esters of 3-hydroxyhexanoic and 3-hydroxyoctanoic acid (cf. Figures 1 and 2).

With this complex ester composition the aroma distribution of mountain papaya is different from that of the common papaya fruit, *C. papaya*, in which a few esters represent only minor constituents (Idstein and Schreier, 1984, 1985b). As to the volatiles, the species are connected by the occurrence of benzyl isothiocyanate, a well-known and characteristic constituent of Caricaceae (Tang et al., 1972; Flath and Forrey, 1977; Idstein and Schreier, 1985b). In mountain papayas, additionally, 2-phenethyl isothiocyanate could be identified (cf. Table I).

As to the volatiles listed in Table I, the aroma composition does not show remarkable features; except for the thiophene derivatives, which have been found recently in different tropical fruit aromas (Idstein and Schreier, 1984), the substances often occur among plant volatiles.

Finally, it has to be pointed out that sensory tests during this study were only carried out checking the sample preparation steps. The concentrated distillation extract showed the typical sensory properties of fresh fruit, but they were destroyed after silica gel fractionation. Whereas fraction I was practically odorless, fraction II showed a fruity odor. Fraction III with its heavy, adherent floral–fruity odor was most likely to correspond to the original fruit odor.

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Registry No. Ethyl 3-mercaptopropanoate, 5466-06-8; butyl (*E*)-2-butenoate, 591-63-9; hexyl (*E*)-2-butenoate, 1617-25-0; butyl 2-furoate, 583-33-5; ethyl 2-methylbenzoate, 87-24-1; butyl 2-hydroxybenzoate, 2052-14-4; butyl nicotinoate, 6938-06-3; butyl acetate, 123-86-4; methyl butanoate, 623-42-7; ethyl butanoate, 105-54-4; ethyl acetate, 141-78-6; 3-methylbutyl acetate, 123-92-2; hexyl acetate, 142-92-7; butyl butanoate, 136-60-7; butyl benzoate, 136-60-7; ethyl 3-hydroxyhexanoate, 2305-25-1; ethyl 3-hydroxyoctanoate, 7367-90-0.

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Racemization Kinetics of Amino Acid Residues in Alkali-Treated Soybean Proteins

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Exposing soy proteins to alkaline conditions (pH 8–14), for various time periods (10–480 min) and temperatures (25–95 °C at 10 °C intervals), induced increasing racemization of L-amino acid residues to D isomers. Relative susceptibilities of most amino acids were correlated with a linear free energy relationship based on plots of the ratio of the logarithm of the rate of racemization of any amino acid to that of alanine vs. σ^*, a parameter that measures electron-donating inductive effects of amino acid side chains. Heats and entropies of activation for selected amino acids were obtained from Arrhenius plots. The values for protein-bound amino acids are compared to corresponding values for free amino acids. Mechanistic rationalizations are offered to account for the observed influence of these variables on racemization kinetics. The possible relevance of these findings to food processing and nutrition is also discussed.

INTRODUCTION

Processed proteins are increasingly used to meet human dietary needs. Alkali treatment of plant (corn, soy) and animal (casein) proteins brings about desirable changes in flavor, texture, and solubility. Such treatments also destroy toxins and trypsin inhibitors and are used to prepare protein isolates.

Treating food proteins with alkali and heat may produce undesirable, as well as desirable, changes in the constituent amino acids, however. Chemical changes that may be undesirable include cross-linking (Provanasal et al., 1975; Finot, 1983), degradation (Asquith and Otterburn, 1977; Sen et al., 1977), Maillard browning reactions, (Finot, 1982), and racemization (Masters and Friedman, 1979,

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Table I. Effective pH Values at 25 and 75 °C

borate buffers (0.05 M)		0.1 N NaOH		1 N NaOH	
pH 25 °C	pH 75 °C	temp, °C	pH	pH 25 °C	pH 75 °C
		25	13.0	14.0	12.3
8.0	7.7	35	12.7		
9.0	8.6	45	12.3		
10.0	9.6	55	12.0		
11.0	10.1	65	11.7		
12.0	10.6	75	11.3		
		85	11.0		
		95	10.7		

1980; Friedman et al., 1981; Friedman and Masters, 1982; Liardon and Ledermann, 1981, 1984; Liardon et al., 1981; Liardon and Hurrell, 1983).

Racemization of L-amino acids to D isomers in food proteins may impair biological value and safety of foods by generating nonmetabolizable and nonutilizable forms of amino acids, creating peptide bonds that are slowly hydrolyzed by the proteolytic enzymes, and forming unnatural amino acids which may be nutritionally antagonistic or toxic.

Understanding the factors that influence the racemization of amino acid residues in food proteins under food processing conditions may permit minimizing undesirable changes in foods, thus helping the formulation of foods that are nutritionally optimal and safe.

This paper extends our previous studies by reporting on the influence of pH, time, and temperature on the extent of racemization of amino acid residues in soybean proteins.

