatment as shown by the data for three animal and two plant protein preparations (Figure 1, Table II). In several studies, digestibilities were determined in quadruplicate and results indicated a standard error of about 5%, which is similar to that observed by Porter et al. (1984). Even a 2-h exposure to high pH at 40 °C caused losses in digestibility ranging from 7% for β-lactoglobulin to 24% for soy protein isolate.

Alkali treatment of proteins can result in both racemization of amino acid residues and cross-linking of the polypeptide chain due to formation of LAL, each of which can reduce the protein's digestibility. Data in Table II and Figure 2 show that appreciable amounts of both reactions occurred under the conditions used. Comparison of Figures 1 and 2 indicates that the degree of these reactions in the alkali-treated proteins paralleled the loss in digestibility. The data for racemization of Phe in these samples was previously reported (Chung et al., 1985) but are included (Table II) for purposes of comparison. The relative rates of racemization of various residues follow a linear free energy relationship with the inductive nature of the side chain attached to the asymmetric carbon; therefore, in general, the order followed is Ser, Asp > Cys > Phe, Met, Glu, Thr > Ala, Tyr > Leu > Val, Ile (Friedman and Liardon, 1985; Jenkins et al., 1984; Schwass and Finley, 1984; Liardon and Hurrell, 1983; Masters and Friedman; 1979). In this study, Phe was selected as an indicator of racemization because it represents an intermediate rate and it was readily measured by the method used.

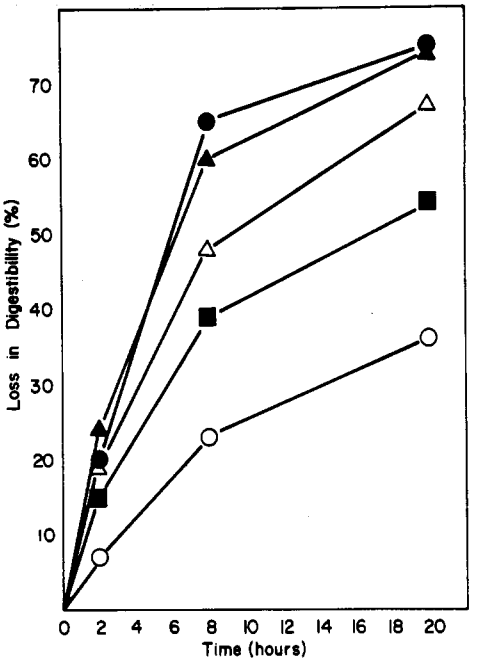

Figure 1. Effect of alkali treatment on protein digestibility as determined with the IDEA system. Proteins (3 mg/mL, except wheat protein isolate) were incubated in 0.2 M NaOH at 40 °C. Key: ●, α-lactalbumin; ○, β-lactoglobulin; ▲, soy protein isolate; △, bovine serum albumin; ■, wheat protein isolate.

Effects of Maillard Reactions. Data in Figure 3 indicate that appreciable losses of digestibility and available lysine occurred during incubation of food protein in 0.5 M fructose at 90 °C. For example, only a 2-h exposure

Table II. Effect of Alkali Treatment of Food Proteins[a]

protein	treatment time, h	digestibility[b]	% racemization[c] (Phe)	LAL[b] mg/g of protein	LAL[b] mol/mol of Lys
α-lactalbumin	0	0.51	0	0	0
	2	0.41	10	66.1	0.50
	8	0.18	20	78.3	0.60
	20	0.13	45	83.4	0.64
β-lactoglobulin	0	0.44	0	0	0
	2	0.41	8	13.9	0.11
	8	0.34	15	21.5	0.16
	20	0.28	40	28.1	0.21
BSA	0	0.54	0	0	0
	2	0.44	10	50.7	0.34
	8	0.28	16	55.8	0.37
	20	0.18	41	64.7	0.43
soy protein isolate	0	0.42	0	0	0
	2	0.32	21	5.2	0.06
	8	0.17	40	8.2	0.10
	20	0.11	53	11.6	0.14
wheat protein isolate	0	0.46	0	0	0
	2	0.39	14	3.1	0.14
	8	0.28	32	4.3	0.19
	20	0.21	48	5.3	0.24

[a] Proteins were incubated in 0.2 M NaOH at 40 °C. [b] Average of duplicate determinations. [c] From Chung et al. (1985). Calculated from the relationship $2D/(D + L) \times 100$.

