4-carbonitrile, 115363-81-0; phenyl isocyanate, 103-71-9; 5-cyano-4-imidazolecarbonyl chloride, 115363-80-9; 4-imidazolecarbonyl chloride, 56460-32-3.

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REVIEW

Chemistry, Analysis, Nutritional Value, and Toxicology of Tryptophan in Food. A Review

Mendel Friedman* and Jean-Louis Cuq

The complex transformations of tryptophan in foods and the great diversity of derivatives formed as well as possible antinutritional and toxic manifestations are reviewed. The stability of free or protein-bound tryptophan during processing and storage depends on temperature and the presence of oxygen or other oxidizing agents, especially lipid peroxides, and radiation. In the absence of oxidizing agents, tryptophan is a stable amino acid, even in strongly basic or acidic conditions. Free or bound tryptophan is relatively stable during heat treatments such as industrial or home cooking in the presence of air or steam sterilization. Only severe treatments cause a significant degradation of this amino acid. In the presence of carbonyl compounds or/and at high temperatures, however, carboline formation occurs. Both carbolines and tryptophan-derived nitroso compounds are potential carcinogens. Tryptophan losses during food processing cannot always be monitored because of the lack of reliable analytical methods. These considerations suggest research needs for better methodology to measure tryptophan in complex foods and for new ways to prevent the formation of tryptophan-derived antinutritional and potentially toxic compounds in foods.

The nutritionally essential amino acid tryptophan was discovered by F. G. Hopkins and S. W. Cole in 1901 [for a historical account, see Curzon (1987)]. This amino acid is exceptional in its diversity of biological functions. It contributes importantly to normal growth and protein synthesis in a number of tissues (Majumdar, 1982) and regulates numerous physiological mechanisms. For example, tryptophan is the precursor of the neurotransmitter serotonin (5-hydroxytryptamine) and therefore is important in brain function (Anonymous, 1987; Wurtman, 1982). It can influence sleep in man (Pollett and Leathwood, 1983; Yuwiler et al., 1981; Trulson and Sampson, 1987) and the aging process of rats (Ooka et al., 1988). Tryptophan and some of its derivatives also alter behavior (Anonymous, 1981; Lieberman and Wurtman, 1986), particularly the regulation of the intake of food and drink (Leathwood and Ashley, 1981; Pollock and Rowland, 1981; Threatte et al., 1980). Tryptophan also serves as the in vivo precursor for the vitamin niacin (Umezawa, 1989), stimulates insulin and

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growth hormone secretion and prevents the development of cortisone-induced hypertension (Fregly et al., 1987), interacts with carbohydrates (Jordan et al., 1977; Gibson and Svensson, 1987) and mycotoxins (Sashidhar et al., 1988a,b; Cavan et al., 1988), and participates in the formation of enzyme–enzyme inhibitor complexes (Laskowski, 1986).

The stability during food processing of free or protein-bound tryptophan has been little studied, primarily due to the difficulties of analysis (Fontana, 1984; Friedman and Finley, 1971, 1975; Friedman et al., 1984), and also because lysine and methionine are the most limiting amino acids in most food proteins. However, new data show that reactions of tryptophan during food processing, especially during thermal treatments, could be of nutritional and toxicological importance.

Since tryptophan is a nutritionally second-limiting amino acid in maize (Benevenga and Cieslak, 1978; Bozzini and Silano, 1978; Hassen et al., 1986), and since cereals and processed foods are increasingly used to meet human dietary needs, it is of paramount importance to develop an understanding of thermally induced changes in tryptophan in order to improve the quality and safety of our food supply. In this paper, we describe chemical transformations of tryptophan induced by heat, acids, bases, carbonyl compounds, carbohydrates, halogens, lipids, nitrites, oxidizing agents, sulfites, and other food ingredients and some of the resulting nutritional and toxicological consequences. With increased understanding of such changes, it should be possible to devise conditions to minimize antinutritional and other deleterious effects in foods.

CHEMISTRY OF TRYPTOPHAN IN FOOD

Chemical Reactions of Tryptophan during Processing and Storage. Chemical transformations of tryptophan are essentially a function of the temperature and the duration of treatments. However, specific modifications can be induced by the presence of oxygen, water (Leahy and Warthesen, 1983; Kanner and Fennema, 1987), other food-oxidizing lipids (Krogull and Fennema, 1987; Nielsen et al., 1985a–d; Yong et al., 1980), vitamins (Kanner and Fennema, 1987), reducing sugars (Ashoor and Zent, 1984; Brautigam and Severin, 1974; Dworschak and Orsi, 1977; Finot et al., 1982; Nyhammar and Pernemalm, 1985; Orsi, 1985; Orsi and Dworschak, 1978; Sgarbieri et al., 1973; Tanaka et al., 1975), carbonyl compounds (Arai, 1980; Chu and Clydesdale, 1976; McLaren, 1977; Previero et al., 1972), nitrites (Bonnett and Holleyhead, 1974; Nakai et al., 1978; Ito et al., 1979; Gruenwedel et al., 1984; Hotchkiss, 1984; Kinae, 1986; Mellet et al., 1986; Ochiai et al., 1986), halogens (Bercz and Bawa, 1986), radiation (Garrison, 1987), and sulfites (Yang, 1973).

The chemical properties of this amino acid have been the object of many investigations, primarily because of the particular reactivity of the indole ring (Fontana, 1984). This aromatic, electron-rich nucleus is susceptible to oxidative cleavage and to substitutions by several reagents. The ring NH group is an extremely weak nucleophile because its unshared electron pair is delocalized by resonance with the indole ring. The indole ring can react as an electron donor with aldehydes or carbocations.

Very hydrophobic tryptophan residues are often located in the interior of food protein molecules and are unaffected by most other food components. These residues contribute to the optical and fluorescent properties of proteins; their spectral characteristics are considered to be important contributors in the study of protein conformation (Jardetzky et al., 1986; Wolfenden, 1986; Holmquist, 1975).

Stability of Free Tryptophan at Room Temperature. At room temperature in the presence of air, solutions of pure tryptophan in dilute hydrochloric acid (0.1 N) are particularly stable (Hugli and Moore, 1972). However, in the presence of other common amino acids, starch, or citrate buffer, the tryptophan content of the solution decreases with time. In a citrate buffer solution, the destruction of this amino acid at pH 2.2 is significant. Stability is enhanced by increasing the pH, by changing from a citrate buffer to a sodium phosphate buffer, or by reducing the temperature (Nielsen and Hurrell, 1985). Losses were also observed when a solution of pure tryptophan in dilute sodium hydroxide was stored in the presence of air.

On the basis of these observations, it appears that tryptophan solutions or protein hydrolysates should be analyzed as soon as possible after preparation.

Stability of Free Tryptophan at Temperatures Near 100 °C. Few data are available concerning the effects of thermal treatments at temperatures near 100 °C, corresponding to industrial or home cooking with air or steam sterilization.

Yamakawa et al. (1979) have demonstrated that indole-3-acetic acid is stable to autoclaving within pH 4–10 (130 °C, 30 min) and also to atmospheric oxygen at ambient temperature. These results indicate that the indole ring is stable during thermal treatments performed in the absence of oxygen.

Using a spectrofluorimetric method of analysis, Stewart and Nicholls (1972, 1974) established the susceptibility of tryptophan to autoxidation at 100 °C in the range pH 2–7 and in both strongly acidic and strongly basic solutions. When oxygen was removed, the rate of tryptophan decomposition dropped sharply, suggesting that the amino acid is oxidized. A free-radical mechanism is involved in the reaction. This is also shown by a decrease in rate in the presence of thioglycollic acid and phenylmethanethiol, known inhibitors of free-radical chain reactions.