EXPERIMENTAL SECTION

Alkali Treatments. Soy protein isolate (0.5 g, Promine-D, U.S. Biochemical Co., Cleveland, OH) was suspended in 50 mL of solvent (0.05 M borate buffer of appropriate pH, 1 N NaOH for pH ~14, or 0.1 N NaOH for pH ~13). The solvents were preheated in the time and temperature studies. Samples in the pH study were mixed at room temperature, so that the pH could be accurately adjusted before heating. Each flask was stoppered with a Nalgene cap covered with aluminum foil to prevent the tops from popping. The flasks were placed into a water bath at the appropriate temperature. At the end of the treatment, the flasks were cooled under running water and the final pH measured with a Beckman PHM 84 research pH meter. The contents in Spectra/Por 4 dialysis tubing, molecular weight cutoff 12000 (Spectrum Medical Industries, Los Angeles, CA), were then immediately dialyzed against 0.25% acetic acid for one day, and thereafter against distilled water for two days. The samples were then lyophilized.

Temperature Dependence of pH. The dependence of pH on temperature must be taken into account in studies of effects of both pH and temperature on racemization. Attempts were, therefore, made to determine effective pH values of the various samples at the different temperatures of this study. These adjusted pH values have been obtained experimentally for the borate buffer (pH 8–12) and calculated for the NaOH suspensions according to Bates (1964), as shown in Table I.

Isomeric Analyses. Isomeric analyses were carried out by a gas chromatographic, mass spectrometric procedure described by Liardon and Hurrell (1983). The reported racemization values are corrected for acid hydrolysis induced contributions as measured according to the procedure described by Liardon et al., (1981), except for tyrosine, for which the correction procedure was not applicable. The data reported for tyrosine include both alkali-treatment and hydrolysis contributions. Unless otherwise indicated in Tables II-VII, all determinations were carried out in duplicate. Table II shows that the untreated soy protein isolate contains a mixture of D- and L-Orn isomers.

RESULTS AND DISCUSSION

pH Effects. Base-catalyzed racemization of an amino acid is postulated to proceed by an abstraction of the proton from the asymmetric carbon atom to give a negatively charged, optically inactive planar carbanion. This carbanion can then be reprotonated on either side, regenerating an equal mixture of both D and L enantiomers (Masters and Friedman, 1980). These considerations suggest that the extent of racemization, defined as (D/D + L)100, can theoretically reach a maximum of 50%. At the equilibrium point, the extent of inversion of L to D enantiomer equals the corresponding inversion of D to L enantiomer.

Inspection of Table II reveals that exposing soybean proteins at 75 °C for 3 h in solvents of increasing effective pH induces a progressive increase in the extent of racemization of all amino acid residues. Compared to the untreated soy protein isolate, little racemization takes place at pH 8, (effective pH, 7.7) on the order of 1% or less for most amino acids. The extent of racemization rises sharply above pH 10.

Table II. Effect of pH on the Racemization of Alkali-Treated Soybean Protein[a-d]

amino acid	untreated soy bean isolate	effective pH at 75 °C[e] 7.7	8.6	9.6	10.1	10.6	11.3	12.3
Ala	0.17 (0.02)	0.24 (0.08)	0.26 (0.01)	0.69 (0.06)	1.53 (0.06)	3.8 (0.1)	17.9 (0.2)	44.4 (0.1)
Val	0.03 (0.02)	0.0	0.0	0.11	0.23 (0.05)	0.49 (0.01)	2.81 (0.08)	22.8 (0.4)
Leu	0.19 (0.03)	0.26 (0.01)	0.3	0.39 (0.03)	0.61 (0.02)	1.41 (0.01)	6.7 (0.2)	33.8 (0.2)
Ile	0.2 (0.1)	0.0	0.2	0.0	0.43 (0.03)	0.90 (0.01)	4.1 (0.3)	31.3 (0.3)
Cys	0.8 (0.1)	0.0	3.6	8.3 (1.6)	9.7	13.1 (2.0)	24.2 (0.8)	33.5 (0.5)
Met	0.33 (0.01)	0.0	0 (0.3)	1.48 (0.02)	3.1 (0.2)	8.0 (0.05)	27.0 (0.05)	44.9 (0.4)
Phe	0.07 (0.03)	0.53 (0.08)	0.84 (0.09)	1.35 (0.01)	3.6 (0.05)	8.7 (0.05)	28.1 (0.3)	45.4 (0.3)
Lys	0.19 (0.05)	0.30 (0.03)	0.44 (0.01)	1.09 (0.03)	1.85 (0.04)	3.5 (0.1)	12.3 (0.1)	41.2 (0.2)
Asp	0.5	1.6 (0.3)	2.5 (0.4)	6.3 (0.3)	12.2 (0.2)	21.9 (0.4)	32.0 (0.6)	44.3 (0.7)
Glu	0.0	0.2	0.2	1.30 (0.01)	3.8 (0.05)	9.0 (0.2)	26.4 (0.4)	43.8 (0.8)
Ser	0.11 (0.01)	1.6 (0.1)	3.2 (1.1)	13.4 (3.0)	23 (5)	37.2 (0.6)	42.1 (0.4)	40
Thr	0.0	0.0	0.0	3.6 (0.3)	7.1 (0.1)	18.9 (0.2)	28.5 (0.05)	21.7 (0.8)
Pro	0.0	0.0	0.0	0.0	0.0	0.0	0.7 (0.3)	3.9 (1.1)
Tyr	2.44 (0.05)	1.61 (0.03)	1.2	1.9 (0.2)	3.4 (0.2)	5.7 (0.1)	18.4 (0.9)	46 (3)
Orn	40	*f*	*f*	42	34	29.8 (1.3)	30.5 (0.8)	43.3 (0.1)

[a] Values are listed as 100D/(D + L). [b] Conditions: 75 °C, 3 h. [c] Values in parentheses are standard deviations from the mean for duplicate analyses. No value indicates a single determination. [d] Racemization data for tyrosine include hydrolysis-induced inversion. [e] See Table I for corresponding pH values at 25 °C. [f] Not detected.