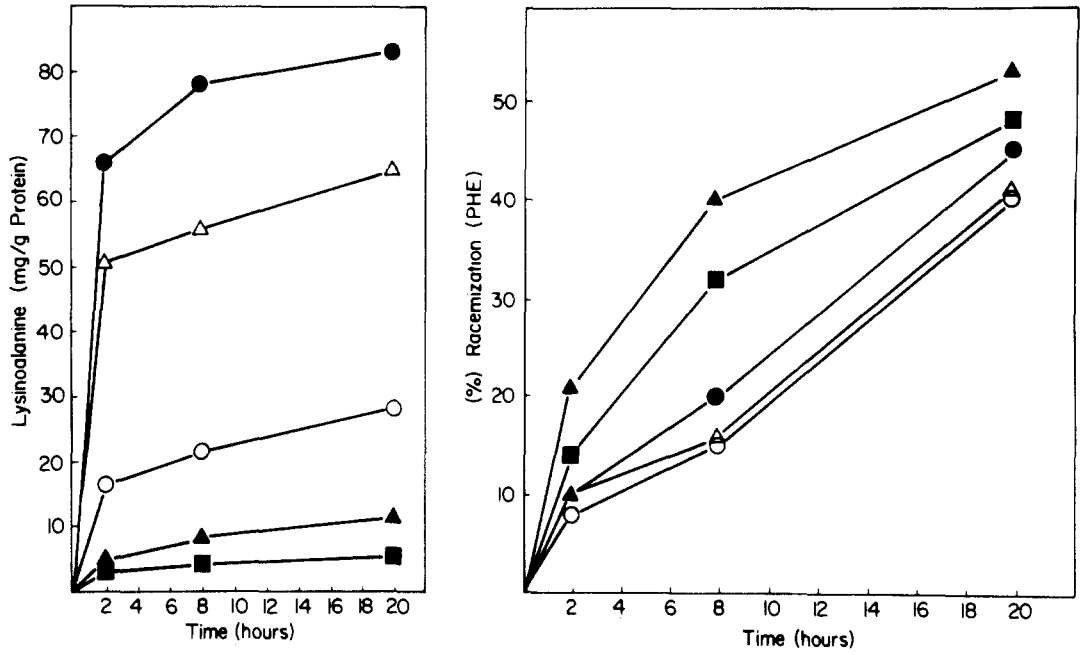

Figure 2. (Left) Formation of lysinoalanine (LAL) due to alkali treatment of proteins by incubation in 0.2 M NaOH at 40 °C. LAL was determined by standard amino acid analysis of acid hydrolysates. Key: ●, α-lactalbumin; ○, β-lactoglobulin; ▲, soy protein isolate; △, bovine serum albumin; ■, wheat protein isolate. (Right) Racemization of Phe due to alkali treatment. Data were taken from Chung et al. (1985). Legend is the same as that in the left panel.