Using HPLC and UV detection or spectrofluorimetry to determine tryptophan, Cuq and Cheftel (1983) demonstrated that tryptophan is degraded only in the presence of oxygen. No amino acid loss was observed in tubes sealed under nitrogen and heated at 110, 125, or 140 °C for 24 h. This confirms that the indole ring is heat stable in the absence of oxygen. In the presence of air or oxygen, the extent of tryptophan degradation increases with time and with temperature (Figure 1); partly water-insoluble brown products are formed. Under the conditions used, tryptophan thermal destruction is greater in the presence of pure oxygen than in the presence of air. The reaction kinetics can be written schematically as follows:

$$\text{Trp} + O_2 \rightarrow \text{oxidized deriv(s)} + \text{insol brown products}$$

$$\frac{\text{d[oxidized deriv(s)]}}{\text{d}t} = k[\text{Trp}][O_2]$$

Under nitrogen, $[O_2] = 0$ and the rate of reaction is zero. In the presence of air or oxygen, if the concentration of dissolved oxygen remains constant during the reaction, one can write the apparent first-order kinetic equations

$$-\text{d[Trp]}/\text{d}t = k_{app}[\text{Trp}]$$

$$\ln [\text{Trp}] = -k_{app}t + \ln [\text{Trp}]_0$$

The variations of the pseudo-first-order rate constant (k_{app}) with temperature and in the presence of air or oxygen are shown in Table I.

The energy of activation of the reaction, calculated with the Arrhenius equation, is equal to 80.5 and 78.9 kJ mol^{-1}

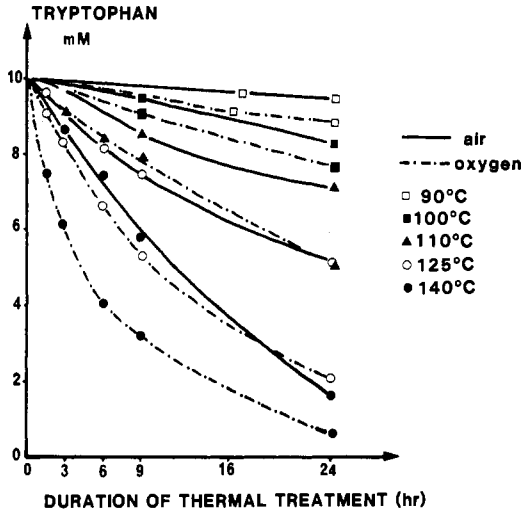

Figure 1. Thermal degradation of tryptophan in the presence of air or oxygen as a function of time and temperature, determined by HPLC with UV detection (278 nm) (Cuq and Cheftel, 1983).

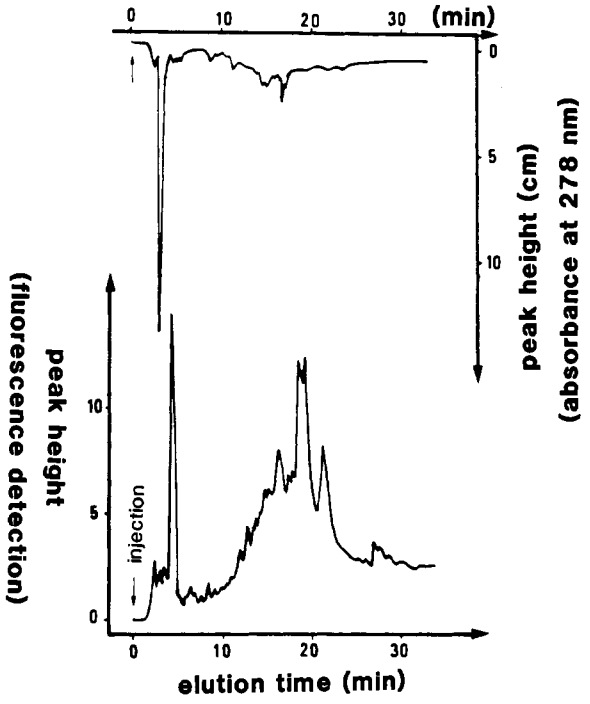

Figure 2. HPLC chromatograms of heated tryptophan solutions (SP 8000; RP 18 column; 10-μL sample of tryptophan (10 mM) heated for 24 h at 140 °C in air). Elution with a gradient of methanol (A) and 0.01 M sodium acetate buffer (pH 4) (B): time 0 min, 40% A, 60% B; 5 min, 60% A, 40% B; 10 min, 80% A, 20% B; 15 min, 90% A, 10% B; 20 min, 100% A. Flow rate 1 mL min^{-1}. Simultaneous absorbance (278 nm) and fluorescence detections. Excitation wavelength 365 nm; emission wavelength 418 nm (Cuq and Cheftel, 1983).

in the presence of air and oxygen, respectively.

HPLC chromatograms show that many products appear as tryptophan is degraded (Figure 2). Some of these are detected by their fluorescence. Measurements of the absorbance of the HPLC eluates at 350 nm (maximum-absorbance wavelength of kynurenine) failed to detect kynurenine or *N*-formylkynurenine. The fluorescence spectra of 10 mM tryptophan solution heat-treated at 140 °C in air for 24 h is shown in Figure 3. Many fluorescent compounds with longer excitation and emission wavelengths than tryptophan are formed. In the same way, the absorbance spectra show that the intensity of the 278-nm

Table I. Influence of Temperature and Presence of Air or Oxygen on the Rate of Decomposition of Tryptophan (10 mM in Water)[a]

temp, °C	k_{app}, $h^{-1} \times 10^{-3}$ air	oxygen
90	2.5	5.1
100	8	11.3
110	15	27.5
125	27.9	66.2
140	78	114

[a] Cuq and Cheftel (1983).

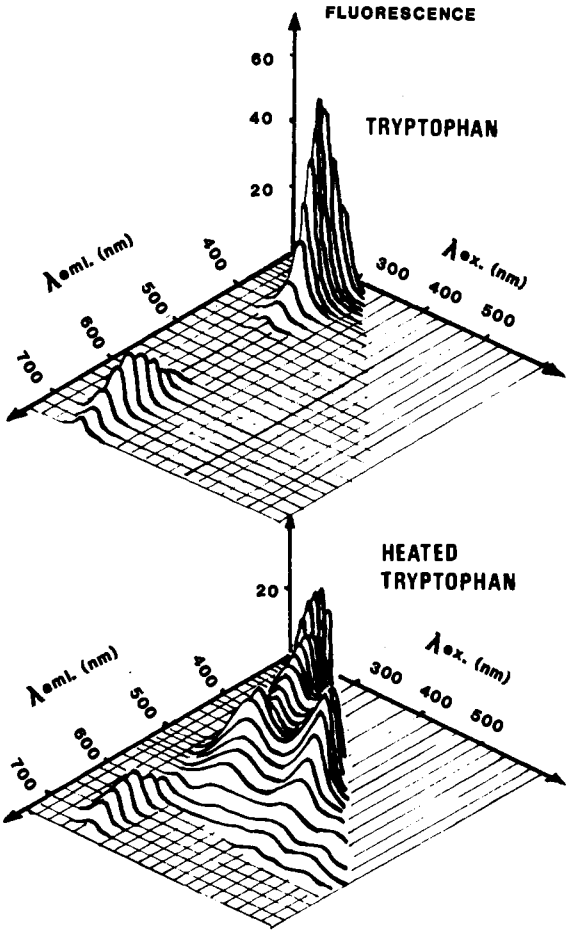

Figure 3. Fluorescence spectra of 10 μM methanolic solutions of tryptophan and heated tryptophan (10 mM, 140 °C, air, 24 h) (Cuq and Cheftel, 1983).

absorbance maximum characteristic of this amino acid decreases with heating time. Simultaneously, the 320–450-nm absorbance increases, reflecting the formation of brown derivatives. These observations indicate that it is not possible to assess tryptophan degradation quantitatively by measurement of the 278-nm absorbance of the solutions (Figure 4). Thermal treatment is accompanied by the liberation of ammonia. It is likely that oxidative deamination of free tryptophan occurs at the primary amino group of the molecule, leading to the formation of indole-3-pyruvic acid, itself unstable.

Some possible mechanisms involved in tryptophan degradation during heat treatment in the presence of oxygen are shown in Figure 5. In the presence of an unknown initiator (R• = HOO•, for instance), the N–H bond, protonated or not depending on pH, is homolytically cleaved to give free radicals that can exist in the mesomeric forms **2** and **3**. In the presence of ammonia, condensation of **3** with oxygen converts either **1** to *N*-formylkynurenine (**5**) and then to kynurenine (**6**) or **2** to quinazoline (**7**) or

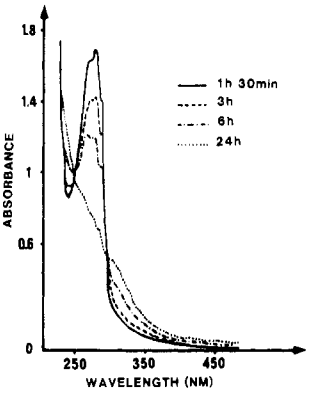

Figure 4. UV absorption spectra of 10 mM tryptophan solutions heated at 140 °C in oxygen for various lengths of time. Measurement after 1/30 dilution with distilled water (Cuq and Cheftel, 1983).