Table III. The Dependence of Amino Acid Racemization Rate Constants on pH[a-c]

amino acid	effective pH at 75 °C						
	7.7	8.6	9.6	10.1	10.6	11.3	12.3
Ala	c	c	0.6	1.4	3.6	20.5	101
Val	c	c	c	0.2	0.5	2.7	28.2
Leu	c	c	c	0.6	1.3	6.7	52.2
Ile	c	c	c	0.5	1.0	4.6	57.2
Met	c	0.4	1.4	2.9	8.1	35.9	106
Phe	0.4	0.8	1.3	3.4	8.8	38.3	111
Lys	c	0.4	1.0	1.7	3.3	13.1	80.6
Glu	c	c	1.2	3.6	9.2	34.88	96.6

[a] Values are listed as $10^{-6}k$ (s^{-1}). Rate constants are based on single racemization values at 75 °C. [b] Listed values have been corrected for temperature dependence of pH, as described in the text. [c] Not significant.

A significant fraction of most amino acids, except the neutral ones such as Pro, Val, Ile, and Leu, is racemized in 0.1 N NaOH corresponding to an effective pH 11.3 at 75 °C (Table I). For Ser, the most strongly affected, the value of 42.1% approaches the equilibrium value of 50.0%. D/L ratios for all amino acids normalized to that of Ala, the simplest amino acid with an asymmetric carbon atom, are as follows: Ser, 235; Asp, 179; Orn, 170; Thr, 159; Phe, 157; Met, 151; Glu, 147; Cys, 135; Lys, 123; Tyr, 103; Ala, 100; Lue, 37; Ile, 23; Val, 16; Pro, 4. Thus, some of the nutritionally essential amino acids such as Thr, Phe, Met, and Lys rank high on this relative racemization scale.

Table III and Figure 1 show the pH dependence for the racemization rate of selected amino acid residues in soybean proteins. These results show an initial slow inversion rate up to an effective pH of 9–9.5 followed by a steep increase in the racemization kinetics. The striking parallelism in the slopes of the rate vs. pH curves suggests that although the absolute rate constants for the racemization of structurally different amino acid residues differ widely as discussed earlier, the pH dependence of the rates do not.

Arg and His racemization could not be measured by the present technique.

Time Effects. Heating of 1% solution of soy protein in 0.1 N NaOH at 75 °C for periods increasing from 10 to 480 min progressively increased racemization of all amino acid residues (Table IV). Significant amounts of D-amino acids were formed after only 10 min. Thus, 13.5% of Asp and 27.1% of Ser were in the D form after 10 min. The initial rapid rate of racemization seems to level off after about 1–2 h.

Figures 2 and 3 show plots of the extent of racemization against time. These plots reveal two distinct kinetic behaviors. Most amino acids exhibit biphasic racemization curves, with the slope changing after about 2 h of treatment. The ratio of the two slopes is approximately 2. With

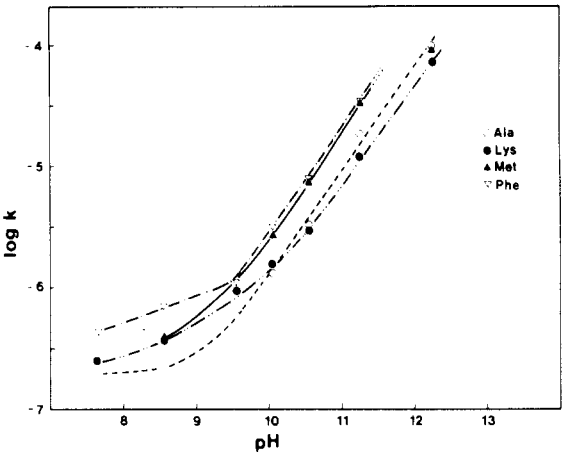

Figure 1. The pH dependence of Ala, Lys, Met, and Phe racemization in soybean proteins at 75 °C in the pH range 8–14. Effective pH values were calculated as described in the text.

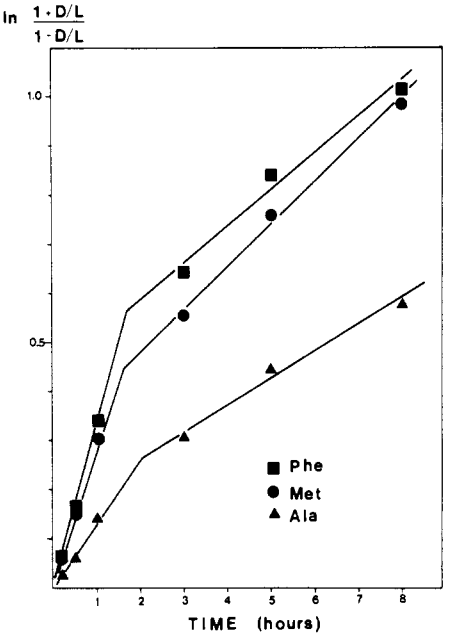

Figure 2. Time course of racemization of Ala, Met, and Phe in 0.1 N NaOH at 75 °C. Note that the lines were drawn to indicate that the slopes change approximately at the 2-h alkali treatment time. The data do not permit a more precise estimate of the time when the change actually occurs.