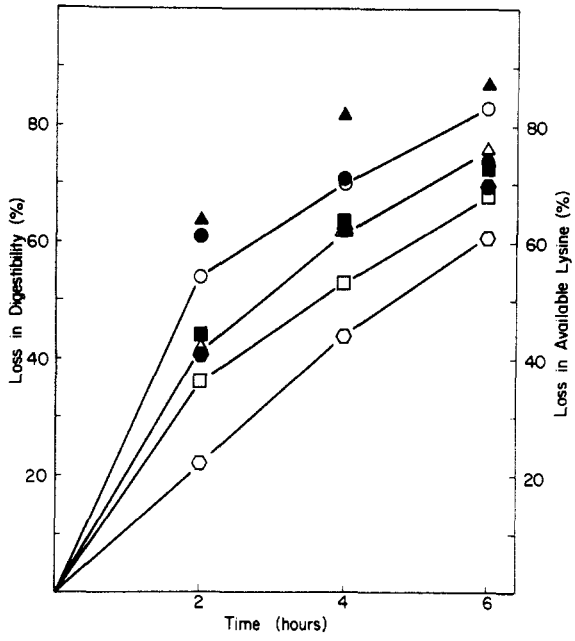

Figure 3. Effect of Maillard reactions on digestibility as determined with the IDEA system and on available lysine. Proteins (3 mg/mL) were incubated with 0.5 M fructose at pH 7.0 for various times at 90 °C. Open symbols denote percent loss in digestibility and closed symbols the percent loss in available lysine. Key: ○,●, soy protein isolate; △,▲, bovine serum albumin; □,■, β-lactoglobulin; ○,●, α-lactalbumin. For the sake of clarity, lines are used only to connect percent loss in digestibility data points.

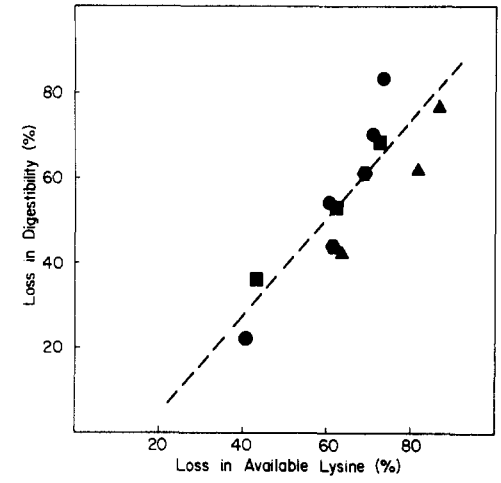

Figure 4. Relationship between percent loss in digestibility as measured with the IDEA system and percent loss in available lysine. Key: ●, soy protein isolate; ▲, bovine serum albumin; ■, β-lactoglobulin; ●, α-lactalbumin. The dashed line represents the linear regression given by $Y = -19.2 + 1.14X$, correlation coefficient 0.87.

resulted in losses of digestibility ranging from 22% to 54% for α-lactalbumin and soy protein isolate, respectively. The relationship observed between loss in digestibility and loss of available lysine is shown in Figure 4. A direct relationship between these two parameters is apparent, with a linear correlation coefficient of 0.87 having been obtained.

DISCUSSION

Both the formation of cross-links in the protein chain and the racemization of amino acid residues, which are catalyzed by exposure to alkali (Friedman, 1977; Masters and Friedman, 1979; Maga, 1984), would be expected to inhibit the ability of proteinases and peptidases to catalyze peptide bond hydrolysis. Alkali conditions employed in this study caused appreciable amounts of both reactions to occur: 0.06–0.64 mol of LAL/mol of Lys and 8–53% racemization of Phe. Considerable variation between proteins was observed in the amounts of LAL formation and racemization. Thus, the two plant proteins, soy protein and wheat protein isolate, racemized more rapidly, whereas α-lactalbumin and BSA exhibited more rapid formation of LAL. Variation of the relative rates of these reactions among individual proteins was previously recognized; for example, it was established that while the relative rates of racemization of individual residues followed linear free energy relationships within a particular protein, the relationship could not be used to predict the

rate of racemization of that residue in another protein (Masters and Friedman, 1979; Masters and Friedman, 1980; Schwass and Finley, 1984; Friedman and Liardon, 1985). Thus, the lower rate of racemization of residues in α-lactalbumin as compared to soy protein was noted previously (Masters and Friedman, 1979, 1980).