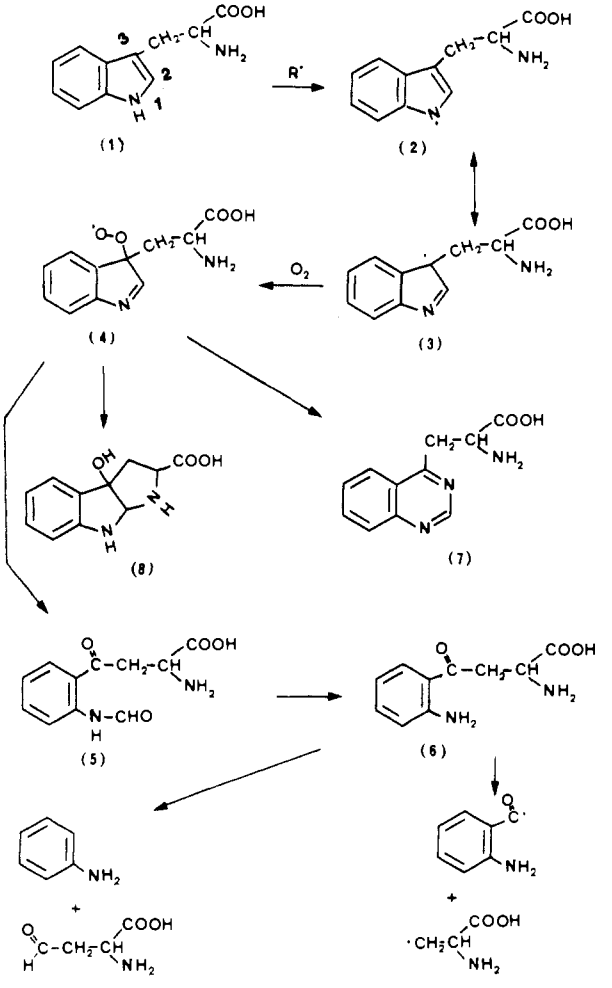

Figure 5. Proposed scheme of some reactions involved in the thermal degradation of free tryptophan (Cuq and Friedman, 1989).

hexahydropyrroloindole (8).

Tryptophan can also condense with aldehydes (Figure 6) to give a 2-acyl derivative (9) which cyclizes to the dihydro-β-carboline (10). This is then aromatized by dehydrogenation to β-carboline 11. Maillard-like reactions can also occur between the aldehydes formed during the "free-radical degradation" and the α-amino group of the remaining tryptophan. This reaction explains the browning that follows thermal treatment of free tryptophan in the presence of oxygen.

One of the condensation products formed could be the (indolylmethyl)tetrahydro-β-carboline (12) shown by

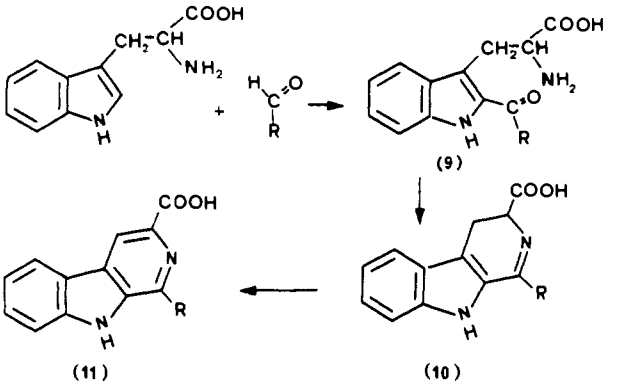

Figure 6. Postulated formation of β-carboline during thermal treatment of free tryptophan.

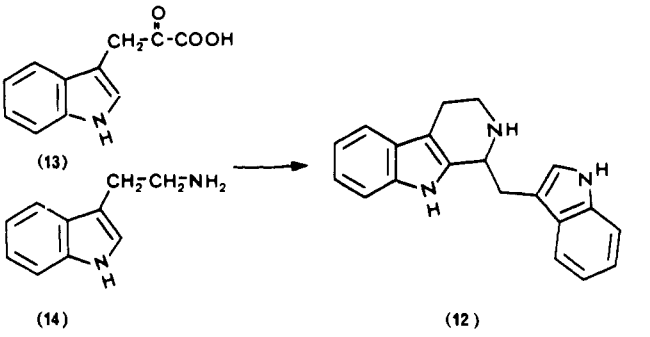

Figure 7. Postulated scheme for the formation of (indolylmethyl)tetrahydro-β-carboline (Cuq and Friedman, 1989).

Gaignault et al. (1978) to result from the condensation of indolyl-3-pyruvic acid (13) and tryptamine (14) derivatives (Figure 7). Indolyl-3-pyruvic acid and tryptamine result from the oxidative deamination and decarboxylation of tryptophan, respectively.

Although the indole ring of tryptophan is probably modified by thermal treatment in the presence of oxygen, no products from such a reaction have been found. Various authors (Fontana, 1984; Previero et al., 1967) have shown that strong oxidizing agents split the C_2–C_3 bond of the indole ring, with the formation of *N*-formylkynurenine and then of kynurenine. These two compounds were not found, probably because they were destroyed during the heat treatment.

The rate of degradation of free tryptophan during thermal treatment in a 90–140 °C temperature range is a function of pH (Leahy and Warthesen, 1983). In the presence of formate buffer, the maximum rate of decomposition occurs at pH 5.3. In the presence of sodium phosphate buffer, increasing pH from 5 to 8 increases degradation from 38 to 48% of the initial contents after 24 h at 121 °C.

Effect of Acids and Bases on the Stability of Free and Protein-Bound Tryptophan. Amino acids in food proteins are now generally measured by high-performance liquid or ion-exchange chromatography after hydrolysis. Tryptophan, however, is completely destroyed during hydrochloric acid hydrolysis, yielding ammonia and many unidentified derivatives. Although alternative methods have been proposed (Friedman and Finley, 1975; Friedman et al., 1984), the determination of tryptophan in proteins is still a problem (McNab and Scougall, 1982; Williams et al., 1982; Sarwar et al., 1983). The principal challenge is to minimize losses of tryptophan during acid or alkaline hydrolysis.

Hydrochloric Acid. In the presence of hydrochloric acid and oxygen, tryptophan degradation increases with

both time of heating and temperature (Stewart and Nicholls, 1972). When oxygen is removed by evacuation, the rate of decomposition decreases dramatically. These results show that oxygen concentration during hydrochloric acid hydrolysis is the main contributor to the destruction. When hydrolysis is performed in sealed tubes under vacuum or nitrogen, traces of oxygen in the medium can contribute to the destruction. Under these attempted "oxygen-free" conditions, addition of thioglycolic acid (Stewart and Nicholls, 1972; Matsubara and Sasaki, 1969) or phenylmethanethiol (Stewart and Nicholls, 1972) decreases the occurrence of free-radical chain reactions.

Most of the common amino acids do not affect the stability of tryptophan during hydrochloric acid hydrolysis (Gruen, 1973; Gruen and Nicholls, 1972), but tyrosine has a small influence and cystine results in substantial loss of tryptophan. In the presence of cystine, tryptophan reacts to give tryptathionine, which is subsequently converted into 2,3-dihydro-2-oxotryptophan, cysteine, and cystine. In the course of this transformation, two compounds are formed, one being bis(2-amino-2-carboxyethyl) trisulfide (Ohta and Nakai, 1979). These data suggest that sulfenic or sulfinic acids, arising from cystine oxidation in the presence of traces of oxygen, are mainly responsible for the loss of tryptophan during the hydrochloric acid hydrolysis of proteins. Oxidized chlorine derivatives (Ohta et al., 1981) and carbohydrates (Olcott and Fraenkel-Conrat, 1947) may also contribute to the degradation of this amino acid.