Table IV. Effect of Time of Treatment on the Enantiomeric Composition of Alkali-Treated Soybean Proteins[a-c]

amino acid	time, min					
	10	30	60	180	300	480
Ala	1.31 (0.01)	3.01 (0.09)	6.63 (0.07)	13.1 (0.2)	17.9 (0.4)	21.9 (0.7)
Val	0.18 (0.03)	0.31 (0.01)	0.81 (0.02)	1.9 (0.1)	2.7 (0.1)	4.2 (0.05)
Leu	0.65 (0.01)	1.03 (0.05)	2.41 (0.04)	4.96 (0.02)	7.2 (0.2)	9.4 (0.2)
Ile	0.20 (0.06)	0.62 (0.05)	1.2 (0.1)	3.0 (0.2)	3.9 (0.2)	5.6 (0.05)
Cys	10.6 (2.2)	13.8 (0.7)	14.7 (0.9)	21 (3)	24.9 (0.4)	31 (4)
Met	2.9 (0.4)	7.0 (0.05)	13.1 (0.1)	21.3 (1.0)	26.6 (0.1)	31.2 (0.7)
Phe	3.1 (0.1)	7.6 (0.1)	14.5 (0.7)	23.8 (0.1)	28.5 (0.4)	31.9 (0.05)
Lys	0.8 (0.05)	2.0 (0.05)	4.3 (0.2)	9.1 (0.1)	12.3 (0.2)	16.3 (0.3)
Asp	13.5 (0.05)	20.9 (0.1)	25.4 (0.1)	29.6 (0.4)	32.0 (2.0)	36.1 (0.6)
Glu	2.6 (0.1)	6.1 (0.1)	11.9 (0.1)	19.6 (0.05)	24.3 (0.2)	28.0 (0.1)
Ser	27.1 (0.4)	38.4 (0.5)	40 (3)	44.0 (0.2)	43.9 (1.0)	43.8 (1.4)
Thr	6.5 (0.4)	12.3 (0.3)	18.3 (0.8)	22.6 (0.2)	22.6 (0.2)	26.0 (0.05)
Pro	0.0	0.0	0.0	0.03	0.2	0.4
Tyr	3.8 (0.1)	4.8 (0.1)	8.4 (0.1)	14.5 (0.2)	19.0 (0.4)	23.1 (0.8)
Orn	36	33.2 (0.05)	25.1 (0.8)	26.6 (0.5)	33.0 (0.8)	37.0 (0.4)

[a] Racemization values are given as 100D/(D + L). [b] Conditions: 0.1 N NaOH; 75 °C. [c] See footnotes *c* and *d* in Table II.

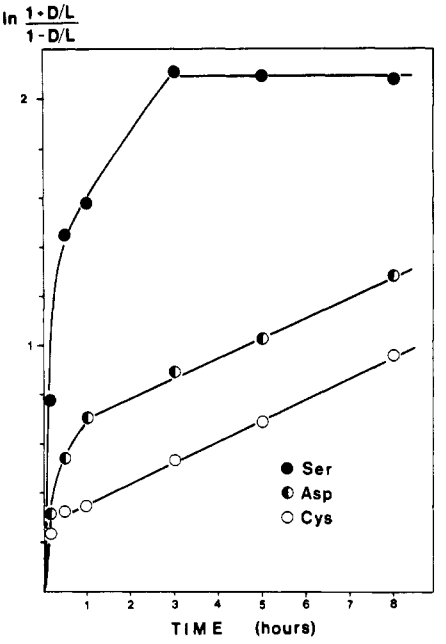

Figure 3. Time course of racemization of Cys, Asp, and Ser residues in soybean proteins treated in 0.1 N NaOH at 75 °C.

Table V. Inversion Rate Constants for the Amino Acid Racemization in Alkali-Treated Soybean Proteins (0.1 N NaOH, 75 °C)

amino acid	rate constants,[a] $10^{-6}s^{-1}$
Ala	19.4 (1.1)[b]
Val	2.2 (0.5)
Leu	6.2 (1.3)
Ile	3.92 (0.4)
Cys[c]	>200, 12.3 (0.4)
Met	37.3 (0.9)
Phe	46.9 (1.1)
Lys	12.4 (1.2)
Asp[c]	>260, 11.2 (0.5)
Glu	37.2 (1.3)
Ser	>650
Thr	52 (3)
Tyr	18 (4)

[a] Based on time study (0–60-min treatments). [b] Values in brackets are standard deviations. [c] First value is based on racemization induced by a 10-min alkaline treatment; second values correspond to the slope of the racemization curve at longer treatment.