The rate of LAL formation vis-a-vis racemization is inversely related for the protein studied. The animal proteins BSA and α-lactalbumin rapidly formed large amounts of LAL (0.34 and 0.50 mol/mol of Lys at 2 h, respectively) compared to the plant proteins, soy protein, and wheat protein isolate (0.06 and 0.14 mol/mol of Lys at 2 h, respectively), whereas the rate of Phe racemization was greater for the plant proteins as compared to the animal proteins. Others have noted similar differences in the susceptibility to LAL formation between soy protein and milk proteins (Masters and Friedman, 1979; de Rham et al., 1977; Hasegawa et al., 1981; de Groot and Slump, 1969; Schwass and Finley, 1984). The reason for this inverse relationship between rates of racemization and extents of LAL formation is not completely clear; however, it has been suggested that fully denatured proteins racemize more readily, whereas some structure promotes LAL formation (Schwass and Finley, 1984). The asymmetric carbon would be completely accessible to nucleophilic attack and subsequent proton addition from either side, thus favoring racemization in a random-coil conformation. On the other hand, local concentrations of ε-amino groups and dehydroalanyl residues (formed from cysteine, cystine, or phosphoserine) potentially could be greater when some structure exists. Thus, α-lactalbumin, β-lactoglobulin, and BSA, which are relatively stable proteins, would be expected to have more structure under the alkaline conditions than wheat or soy proteins. However, factors other than structure could contribute to differences in local effective concentrations of ε-amino groups and dehydroalanyl residues. For example, both soy and wheat protein isolates have low lysine concentrations. However, the lysine, half-cystine, and serine concentrations of β-lactoglobulin are similar to those of α-lactalbumin (Swaisgood, 1982), but its susceptibility to LAL formation is similar to that of soy protein (0.11 mol/mol of Lys at 2 h).

Earlier studies have noted reductions in digestion of alkali-treated proteins by pepsin (Hayashi and Kameda, 1980), digestion by trypsin and chymotrypsin (Friedman et al., 1981), or digestion with a mixture of proteinases (Bunjapamai et al., 1982). Comparison of the data in Figures 1 and 2 suggests that both racemization and cross-linking contribute to loss of digestibility as expected. Thus, α-lactalbumin and soy protein isolate lost digestibility most rapidly; that of the former apparently due to cross-linking while that of the latter was more affected by racemization. The possible additivity of these two effects was tested by examining the relationship between loss of digestibility and the sum of the percent racemization and the percent lysine converted to LAL. This relationship, shown in Figure 5, is characterized by a linear correlation of 0.84 and an intercept of approximately zero, which suggests that these two effects are additive and that both of these alterations in a protein's structure would affect its biological availability.

Conditions used to enhance carbohydrate–protein reactions should not have caused significant racemization or cross-linking (Liardon and Hurrell, 1983). Thus, the observed decreases in digestibility are a result of Maillard reactions. In contrast to the effects of racemization and cross-linking, a lag between loss in digestibility and loss in available lysine due to Maillard reactions was observed

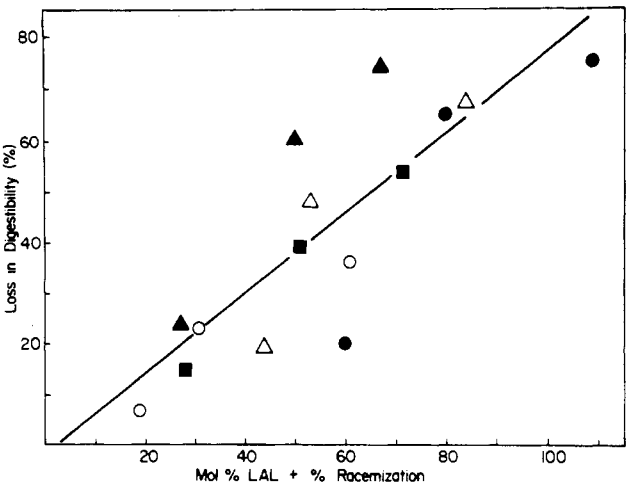

Figure 5. Relationship between percent loss in digestibility as measured with the IDEA system and the sum of effects of LAL formation and racemization. The sum of these effects was calculated as the mol % of LAL plus percent racemization of Phe. Key: ○, β-lactoglobulin; ●, α-lactalbumin; △, bovine serum albumin; ▲, soy protein isolate; ■, wheat protein isolate. The line represents the linear regression given by $Y = -2.2 + 0.789X$, correlation coefficient 0.84.