An interesting procedure for the analysis of tryptophan and the total amino acid composition of protein is based on the reduction of tryptophan residues by pyridine borane before hydrochloric acid hyrolysis (Wong et al., 1984). The reduced tryptophan is stable to hydrolysis in 6 N HCl, and the reduction does not affect the other amino acids [cf. also Chauffe et al. (1975)]. Similarly, periodate oxidation of tryptophan leads to kynurenine residues that are stable to 6 N HCl at 108 °C under vacuum (Inglis et al., 1979).

Another analytical method is based on addition of trifluoroacetic acid to hydrochloric acid/thioglycolic acids. This reduces hydrolysis times and increases the rate of cleavage of the hydrophobic peptide bonds, particularly those with tryptophyl residues (Yokote et al., 1986).

Organic Acids. Organic acids, such as *p*-toluenesulfonic acid (Liu, 1972; Liu and Chang, 1971), mercaptoethanesulfonic acid (Creamer and Matheson, 1976; Penke et al., 1974), and methanesulfonic acid (Simpson et al., 1976) may be suitable for determining tryptophan under acid hydrolysis conditions.

Thus, free tryptophan remains stable when heated at 110 °C under nitrogen for various lengths of time (24, 48, 72 h) in the presence of 4 N methanesulfonic acid containing 0.2% of tryptamine, as determined by HPLC (Cuq et al., 1983). The tryptamine probably acts as a free-radical scavenger during the treatment, preventing degradation of tryptophan by any residual oxygen in the hydrolysis tube. The addition of cysteine or of a mix of the common amino acids does not influence the stability of tryptophan. On the other hand, the addition of glucose or starch causes a significant loss.

To assess the utility of a number of reported methods for the analysis of tryptophan in complex foods, Friedman and colleagues (1984) evaluated six hydrolytic and one nonhydrolytic acid–ninhydrin methods with three proteins and several carbohydrate-containing food products. For the acid–ninhydrin method, a standard curve for lysozyme was used as a reference to calculate the tryptophan content of the other materials tested. The results (Tables II and III) show that for carbohydrate-containing foods (a) the thioglycolic and organic acid methods appear ineffective, (b) basic hydrolysis by NaOH and $Ba(OH)_2$ appears preferable to hydrolysis in organic acids, and (c) the acid–ninhydrin method merits further study to assess its general applicability to complex foods because it gave good reproducibility with a wide range of commonly used food products.

Table II. Tryptophan Content of Proteins and Foods[a–c] (Values in g/16 g of N)

analytical method	lysozyme[d]	casein	milk powder	soybean protein	soybean flour
thioglycolic acid	5.84	1.27	nd[e]	0.33	nd[e]
mercaptoethanesulfonic acid	6.69	1.26	0.09	1.24	0.31
methanesulfonic acid	5.72	0.71	nd[e]	0.65	nd[e]
p-toluenesulfonic acid	6.64	1.07	nd[e]	1.01	nd[e]
NaOH	5.65	1.10	1.26	1.01	1.07
$Ba(OH)_2$, amino acid analysis		1.30	1.25	1.81	0.81
$Ba(OH)_2$, spectrophotometric	6.54	1.25	1.03	0.75	1.12
acid ninhydrin	7.66	1.70	1.73	1.36	1.91

[a] Friedman et al. (1984). [b] Values are averages from three separate determinations except for a single assay by the acid ninhydrin procedure. [c] Nitrogen content (%): lysozyme, 16.2; casein, 13.6; milk powder, 5.32; soybean protein, 14.0; soybean flour, 8.32. [d] The tryptophan content of lysozyme was calculated to be 7.66 g/16 g of N from its known amino acid sequence and determined nitrogen content. Observed values were corrected for contributions of tyrosine to the absorbance at 385 nm by (TRP(obsd)/TRP(calcd))1.013 + 0.0335Tyr/Trp. [e] Not detected.

Table III. Tryptophan Content of Flours (Values in g/16 g of Nitrogen)

		acid ninhydrin method[a–c]		
protein source	N, %	flour	protein extr of flour[b]	$Ba(OH)_2$ hydrolysis[d]
barley	1.26	1.553	1.271	1.69, 1.83
lima beans	3.32	1.422	1.305	1.62, 1.69
beef, minced	13.57	1.250	1.402	1.58, 1.61
corn, high Lys	1.59	1.795	1.537	1.46, 1.44
corn	1.46	1.853	1.277	1.12, 0.98
cottonseed	10.01	1.373		1.45, 1.56
rice	0.98	1.724	1.371	1.73, 1.82
wheat	2.22	1.557	1.115	1.54, 1.58
soybeans	8.32	1.430	1.425	1.12, 1.13
oats	2.56	1.682	1.329	

[a] Friedman et al. (1984). [b] Proteins were extracted by the procedure described by Concon (1975). [c] Listed values are not corrected for tyrosine absorption. [d] The basic hydrolyzate was treated with *p*-(dimethylamino)benzaldehyde, and the derivatized tryptophan was determined spectrophotometrically.

It appears that protein hydrolysis with methanesulfonic acid as a catalyst cannot be used for tryptophan analysis when carbohydrates are associated with proteins (Friedman et al., 1984; Cuq et al., 1983; Simpson et al., 1976), a situation often encountered in food.

Basic Solutions. Without oxygen, free tryptophan heated in the presence of sodium hydroxide remains stable, as determined by HPLC (Cuq et al., 1983), ion-exchange chromatography, fluorimetry, or colorimetry (Robin and Robin, 1971; Stewart and Nicholls, 1972; Spies and Chambers, 1949). Under the same conditions, but in the presence of air, tryptophan is degraded (Stewart and Nicholls, 1972). The rate of this reaction is dependent on the alkali concentration (Blackburn, 1978). The addition of cysteine, starch, or the other amino acids does not markedly influence tryptophan recovery.

Stability of Tryptophan Residues in Heated Model Compounds and Food. The stability of protein-bound

Table IV. Tryptophan Losses after Heat Treatment at 125 °C of a Glycyl-L-tryptophanglycine Solution, pH 8, Determination by HPLC after Hydrolysis with Methanesulfonic Acid[a]

	Trp loss, %			
atmosphere	3 h	9 h	24 h	48 h
air	9.2	15.9	32.1	71.8
oxygen	18.1	22.2	56.1	86.4

[a] Cuq and Cheftel (1983).

Table V. Tryptophan Losses after Heat Treatment at 125 °C of a Casein Solution[a]

	Trp loss, %			
atmosphere	3 h	9 h	24 h	48 h
air	9.8[b]	15.8		36
	15.4[c]	16.9	41.5	51.5
oxygen	15.8		51.1	79.7
	24.6		73	96
nitrogen			19	14

[a] Determined by HPLC after hydrolysis with methanesulfonic acid or sodium hydroxide. Adapted from Cuq and Cheftel (1983) and Cuq et al. (1983). [b] 5% solution, pH 8. [c] 4% solution, pH 7.

tryptophan during moderate heat treatments, such as domestic cooking or industrial sterilization, has been investigated very little, essentially due to difficulties encountered with the analytical methods. The chemistry of the reactions involved is also very complex (Ziderman and Friedman, 1985; Ziderman et al., 1987; Friedman et al., 1987).

With glycyl-L-tryptophylglycine as a model, it appears that heating for various lengths of time in the presence of air or oxygen leads to the destruction of the tryptophan residue (Cuq et al., 1983). This degradation is greater in the presence of oxygen than in the presence of air (Table IV).

At 125 °C, the pseudo-first-order rate constants are equal to 26 and 40 × 10^{-3} h^{-1}, respectively, in the presence of air and oxygen. The corresponding values for free tryptophan are similar (Cuq and Cheftel, 1983). From these results, it can be calculated that the sterilization of a solution of the tripeptide at pH 8 and at 125 °C for 15 min in the presence of air would lead to a negligible loss of tryptophan.

When a casein solution at pH 8 is heated at 125 °C for up to 48 h, the tryptophan loss reaches 80% under oxygen, while it is only 36% and 14% under air and nitrogen, respectively (Table V). Calculations suggest that only a 1% trytophan loss would result from sterilization of casein solution at 125 °C for 30 min in the presence of air, a value too small to detect by the present methods of analysis. When casein was heated at 80 °C for 4 h in 0.15 M sodium hydroxide, the tryptophan fell by 10% (Nielsen et al., 1985).