Cys, Asp, Ser, and Thr, however, very rapid racemization begins during the first few minutes of treatment, leveling off after about 2–3 h.

Racemization or inversion rate constants were calculated as described previously (Masters and Friedman, 1979; Liardon and Hurrell, 1983). [For a mathematical derivation of the kinetic equations describing amino acid racemization, see Masters and Friedman, 1980.] The values shown in Table V were calculated for the first set of amino acids on the basis of the initial racemization curves (up to a 60-min treatment). For Cys, Asp, and Ser, the rate constants represent the lower limit for the initial inversion rate and were calculated from the extent of racemization after 10-min treatments.

The calculated rate constants in Table V were plotted against inductive parameters for amino acid side chains described by Hansch and Leo (1979). The linear plot in Figure 4 represents a free energy relationship that correlates the rate of racemization with the σ^* constant, a parameter that gives a quantitative measure of the inductive or electron-donating nature of the amino acid side chain R attached to the asymmetric carbon atom compared to

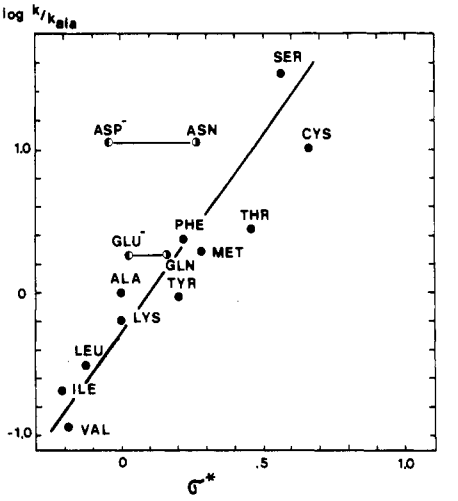

Figure 4. Relationship between the inductive constants (σ^*) of the amino acid side chains and the logarithms of the racemization rate constants (k) relative to alanine (k_{ala}) for Val, Ile, Leu, Lys, Tyr, Glu^-, Gln, Met, Phe, Thr, Asp^-, Asn, Cys, and Ser residues in soybean proteins treated in 0.1 N NaOH at 75 °C.

Ala, the simplest optically active amino acid. The equation for the line in Figure 4 is

$$\log\left[\frac{k(\text{any amino acid})}{k(\text{alanine})}\right] = (2.25 \pm 0.29)\,\sigma^* - (-0.3 \pm 0.1) \quad (1)$$

with a regression coefficient $r = 0.93$.

Asp and Glu were not included in the regression calculation because of uncertainties about the values for their inductive constants.

Similar correlations have recently been demonstrated for amino acid racemization rates in casein (Friedman and Masters, 1982), lactalbumin, and lactoglobulin (Liardon and Ledermann, 1984) under moderately mild conditions of alkali treatment. Although the regression coefficients usually exceeded 0.90, the regression parameters calculated for these and several other proteins differed significantly (Liardon, 1984).

Because the regression parameters for the linear correlations vary among proteins, any predicted racemization rates calculated from the equations for the linear plots are probably applicable only for the same protein. For example, although the extent of racemization of Trp in soy protein could not be measured because this amino acid is degraded during exposure to the strong acid conditions used to hydrolyze the proteins, the reported σ^* value for Trp of zero (Charton, M., 1981) makes it possible to predict that the extent of racemization of Trp residues in soy proteins should be identical with that for Ala, whose inductive constant is also zero. Similarly, it is possible to calculate predicted rate constants with the aid of eq 1 for the racemization of Arg and His residues. As mentioned earlier, the D/L ratios for these amino acids could not be determined by our technique.

These considerations imply that the main utility of the free energy relationships is in (1) calculating racemization rates of various amino acids when experimental data are available for only one or two amino acids and (2) calculating predicted rates of racemization for amino acids whose racemization cannot be experimentally measured. Furthermore, since the time course of racemization (Table IV, Figures 2 and 3) is generally not a linear function, relative rate constants may not necessarily correspond to relative extents of inversion.

Table VI. Effect of Temperature on the Racemization of Amino Acid Residues in Alkali-Treated Soybean Proteins[a-c]