(Figure 4). Thus, it appears that some threshold level of modification must occur before digestibility significantly decreases. Linear extrapolation suggests a value of about 20% loss in lysine. The apparent difference between the effects of Maillard reactions and those caused by alkali treatment can be rationalized by noting that racemization or cross-linking would affect hydrolysis of residues greatly extended beyond the site of modification due to steric factors and the extended binding sites of many proteinases; however, modification of a lysyl residue largely affects only trypsin until a sufficient number are modified to sterically limit access to the protein's surface.

In vitro assays have been used in attempts to predict both the degree of protein modification resulting from processing and the digestibility of processed proteins. A mixture of Pronase and kidney microsomal peptidases has been used to indicate the extent of Maillard reactions and to prepare hydrolysates in which lactuloselysine and fructoselysine were identified following storage of ultra-high-temperature processed milk (Moller et al., 1977 a,b). Anderson et al. (1984) reported a correlation between results from in vitro digestion with Pronase and in vivo digestibility of proteins heated in the presence of gossypol. However, their method required release of free lysine, and therefore the assays consistently underestimated the in vivo digestibilities. A method for assessment of protein digestibility by enzymatic hydrolysis, which used simultaneous dialysis to quantitate the digestion products, has been reported (Gauthier et al., 1982). The calculations were based on the amount of nitrogen appearing in the dialysate following a 24-h digestion with pancreatin of a sample pretreated with pepsin. However, the 24-h value appears to be an arbitrary point obtained from a nonlinear progress curve. Furthermore, flux through the membrane would depend upon both the concentration gradient across the membrane and the size of the peptides released and, thus, on the rate of dialysate renewal and the primary structure of the particular protein being hydrolyzed. In addition, 8% of the protein included in their digestibility assay was the proteolytic enzymes; hence, the accuracy would be affected by autolysis. Finally, the changes in digestibility due to exposure to pH 12 for 4 h at 80 °C were much less than those noted previously (Friedman et al.,

1981) or that expected from the amount of racemization and LAL formation occurring under similar conditions (Jenkins et al., 1984; Friedman and Liardon, 1985; Schwass and Finely, 1984).

Previously, we have shown a linear relationship between digestibilities determined with the IDEA system and in vivo digestibilities for several plant and animal proteins (Porter et al., 1984). The data provided here indicate that the IDEA system also might be useful for prediction of in vivo digestibilities of processed proteins. This system eliminates the disadvantages of the enzymatic assays discussed above, namely (1) there is no autolysis so autolysis products do not interfere with the assay, (2) the actual fraction of peptide bonds hydrolyzed is measured and thus more accurately reflects digestion than does release of a specific free amino acid or diffusion rates through membranes, (3) the extent of hydrolysis is measured rather than rate so the method is not dependent on ratios of enzyme activities, and (4) the changes in digestibility recorded by this assay correlate with known degrees of protein modification.

The durability of the IDEA reactor system is affirmed by the data in Table I, which indicate that trypsin, chymotrypsin, and intestinal peptidases maintained greater than 80% of their original activity and pepsin 70% of its initial activity after 35 assays. Furthermore, these reactors were used over a period of several years. The stability of these reactors suggests the possibility of using human enzymes in routine assays that might more nearly reflect bioavailability in the human.

Registry No. LAL, 23250-50-2; NaOH, 1310-73-2; L-Phe, 63-91-2; L-Lys, 56-87-1; pepsin, 9001-75-6; trypsin, 9002-07-7; chymotrypsin, 9004-07-3; peptidase, 9031-96-3; D-fructose, 57-48-7.

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