These results with model systems suggest that only severe heat treatments in the presence of oxygen significantly degrade protein-bound tryptophan. The destruction of tryptophan in various legume proteins during home-boiling or pressure-cooking is about 5% (Goyal and Mathews, 1985; Abdel-Rahman, 1983; Geervani and Theophilus, 1980). But after roasting or baking, the tryptophan loss is more than 15% (Purcell and Walter, 1982; Hayase et al., 1979). In related studies, (a) Devi and Geervani (1983) found a 9 and 22% loss of tryptophan following baking or deep fat frying of wheat products, respectively; (b) Landry et al. (1988) describe improved procedures for measuring tryptophan in feedstuffs based on hydrolysis by barium hydroxide; (c) Tschitel and Karnauschenko (1987) observed that freezing causes a decrease in the tryptophan content of bread products; (d) Orsi (1985) found an activation energy of 105 kJ mol^{-1} for the decomposition of tryptophan in an infant formula stored for 8 h in the temperature range of 40–100 °C; (e) Pearce et al. (1988) report a ninhydrin color yield of 0.88 for tryptophan compared to 1.0 for leucine; and (f) Voutsinas and Nakai (1979) described a method for covalently attaching tryptophan to soy proteins.

Racemization. The effects of heating time and temperature on racemization of free tryptophan in strongly basic solutions were studied by Spies and Chambers (1949). Complete racemization of free tryptophan occurred at 151 and 185 °C in 8 and 2 h, respectively. After 18 h at 100 °C, isomerization of free tryptophan was only 11%.

Under moderate alkaline treatment (pH 9, 83 °C) for times ranging from 0.5 to 96 h, it appears (Liardon and Lederman, 1986) that racemization of free tryptophan occurs with an inversion rate constant equal to 2.7 × 10^{-8} s^{-1}. Thermal treatment of a 10 mM aqueous tryptophan solution at 140 °C for 24 h in the presence of air isomerized only 5% of the remaining tryptophan to the D configuration. In strong mineral acid conditions, such as those used for standard protein hydrolysis, the inversion rate constant of free tryptophan is lower than for many of the other free amino acids (Liardon and Jost, 1981; Friedman and Liardon, 1985). This rate is related to the electron-withdrawing capacity of the side chain (negative inductive strength).

The power of the linear free energy correlations is illustrated by the fact that although the rate of racemization of tryptophan residues in soybean proteins could not be measured, Friedman and Liardon (1985) predicted that its rate should be similar to that of alanine, since the inductive constants of both amino acids are also similar. This general prediction was confirmed by Nielsen et al. (1985a), who showed that the (D/(D + L)) ratios for alanine and tryptophan in casein treated with alkali are identical within experimental error. It remains to be shown whether this is also true for other proteins.

Protein-bound tryptophan is known to racemize under various conditions of temperature and pH. In strongly alkaline solution, such as that used for protein hydrolysis, complete isomerization (racemization) occurs in proteins after 18 h at 100 °C (Spies and Chambers, 1949). At pH 9 and 83 °C, the inversion rate constant (Liardon and Ledermann, 1986) of tryptophan in lysozyme reaches 7 × 10^{-8} s^{-1}. In a concentrated acid solution, such as that used for protein acid hydrolysis, isomerization is slow (Liardon and Jost, 1981). The probable reason why protein-bound tryptophan isomerizes more rapidly than free tryptophan is that the reaction proceeds via the abstraction of the α-hydrogen and the formation of a carbanion. This asymmetrical intermediate may equilibrate with a symmetrical enolate tautomer. Reprotonation of the L or D carbanion yields L- or D-tryptophan (Figure 8). Any factor tending to modify the equilibrium would be expected to affect the extent of the isomerization. Thus, the inversion rate is directly related to the structural and electronic characteristics of the side chain and also to its microenvironment (Friedman and Liardon, 1985; Liardon and Friedman, 1987).

Carboline Formation. At temperatures above 200 °C, often reached in grill-cooking of meat and fish, various tryptophan derivatives can form. Most of the tryptophan derivatives, including the carbolines, have been found in cooked, broiled, or grilled foods such as beef, chicken, and sardines, and in beer and wine (Barnes et al., 1983; Nagao

Table VI. Approximate Yield of Some Derivatives Found in Free Tryptophan or Protein Pyrolyzates (μg/g of Free or Bound Tryptophan)[a]

	Norharman	Harman	Trp-P-1	Trp-P-2	A-α-C	Me-A-α-C
tryptophan	27000	17000	7	10	250	20
casein	2800	500		5	300	
albumin	2200	1100		6	4300	
zein				0	tr	

[a] Adapted from Sugimura (1985, 1986).

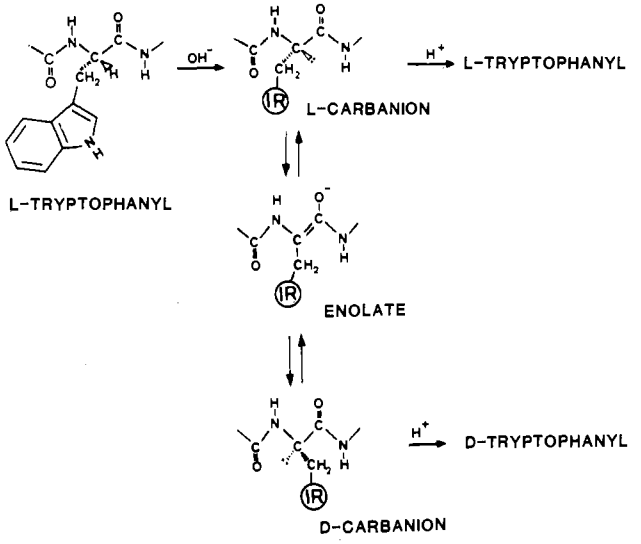

Figure 8. Isomerization of protein-bound tryptophan. IR = indole ring.

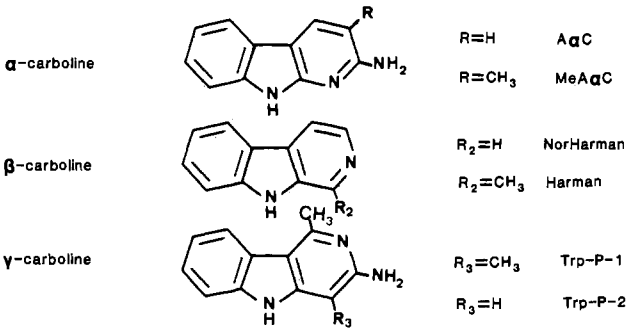

Figure 9. Formulas of some derivatives found in the basic fraction of tryptophan or protein pyrolysates.

et al., 1983; Bosin et al., 1986; Milon et al., 1987).

Among these compounds, α-, β-, and γ-carbolines have been identified from the basic fraction of the pyrolysates (Sugimura, 1986) (Figure 9). The yields of these compounds are a complex function of temperature and heating time (Yoshida and Matsumoto, 1979; Yoshida et al., 1979, 1980; Nishigata et al., 1980). They also depend on the heating atmosphere, i.e., air or nitrogen. Heating produces much larger amounts of β-carbolines than α- or γ-carbolines (Table VI). The probable mechanism of formation of β-carbolines varies for free or bound tryptophan (Figure 10). Thus, the formation of β-carbolines during the treatment of free tryptophan requires organic derivatives such as aldehydes. The chemical condensation between these compounds, resulting from the degradation of the amino carboxylic chain, gives carbonylamine. After H_2O loss, the Schiff base created can react with the C_2 electron donor of the indole ring to give a hydrocarboline. The formation of β-carboline then occurs by dehydrogenation. From bound tryptophan, direct cyclization between the C_2 of the indole ring and the "carbonyl" group of the peptide bond may be the first stage of the reaction. But it is more probable that β-carboline formation requires the