amino acid	temp, °C							
	25	35	45	55	65	75	85	95
Ala	0.29 (0.01)	0.54 (0.02)	2.00 (0.09)	3.88 (0.07)	9.93 (0.01)	17.0 (0.2)	24.5 (0.7)	32.7 (0.2)
Val	0.06 (0.06)	0.11 (0.02)	0.31 (0.05)	0.46 (0.01)	1.03 (0.01)	2.3 (0.2)	4.19 (0.01)	8.6 (0.4)
Leu	0.18 (0.05)	0.26 (0.02)	0.85 (0.18)	1.55 (0.01)	3.11 (0.07)	6.5 (0.3)	10.4 (0.5)	16.8 (0.2)
Ile	0.03	0.20 (0.01)	0.55 (0.05)	0.88 (0.11)	1.77 (0.04)	3.4 (0.1)	6.5 (0.2)	11.4 (0.5)
Cys	6.4 (2.0)	10.4 (3.4)	11.2 (0.1)	13.1 (1.7)	14.4 (0.3)	22.3 (0.1)	25.6 (2.2)	29.4 (1.1)
Met	0.98 (0.02)	1.6 (0.1)	5.1 (0.2)	9.8 (0.1)	18.2 (0.1)	27.1 (0.05)	32.3 (0.1)	39.4 (0.8)
Phe	0.80 (0.06)	1.78 (0.07)	5.2 (0.4)	10.8 (0.1)	19.7 (0.5)	27.9 (0.5)	33.3 (0.4)	39.3 (0.2)
Lys	0.26 (0.08)	1.51 (0.02)	1.42 (0.02)	3.0 (0.1)	6.7 (0.1)	11.9 (0.1)	17.8 (0.1)	27.0 (1.1)
Asp	5.91 (0.04)	9.3 (1.3)	17.1 (0.2)	20.1 (2.0)	27.7 (1.6)	31.6 (0.7)	34.8 (1.1)	41.2 (0.9)
Glu	0.44 (0.03)	1.42 (0.03)	4.8 (0.3)	9.1 (0.1)	16.2 (0.2)	22.5 (0.3)	28.8 (0.1)	36.9 (0.1)
Ser	13.8 (0.8)	20.4 (4.4)	33.9 (0.5)	38.9 (0.1)	42.2 (0.5)	40.5 (1.0)	42.8 (0.5)	41
Thr	2.8	6.5 (1.4)	11.3 (1.4)	15.8 (0.3)	21.9 (2.0)	23.3 (0.2)	21.0 (0.2)	31 (2)
Pro	0.0	0.0	0.0	0.0	0.0	0	0.49 (0.01)	1.22 (0.06)
Tyr	1.7 (0.3)	2.6 (0.05)	3.8 (0.4)	5.8 (0.3)	10.8 (0.1)	16.8 (0.4)	24.1 (0.05)	33.6 (0.1)
Orn	*d*	*d*	39 (2)	30 (3)	24.9 (0.3)	32	36.5 (0.1)	40.5 (0.7)

[a] Values are listed as 100D/(D + L). [b] Conditions: 0.1 N NaOH; 3 h. [c] See footnotes *c–e* in Table II. [d] Not detected.

The linear free energy relationship illustrated in Figure 4 has both theoretical and practical significance. On the theoretical side, first, is the fact that racemization rates of amino acid residues can be correlated with a parameter that measures the inductive ability of amino acid side chains attached to the asymmetric carbon atom. This implies that stabilization of the intermediate carbanion formed by abstraction of the C–H proton is the major factor governing racemization. Once this proton is abstracted, reprotonation can occur at either side of the planar carbanion, regenerating a racemic mixture. These considerations also imply that proton abstraction is the slow, rate-determining step and is followed by a rapid reprotonation.

Plots of the exent of racemization against time both in this (Figures 2 and 3) and earlier studies (Masters and Friedman, 1979; Friedman and Masters, 1982; Liardon and Ledermann, 1984) produce curves with two or more slopes. This suggests that additional factors may affect the racemization process at the molecular level. These could include both structural and charge effects. For example, initial exposure of a protein with a thermodynamically stable conformation to negatively charged OH^- ions could result in a rapid abstraction of C–H protons from those sites on the protein that (1) are sterically accessible and (2) have no or minimal negative charge that would electrostatically repel the incoming hydroxide ions. Once these sites were racemized, slower racemization would then proceed at other sites of the protein molecule, which are less sterically accessible and/or have more negative charge. These events are further complicated by alkali-induced conformational changes and partial hydrolysis of the protein, which produces new negatively charged carboxyl ions. Since the extent of such changes is unique for each protein and is governed by the amino acid sequence, racemization rates are expected to vary from protein to protein. It is relevant to note that inductive effects of protein side chains are postulated to influence hydrogen-bond strengths of polypepetide amide groups, the α-helical content, and other properties of amino acids and proteins (Ling, 1964; Friedman, 1967; Friedman and Wall, 1964, 1966; Friedman et al., 1965; Charton and Charton, 1983; Niederhoffer et al., 1984).

Temperature Effects. Table VI shows racemization data for amino acid residues in soybean protein treated for 3 h in 0.1 N NaOH in the temperature range 25–95 °C at 10 °C intervals. The data in this table show that racemization of all amino acids continuously increases with temperature. Selected rate constants derived from this table are plotted in the form of Arrhenius plots in Figure 5. The activation parameters calculated from these plots are listed in Table VII. Values taken from the literature for free amino acids are included for comparison.