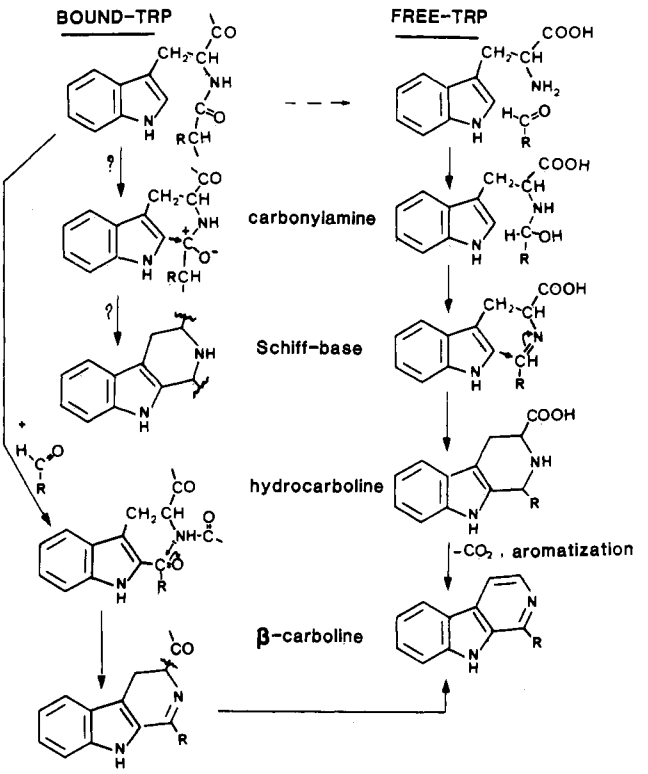

Figure 10. Proposed pathways for the formation of β-carbolines during heating of free or protein-bound tryptophan (Cuq and Friedman, 1989).

presence of carbonyl compounds resulting from the thermal degradation of the protein. In that case, condensation occurs between C_2 of the indole ring and the aldehyde (Figure 10).

Reactions of Tryptophan with Other Food Components or Additives. *Reducing Sugars.* The Maillard reaction between food proteins and reducing sugars causes browning during storage or heating. Whereas the contribution of the ε-NH_2 lysyl group in this reaction is well-documented (Cheftel, 1977; Friedman, 1982; Smith and Friedman, 1984; Ziderman and Friedman, 1985; Ziderman et al., 1987), the reactivity of the indole ring with reducing sugars is less well-known. The α-amino group of the free amino acid reacts with carbonyl compounds such as pentoses or hexoses (Lee et al., 1979). The extent of this Maillard reaction is a function of time, temperature, and water activity (Leahy and Warthesen, 1983).

Free tryptophan in the absence of other reactive compounds is relatively unaffected by heating at temperature near 100 °C for about 10 h. However, in the presence of glucose the loss reaches about 50%. This loss is much greater at a water activity (a_w) of 0.75 than at 0.22. In comparison with the lysine–glucose browning mixture, it appears that at the lower a_w the Maillard reaction is less extensive and that other reactions involving the indole ring take place, leading for instance to the formation of carboline derivatives (Leahy and Warthesen, 1983; Brautigam and Severin, 1974) (Figure 10). When the α-NH_2 of free

tryptophan is acetylated to prevent its reaction with glucose, glucose does not react with the indole nitrogen, showing that the indole ring is stable in the presence of reducing sugars (Finot et al., 1982). The indole nucleus can also react with Maillard derivatives such as the advanced reaction products formed by heating glycine and glucose (Finot et al., 1982).

Despite its low basicity (nucleophilicity), the NH group of the indole ring participates in the Maillard reaction, in contrast to the suggestion of some authors (Finot et al., 1982; Dworschak and Orsi, 1977). Thus, in pure acetic acid, N^{α}-acetyl-1-(β-D-glycopyranosyl)-DL-tryptophan amide has been identified following the reaction of N^{α}-acetyl-DL-tryptophan amide with either D-xylose or D-glucose at 100 °C (Nyhammar and Pernemalm, 1985). The different findings may be due to the fact that Finot et al. (1982) carried out their experiments in water and Nyhammar and Pernemalm (1985) in pure acetic acid.

The behavior of protein-bound tryptophan during the Maillard reaction has been studied very little, essentially due to the difficulties of adequate analysis. As observed with the α-NH_2-protected free tryptophan, the thermal degradation of protein-bound tryptophan, measured by HPLC after alkaline hydrolysis, apparently is not significantly influenced by the presence of reducing sugars (Abdel-Rahman, 1983, Nielsen et al., 1985; Hayase et al., 1979).

Aldehydes and Ketones. Both the amino group and the indole ring of tryptophan condense with carbonyl compounds such as ethyl acetoacetate (McLaren, 1977), 1-hexanal (Arai, 1980), pyruvic acid (Chu and Clydesdale, 1976), α-ketoglutaric acid (Chu and Clydesdale, 1976), dehydroascorbic acid (Namiki et al., 1982a,b), aldehydes (Jayasimhulu and Day, 1980), other carbonyl compounds (Saito et al., 1986), and acetyl chloride (Previero et al., 1972) to form β-carboline derivatives. For bound tryptophan, the first reaction occurs between C_2 of the indole ring and the carbonyl compound. For free tryptophan, this reaction can be associated with another condensation between the α-NH_2 group and the carbonyl derivative (Figure 10). Temperature, time, and pH greatly influence the pattern of these complex reactions.

Peroxidizing Lipids. Lipid oxidation products such as hydroperoxides, cyclic peroxides, aldehydes, and ketones react with free and bound tryptophan. For example, reactions between free tryptophan and peroxidizing methyl linoleate at low water activity, involving a free-radical mechanism, result in the degradation of the amino acid. *N*-Formylkynurenine, kynurenine, and diastereomers of dioxindole-3-alanine have been identified among the degradation products (Krogull and Fennema, 1987; Yong et al., 1980). Bound tryptophan, determined after alkaline hydrolysis and HPLC, reacts with oxidizing lipids (Nielsen et al., 1985). The extent of degradation increases with time and temperature. Secondary products of lipid oxidation such as aldehydes or ketones can react with free or bound tryptophan to form carboline derivatives, as indicated earlier.

Krogull and Fennema (1987) showed that the rate of tryptophan oxidation to kynurenine and *N*-formylkynurenine in the presence of methyl linoleate is dependent on pH, ionic strength, water activity, buffer concentration, temperature, and copper ions. These observations imply that both ionic and free-radical intermediates are produced during oxidation. In a related study, Kanner and Fennema (1987) report (a) that riboflavin-catalyzed photooxidation of tryptophan to kynurenine and *N*-formylkynurenine at 20 °C proceeded most rapidly at pH 7.5 and (b) that, of three antioxidants tested, sodium azide and L-ascorbic acid were equally effective in retarding oxidation, with EDTA least effective.

Hydrogen Peroxide. Hydrogen peroxide, which is used to sterilize processing equipment and packing materials, oxidizes tryptophan (Cuq and Cheftel, 1983; Yong et al., 1980; de Weck et al., 1987). However, the rate of this pseudo-first-order reaction is very low, particularly in comparison with that of hydrogen peroxide with methionine. Thus, in the presence of 0.2 M hydrogen peroxide, only 20% of the initial tryptophan is destroyed at 50 °C after 96 h. Under the same conditions, methionine is completely oxidized in less than 10 min. The rate of tryptophan oxidation increases with temperature.

Radiation. Exposure of tryptophan to γ-radiation in the presence of oxygen results in the formation of *N*-formylkynurenine (Garrison, 1987). Just as with hydrogen peroxide, tryptophan is less susceptible than the sulfur amino acids to radiation-induced oxidation (Friedman et al., 1973; Garrison et al., 1987). In view of the proposed use of γ-radiation to sterilize certain foods, this aspect of tryptophan chemistry is of paramount importance for food science. Tryptophan residues in proteins are also susceptible to ultraviolet radiation (Sun and Zigman, 1979; Khan and Ali, 1986; Donoso et al., 1988).

It is also noteworthy that sunlight-induced transformation of tryptophan leads to the formation of off-flavors in milk (Creamer and Matheson, 1976) and yellowing of wool (Friedman and Finley, 1971).

Sulfites. Free tryptophan is rapidly destroyed in the presence of Mn^{2+} during the aerobic oxidation of sulfite ions (Yang, 1973). A free-radical mechanism occurs during this oxidation. The superoxide anion radical ($O_2^{\bullet -}$)–*N*-formylkynurenine has been identified among the many derivatives formed.

Nitrites. An important proportion of the nitrite added to meat products for curing reacts with free or protein-bound tryptophan (Bonnett and Holleyhead, 1974; Nakai et al., 1978; Kinae, 1986; Mellet et al., 1986; Ochiai et al., 1986). With free tryptophan, nitrosation essentially affects only the primary amino group. But when this group is blocked by acetylation, nitrosation of the indole nitrogen is promoted, leading to the formation of the *N'*-nitroso derivative. The extent of this reaction increases with time of exposure to nitrites. In addition, pH is important since N'-nitrosation proceeds rapidly at low pH, slowly at pH 5, and very sluggishly above pH 5.5.

Halogens. The indole ring of tryptophan can participate in electrophilic substitution reactions with halogens. Such chlorination and/or iodination reactions may lead to the formation of mutagens in drinking water treated with chlorine-based disinfectants (Bercz and Bawa, 1986; Fielding and Horth, 1986; Tan et al., 1987). The scope of these transformations and their significance for human health and safety are not well understood.

The reactions previously described can lead to loss of nutritional value or occasionally to the production of potentially toxic compounds.

ANALYTICAL ASPECTS

The most widely studied method for the analysis of protein-bound tryptophan in food and feedstuffs appears to be alkaline hydrolysis (Friedman and Finley, 1971, 1975; Friedman et al., 1984; Hugli and Moore, 1972; Nielsen and Hurrell, 1985; Huet and Pernollet, 1986; Levine, 1982; Sato et al., 1984; Sastry and Murray, 1987). The alkalis used for protein hydrolysis are NaOH, $Ba(OH)_2$, or LiOH. When $Ba(OH)_2$ is used, Ba^{2+} is removed from the hydrolysate as the insoluble barium sulfate, preferably after the

solution is brought to pH 2 to minimize losses due to adsorption of $BaSO_4$ to the glass containers. Starch, maltodextrin, or thiodiglycol reduces the losses of tryptophan when the hydrolysis is made in the near absence of oxygen. Apparently, starch or maltodextrin removes the residual traces of oxygen in the hydrolysis medium by reacting with oxygen. The unknown products formed do not react with tryptophan. When thiodiglycol is used as antioxidant, the resulting sulfoxide acts as a mild oxidizing agent of tryptophan. Tryptophan recovery from protein after alkaline hydrolysis in the absence of starch is essentially a function of the residual oxygen. As free or bound tryptophan shows the same behavior in strongly basic conditions, the destruction observed appears to be linked to the modification of the indole ring. For example, during the alkaline hydrolysis of ^3H-labeled goat casein, many unidentified products are formed in small quantities (Nielsen and Hurrell, 1985). Another reason for the unsatisfactory recovery of protein-bound tryptophan involves the incomplete hydrolysis of tryptophanyl peptide bonds. This conclusion is supported by the observed slow hydrolysis of valyltryptophan and isoleucyltryptophan.

These results indicate that hydrolysis under vacuum with alkali appears to be a useful method for the determination of protein-bound tryptophan. However, modifications of existing methods and the development of new methods are very active areas of tryptophan research (Finley et al., 1975; Sodek et al., 1975; Oste et al., 1976; DeVries et al., 1980; Lucas and Sotelo, 1980; Piombo and Lozano, 1980; Wieser et al., 1983; Eka and Oyeleke, 1984; Maeda et al., 1984; Hewitt and Ford, 1985; Chrastil, 1986; Delhaye and Landry, 1986; Werner, 1986; Scheuermann and Eckstein, 1986; Kirchgessner et al., 1987; Allred and MacDonald, 1988; Gorinstein et al., 1988; Landry et al., 1988).

Because tryptophan has a strong affinity for serum albumin, difficulties are often encountered in measuring serum-bound and free tryptophan in plasma (Friedman, 1984; Labadarios et al., 1986; Gjerde et al., 1987). Additional research is also needed to devise improved methods to differentiate between free and albumin-bound tryptophan in body fluids.

NUTRITION

Bioavailability of Tryptophan in Processed Foods. Contradictory results exist about the bioavailability of tryptophan after heat treatment, such as domestic cooking or industrial sterilization. Autoclaving of rapeseed meal at 121 °C for 6 h caused a 50% decrease in the nutritional availability to rats (Sarwar and Boland, 1976). This decrease, however, could result principally from the reaction of rapeseed proteins with carbohydrates. In casein heated at 130 °C for 8 h, only a 4% decrease in the availability of tryptophan was observed as measured by the growth of *Streptococcus zymogenes* (Osner and Johnson, 1975). No reduction in the availability of this amino acid as measured by *Leuconostoc mesenteroides* was observed when lamb or mutton meat was cooked in an oven (Blum et al., 1966). However, heating cod muscle at 85 °C for 27 h reduced the availability by 16.5% as measured by *S. zymogenes* growth (Miller et al., 1965). General thermal treatment of the protein seems to reduce the availability of this amino acid, allthough there are few changes in amino acid content as determined by physical or chemical methods. Often, the reduction observed in nutritional value is due to reduced digestibility of amino acids (Spies and Chambers, 1949; Tschitel and Karnauschenko, 1986; Nielsen et al., 1985c).

When free tryptophan is submitted to a heat treatment of 140 °C for 24 h in the presence of air, leading to an 80% loss, the derivatives formed apparently do not have any tryptophan nutritional value for and may be toxic to rats. Thus, with a basal diet limiting in tryptophan, the weight gain observed after the ingestion of the heated preparation of tryptophan is not proportional to the remaining tryptophan (Varnish and Carpenter, 1975).

The same phenomenon is observed from heated casein, in which destruction of tryptophan, determined by alkaline hydrolysis and HPLC, reaches 43%. The nutritional utilization of the tryptophan residues in this heated casein was lower than the level of undestroyed tryptophan. This effect can result from the decrease in protein digestibility or from toxic or antinutritional derivatives formed during the heat treatment (Cuq and Gilot, 1985).

The losses in tryptophan bioavailability during heat treatment such as home-cooking or industrial sterilization, however, appear less important when compared to the other detrimental effects, particularly on lysine or methionine.

The rat and the pig seem to be useful animal models for estimating tryptophan bioavailabilities from foods and feeds (Sarwar et al., 1984, 1985; Sato et al., 1987; Heger and Frydrych, 1987). Weggmann et al. (1979) measured the nutritive value of *N*-acetyl-L-tryptophan in man. It is also worth noting that the drug atropine affects the pancreatic secretory response to intestinal tryptophan (Vazquez-Echarri et al., 1986).

Nutritional Availability of Tryptophan Heated in the Presence of Reducing Sugars. The Maillard reaction between proteins or amino acids and reducing carbohydrates causes deterioration of the nutritional quality of foods during storage or processing (Mauron, 1972; Pintauro et al., 1983; Friedman et al., 1987). This reaction is often used to produce desirable flavors, colors, and aromas (Hurrell, 1982; Ashoor and Zent, 1984). It generally occurs between primary amino groups such as the ϵ-NH_2 groups of lysine, the α-NH_2 of the free amino acid, or the α-NH_2 of the terminal amino acid and the reducing sugar. Whereas the biological consequences of this reaction upon lysine have been extensively investigated, the involvement of tryptophan residues is little known. The loss of tryptophan determined both chemically and biologically (rat assay) is only slightly influenced by the Maillard reaction, while the corresponding loss of lysine was substantial (Gilot et al., 1985; Nielsen et al., 1985).

Rats appear to absorb only a small percentage of the Amadori compounds glucose–L-tryptophan and fructose–L-tryptophan, purified from a Maillard reaction between the free amino acid and the corresponding reducing sugar (Sgarbieri et al., 1973; Tanaka et al., 1975; Hurrell et al., 1983).

Nutritional Consequences of Racemization. Many reports have demonstrated that the efficiency of the utilization of D-tryptophan differs considerably among species. Humans (Rose, 1954; Berg, 1959; Young, 1971), mice (MacEwan and Carpenter, 1980; Ohara et al., 1980; Friedman and Gumbman, 1989), rabbits (Loh and Berg, 1971), and chickens (Ohara et al., 1980) utilize D-tryptophan very poorly. Dogs (Czarnecki and Baker, 1982) and pigs (Arentson and Zimmerman, 1985) utilize the D isomer of this amino acid more efficiently than humans, but less efficiently than rats (Kirchgessner and Roth, 1985; Ohara et al., 1980).