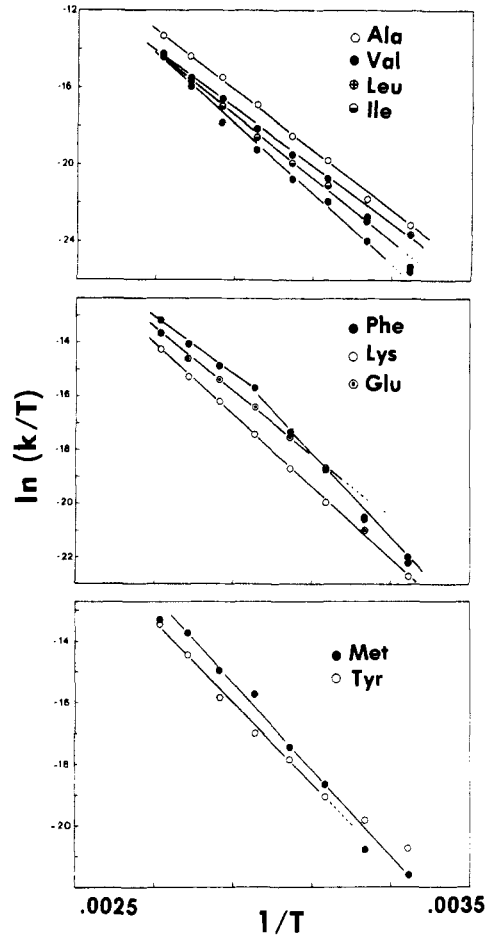

Figure 5. Arrhenius plots for the racemization of amino acid residues in soybean proteins. Racemization rates were determined at 10 °C intervals in the temperature range 25–95 °C (Table VI).

The rate constants used to calculate the activation parameters were obtained by first adjusting the calculated values at different temperatures to a common pH of 12 with the aid of the nomogram shown in Figure 6, as previously described by Masters and Friedman (1980) for casein. One effect of adjusting the rate constants has been to bring the ΔH^* values into much better agreement with the corresponding values for free amino acids taken from the literature (Table VII). On the other hand, it is difficult to make any meaningful conclusions from the listed pos-

Table VII. Activation Parameters for Amino Acid Racemization of Selected Amino Acid Residues in Soybean Proteins in 0.1 N NaOH

	ΔH^*, kcal/mol		ΔS^*, cal/°C mol	
	this work	free amino acids at pH 7.6[b]	this work	free amino acids at pH 7.6[b]
Ala	−31.7 (0.6)[c]	−28.6	13 (2)	−16.3
Val	−34.0 (1.1)	−28.0	16 (3)	−19.8
Leu	−30.2 (0.8)	−27.5	7 (2)	−19.8
Ile	−32.1 (0.7)	−27.1	12 (2)	−21.6
Lys	−25.7 (0.9)		−6 (3)	
Tyr	−25.4 (1.0)		−5 (3)	
Phe[a] (1)	−20.9 (1.1)		−16 (3)	
(2)	−31.1 (1.5)		13 (5)	
Met	−27.8 (0.3)		3 (1)	
Glu	−23.3 (0.4)	−30.9	−11 (1)	−15.1

[a] Double linear plot: (1) high temperature range; (2) low temperature range. [b] From Wonnacot, D. M. (1979). [c] See footnote b, Table II.

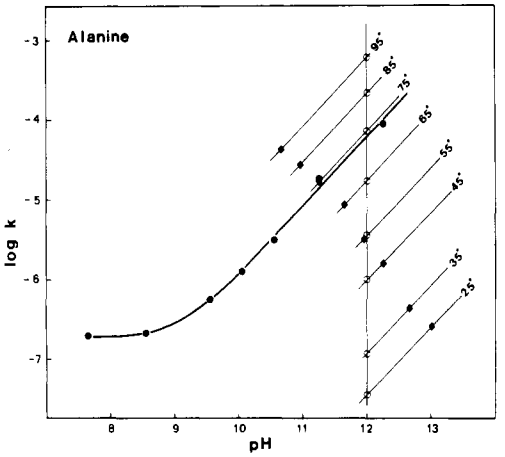

Figure 6. A nomogram for the temperature dependence of pH for the kinetics of racemization of amino acid residues in soybean proteins. Since effective pH is dependent on temperature, all values are adjusted to pH 12 with first-order extrapolation lines, as shown.

itive and negative values for the calculated entropies of activations (ΔS^*).

Possible Effects of Concurrent Reactions. The alkali treatment undoubtedly also hydrolyzes some labile peptide bonds producing small fragments which are lost during dialysis. These peptides could, in principle, racemize at rates different from those reported here for the large protein chains which are retained in the dialysis tubing. About 70% of the original weight was generally recovered after dialysis.

These considerations suggest that the question of concurrent hydrolysis of peptide bonds needs more consideration. For example, could there be any relation between the susceptibility to racemization and the rate of hydrolysis of a nearby peptide bond? Hydrolysis rates depend on (at least) two adjacent residues, giving rise to (at least) 400 or so possibly different rate constants. If there is any correlation with racemization, then the more rapid removal of the more susceptible peptide linkages may affect the later course of racemization. Such a (hypothetical) mechanism could possibly account for the supposedly "biphasic" curves of Figures 2 and 3, in particular because in the experimental workup fragments of M_r <12 000 are dialyzed away; these would be in the sections containing more hydrolyzable peptide bonds. Additional studies are, therefore, needed to clarify the dependence of the extent of racemization on the length of a peptide chain, especially since the observed extent of racemization of the same

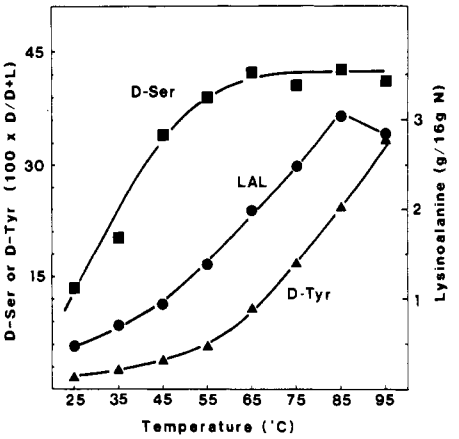

Figure 7. Effect of temperature on D-Ser, D-Tyr, and lysinoalanine content of alkali-treated soybean proteins. D-Ser and D-Tyr data are from Table VI and lysinoalanine values from Friedman et al. (1984).

amino acid residues appears to vary among structurally different proteins (Masters and Friedman, 1979).

Other side reactions besides hydrolysis could influence the kinetics of racemization of certain amino acids. Thus, under alkaline conditions, Cys, Ser, and Thr are subject to both degradation and racemization. The respective D and L isomers, which may be situated in different microenvironments in the protein, may also have different susceptibilities to degradation. In such cases, the cited D/L values would no longer reflect the true relative racemization rates of those amino acids. However, the available data do not show whether degradation affects racemization. A similar situation holds true for Lys, since part of it is transformed to lysinoalanine and part is racemized.

These suggestions are supported by our observations (Tables II and III) that the apparent racemization process for Ser and Thr stops completely after 3 h even though the equilibrium distribution of 50% has not been reached. A similar observation was reported by Liardon and Hurrell (1983) in studies of the racemization of amino acid residues of chicken muscle proteins. Evidently, not all of the potential asymmetric centers appear accessible to attack by OH^- ions.

Nutritional Significance. The nutritional utilization of D-amino acids appears to vary among animals and humans (for reviews, see Masters and Friedman, 1980; Tovar and Schwass, 1983). In addition, some D-amino acids may be nutritionally antagonistic. For example, although D-Phe is nutritionally available, high concentrations of D-Tyr inhibit the growth of mice (Friedman and Gumbmann, 1984a). The growth-depressing effect of D-Tyr can, however, be minimized by increasing the L-Phe or protein content of the diet. Similarly, although L-Cys has a sparing effect on L-Met when fed to mice, D-Cys does not (Friedman and Gumbmann, 1984b). It is also noteworthy that although D-Lys is not utilized as a source of L-Lys (Friedman and Gumbmann, 1981), the nutritional utilization of D-Met by mice as a source of the L isomer is dose dependent, reaching approximately 76% of the value observed with L-Met (Friedman and Gumbmann, 1984b). We also found that synthetic lysinoalanine (an equimolar mixture of L-L and L-D isomers) was about 4% as potent as lysine in promoting weight gain of mice (Friedman et al., 1982). Both D-Ser and lysinoalanine induce histological changes in the rat kidneys (Kaltenbach et al., 1979; Finot, 1983). Note that the three potentially nutritionally antagonistic or toxic unnatural amino acids (D-Ser, D-Tyr, and lysinoalanine) are produced in significant amounts of

soybean proteins under the influence of even short periods of alkaline treatment (Figure 7).

Since racemization also impairs protein digestibility (Hayashi and Kameda, 1980; Friedman et al., 1981; Bunjapamai et al., 1982; Savoie, 1984), assessment of the extent of racemization may be a useful indicator of protein damage. Even low values of racemization could be nutritionally significant since the introduction of D–L, L–D, or D–D peptide bonds along a protein chain could impair its susceptibility to digestion. Table II shows that the extent of racemization increases rapidly above pH 10 (effective pH 9.6).

A major unsolved problem is whether the biological effects of D-amino acids differ depending on whether they are consumed in the free or protein-bound forms. Possible antagonistic and synergistic effects of D-amino acids in vivo also merit further study.

Finally, although alkali treatment is widely used in the food industry (Sternberg and Kim, 1977; Sternberg et al., 1975; Haagsma and Slump, 1978; Raymond, 1980), and D-amino acids are present in some widely consumed food products (Bunjapamai et al., 1982; Friedman and Masters, 1982), additional studies are needed to define the extent of D-amino acid formation during home and commercial food processing and the possible nutritional and antinutritional significance of free and protein-bound D-amino acids as a function of the amounts consumed (Friedman and Gumbmann, 1984a,b).

In conclusion, the present study demonstrates that (1) pH, time, and temperature are major parameters influencing racemization of amino acid residues in soybean proteins, and (2) the relative rates of racemization are correlated with a linear free energy relationship. Conformational and charge effects and concurrent hydrolytic and other alkali-catalyzed reactions are also postulated to influence the kinetics of generation of D-amino acids. Because accurate knowledge of the composition of foods is important in assessing the role played by proteins in the human diet, these fundamental studies should facilitate understanding compositional changes and the resulting nutritonal and toxicological consequences of food processing. Such an understanding should help in formulating nutritionally optimal and safe food products.

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Registry No. L-Ala, 56-41-7; L-Val, 72-18-4; L-Leu, 61-90-5; L-Ile, 73-32-5; L-Cys, 52-90-4; L-Met, 63-68-3; L-Phe, 63-91-2; L-Lys, 56-87-1; L-Asp, 56-84-8; L-Glu, 56-86-0; L-Ser, 56-45-1; L-Thr, 72-19-5; L-Pro, 147-85-3; L-Tyr, 60-18-4; L-Orn, 70-26-8.

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