The level of the other amino acids and the addition of nicotinic acid may influence the utilization of D-tryptophan (Ohara et al., 1980; MacEwan and Carpenter, 1980; Umezawa, 1989).

Table VII. Growth of Mice Fed D- and L-Tryptophan as Part of an Amino Acid Diet[a]

amino acid tested	% in diet	mean wt gain after 14 days, g	% equiv in diet[b]	rel potency,[c] %
L-Tryp	0	−3.5		
	0.022	−1.8		
	0.044	2.2		
	0.087	12.4		
	0.174	15.6		
D-Tryp	0	−3.5		
	0.022	−2.8	0.014	64
	0.044	−1.8	0.024	55
	0.087	−0.4	0.033	38
	0.174	3.8	0.051	29

[a] Friedman and Gumbmann (1989). [b] Percent equivalent in the diet is that concentration of L-amino acid that would produce the same growth observed for the D-amino acid (determined graphically). [c] Relative potency is the percent equivalent in the diet divided by the percent actually fed × 100.

Bound L-tryptophan isomerizes slowly during the thermal treatment of food proteins. The loss observed in available tryptophan could result from the isomerization of the other amino acids, leading to a specific decrease in the enzymatic release of tryptophan. This loss, however, would appear to be less significant when compared with the other detrimental consequences of the thermal effects.

Some of the reported variabilities in the utilization of D-tryptophan could be due to the fact that the value (potency) of D-tryptophan as a nutritional source of L-tryptophan is strongly dose dependent (Table VII).

The biological utilization of tryptophan in different food types as a function of different processing conditions merits further study.

The D isomer of tryptophan and *N*-formylkynurenine are potential sweetening agents (Finley and Friedman, 1973; Yamada et al., 1975). It is also noteworthy that significant amounts of D-tryptophan are biosynthesized in wilting leaves of many plants (Rekoslavskaya et al., 1986).

TOXICOLOGY

Toxicity of Derivatives. Among oxidation products resulting from photooxidation or from the oxidation of tryptophan by strong oxidizing agents such as hydrogen peroxide or peroxidizing lipids are *N*-formylkynurenine, kynurenine, dioxindole-3-alanine, β-carboline, quinazoline, and hexahydropyrroloindole derivatives (Yong et al., 1980; Kanner and Fennema, 1987; Walrant and Santus, 1974; Savige, 1975; Nakagawa and Hino, 1977; Sun and Zigman, 1979). These compounds do not possess any nutritional value, and some exhibit toxicity for bacteria, isolated mammalian cells, and animals (Steinhart and Kirchgessner, 1978; de Weck et al., 1987).

When protein reacts with oxidizing lipids, extensive loss of available tryptophan is observed. This loss is due to the destruction of this amino acid and to the general decrease in protein digestibility (Nielsen et al., 1985; Steinhart and Kirchgessner, 1978). In stored foods, in which such reaction can occur, tryptophan loss probably would be negligible compared to the oxidation of methionine to its sulfoxide and to the loss of lysine (Nielsen et al., 1985).

Toxicology of Carbolines. As indicated earlier, carboline formation occurs when free or bound tryptophan is heated at high temperature. These derivatives also have been found in foods such as commercial beef extracts and fried hamburger (Bjeldanes et al., 1984; Felton et al., 1984), heated milk (Rogers and Shibamoto, 1982), and beer and wine (Bosin et al., 1986). Among these compounds, two γ-carbolines (Trp-P-1, Trp-P-2) and two α-carbolines (A-α-C, Me-A-α-C) show significant mutagenic activity in *Salmonella tryphimurium* tester strains after metabolic activation. Although not mutagenic themselves, the β-carbolines (harman and norharman) enhance the activity of the mutagenic α- or γ-carbolines. There is an abundance of scientific information on the *Salmonella* mutagenicity of these compounds, and some important reviews have been written on this topic (Sugimura, 1985, 1986; Sugimura et al., 1980; Nagao et al., 1983). Feeding mice a diet containing Trp-P-1, Trp-P-2, or other carbolines demonstrated the hepatocarcinogenicity of these compounds (Table VIII).

Table VIII. Mutagenicities and Carcinogenicities of Heterocyclic Amines Isolated from Cooked Foods[a]

heterocyclic amine	mutagenicity (TA98), revertants/μg	mouse carcinogenicity
1Q	433 000	liver, forestomach, lung
MeIQ	661 000	liver, forestomach
dMeIQx	145 000	liver
Trp-P-1	39 000	liver
Trp-P-2	104 200	liver
Glu-P-1	49 000	liver, blood vessel
Glu-P-2	1 900	liver, blood vessel
A-α-C	300	liver, blood vessel
Me-A-α-C	200	liver, blood vessel

[a] Adapted from Ohgaki et al. (1986) and Sugimura (1986).

Risk assessment (Ames et al., 1987; Sugimura, 1986) suggests that it can be assumed that some human cancers are the result of the absorption of carbolines in heated proteinaceous foods. The Maillard reaction, which contributes separately to the formation of some of these carbolines, may be also an important contributor to the carcinogenic potential of our foods (Barnes et al., 1983; Omura et al., 1983; Spingarn et al., 1983; Taylor et al., 1985; Friedman et al., 1988).

In a long-term assay (about 500 days), an intake of 2 g of pyrolysates of tryptophan or arginine–tryptophan per kilogram of diet containing 12% of protein appeared to induce a sex-dependent decrease in the growth of rats. Pyrolysate withdrawal in accustomed rats (second and third generations) demonstrated an acquired inurement to these products. Indeed, their removal caused an increased food consumption and growth (Rabache and Adrian, 1984).

The level of these potential carcinogens in some broiled or roasted foods, such as broiled sardines, is about 10 ng/g. This amount is much lower than those used in the carcinogenicity assays. The long-term cumulative effect of the carboline intake may be of great concern. In fact, tryptophan itself can promote the formation of tumors (Berry and Helmes, 1984; Sidransky, 1987; Williams, 1988), and a tryptophan–riboflavin photoinduced adduct may have a role in the pathogenesis of hepatic dysfunction observed during parenteral nutrition (Donoso et al., 1988).

In view of the extremely high mutagenic activity of the heterocyclic amines derived from browning reactions toward both bacteria (Ames test) and mammalian cell lines, the question arises whether such short-term tests of genotoxicity are good predictors of carcinogenicity. According to Sugimura (1986), Weisburger (1987), and Krone et al. (1986), this is indeed the case. All of the heterocyclic amines tested induce multiple tumors in rats and mice. Target organs include breast, colon, and pancreas, major sites of human cancer throughout the world. It should also be noted that the heterocyclic amines derived from tryptophan were found to induce liver cancer (Table VIII).

Sugimura (1985, 1986) estimates that the average person consumes about 100 μg of heterocyclic amines/day. This is a significant amount, in view of these compounds' re-

ported extremely high mutagenic activities and the possibility that the effects in animals and humans could be cumulative. The presence of tumor promoters in the diet may further enhance the risk of consuming heterocyclic amines.

It is also worth noting that a form of cytochrome P-450 responsible for activation of Trp-1 and Trp-2 in vivo to active carcinogens is inducible by dietary treatment of mice or rats with these compounds (Degawa et al., 1987). These workers also report that the amount of both native and inducible cytochrome P-450 is related to the species, sex, and organ differences in their carcinogenic susceptibility to the tryptophan derivatives.

These considerations suggest an urgent need, repeatedly emphasized by active workers in the field (Sugimura, 1986; Ohgaki et al., 1986; Hatch and Felton, 1986; Weisburger, 1987) to develop new approaches and strategies to prevent the formation during food processing of heterocyclic amines and other browning products.

FUTURE RESEARCH

This survey of heat- and chemical-induced transformations of tryptophan and the nutritional and toxicological consequences suggests an urgent need for additional research (a) to define possible approaches to prevent or minimize the formation of antinutritional and toxic tryptophan condensation and oxidation products in foods; (b) to explore possible beneficial effects of antioxidants such as vitamins C and E, carotenes, flavonoids, indole derivatives, selenium compounds, and sulfur amino acids in enhancing the stability of tryptophan in foods; (c) define conditions to prevent formation in vitro and activation in vivo of carbolines to mutagens and carcinogens; and (d) to develop reliable assays to measure tryptophan in cereals, dairy and meat products, processed foods, and body fluids.

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