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Tryptophan Degradation During Heat Treatments: Part 2—Degradation of Protein-Bound Tryptophan*

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ABSTRACT

The stability of free or protein-bound tryptophan was determined during acid (4 M methane sulfonic acid) or alkaline (4·2 M sodium hydroxide) hydrolysis at 110°C under nitrogen. The influence of time and of glucose, starch or amino acid addition was studied. It was found that hydrolysis with methane sulfonic acid caused tryptophan losses when glucose or starch was present.

The thermal degradation of protein-bound tryptophan was studied using either glycyl-L-tryptophyl-glycine or casein. Analysis of tryptophan was carried out after acid or alkaline hydrolysis, through high performance liquid chromatography and UV detection.

Heat treatment of tripeptide (9·4 mM, pH 8, 125°C, 3 to 48 h) in the presence of oxygen, air or nitrogen resulted in peptide hydrolysis, with the formation of the corresponding dipeptides and of free tryptophan. After acid or alkaline hydrolysis, tryptophan loss was seen to be higher when the initial heat treatment was performed in the presence of oxygen than in the presence of air.

Thermal treatment of 4 or 5% w/v casein solutions (pH 7 or 8) was carried out at 125°C, under oxygen, air or nitrogen. A marked loss of tryptophan was found to occur after 24 h at 125°C when oxygen or air was present. Under nitrogen, protein-bound tryptophan was heat stable, even in the presence of glucose or starch. It is therefore unlikely that the indole ring of protein-bound tryptophan may react with reducing carbohydrates

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through Maillard-type reactions. Oxidative degradation of protein-bound tryptophan is also unlikely to reach any significant extent during the heat processing of foods, unless catalysts or strong oxidizing agents are present.

INTRODUCTION

Heat treatments of varying intensity cause different chemical modifications of protein-bound tryptophan.

Above 200 °C, as in the grill cooking of meat and fish, various derivatives of tryptophan can be formed, such as α or β carbolines (Sugimura *et al.*, 1977). Some of these derivatives possess high mutagenicity according to the Ames test on Salmonella (Moule, 1980; Nishigata *et al.*, 1980; Yoshida *et al.*, 1980). Severe heat treatments can also lead to the isomerization of L into D-tryptophan residues (Hayase *et al.*, 1975, 1979). D-tryptophan can be utilized by rats but has no nutritional value for chickens (Ohara *et al.*, 1980) or for man (Rose *et al.*, 1954). The presence of D-tryptophan residues in a protein may also reduce its digestibility.

The effect of moderate heat treatments, such as domestic cooking or industrial sterilization, are less well known. Sarwar & Bowland (1976) have shown that autoclaving a rapeseed meal at 121 °C for 24 h caused a 50% decrease in the nutritional availability of tryptophan for rats; this decrease, however, could result principally from the reaction of rapeseed proteins with reducing carbohydrates. Osner & Johnson (1975) reported a 4% decrease in the availability of tryptophan, as measured by the growth of *Streptococcus zymogenes*, in casein (6·3% H_2O) heated at 130 °C for 8 h. Donoso *et al.* (1962), using the analytical method of Spies & Chambers (1949), found a 5% loss of tryptophan in meat autoclaved at 110 °C for 24 h. Blum *et al.* (1966) did not find any reduction in the availability of tryptophan (*Leuconostoc mesenteroides* growth) when lamb or mutton meat was cooked in an oven. However, heating cod muscle at 85 °C for 27 h in the presence of 14% water reduced the availability of tryptophan by 16·5% (*Streptococcus zymogenes* growth), while the tryptophan content of cod muscle remained unchanged (method of Spies and Chambers after protein hydrolysis with barium hydroxide) (Miller *et al.*, 1965). When cod muscle was heated at 85 °C for 27 h in the presence of 14% water and 10% glucose, the availability of tryptophan was reduced by 83% without modification of the tryptophan

content (method of Spies and Chambers). More recently, other authors reported on the possibility of Maillard type reactions between reducing carbohydrates and the nitrogen atom of the indole ring (Brautigam & Severin, 1974; Dworschak & Orsi, 1977; Orsi & Dworschak, 1978).

Discrepancies between these various studies could be due to difficulties encountered with the analytical methods for tryptophan determination (Friedman & Finley, 1975). It may be recalled here that tryptophan is degraded during the hydrochloric acid hydrolysis of proteins. This degradation appears to be linked to the transformation of cystine into bis-(2-amino-2-carboxyethyl)-trisulfide, and to the reaction of this compound with tryptophan (Ohta & Nakai, 1979). Oxidized chlorine derivatives may also contribute to the degradation of tryptophan (Ohta *et al.*, 1981).

In the present study, two methods of protein hydrolysis have been utilized: that with methane sulfonic acid (Simpson *et al.*, 1976) and that with sodium hydroxide (Hugli & Moore, 1972). Tryptophan was then determined by HPLC. These two methods have been tested on model proteins with or without the addition of carbohydrates before hydrolysis.

In the second part of this study, the thermal degradation of peptide- or protein-bound tryptophan was assessed using glycyl-tryptophyl-glycine and casein, heated in the presence or in the absence of oxygen and/or glucose.

MATERIAL AND METHODS

Reagents

Tryptophan, tryptamine, methane sulfonic acid, casein (Hammarsten), lysozyme (No. 5282) and α_{S1} casein (No. 2246) are from Merck. Kynurenine and *N*-formyl kynurenine are from Calbiochem. β lactoglobulin (L-2506) and starch (S-4501) are from Sigma. The tripeptide, glycyl-L-tryptophyl-glycine, and the dipeptides, glycyl-L-tryptophan and L-tryptophyl-glycine, are from Interchim. Nitrogen 'U' (less than 5 ppm O_2) and oxygen 'R' come from l'Air Liquide.

Thermal treatments

(1) Glycyl-L-tryptophyl-glycine

Two millilitres of 9·4 mM tripeptide in 0·1 M sodium phosphate buffer, pH 8, are placed in a 180 mm × 18 mm pyrex tube. The tube is heat-sealed at

atmospheric pressure under air, oxygen or vacuum (Cuq & Cheftel, 1982). The tube is then heated in an oven at 125 ± 1·5 °C for 3, 9, 24 or 48 h. After opening by heat shock, the contents are analyzed by HPLC either directly or after hydrolysis with methane sulfonic acid.

(2) Casein

Four or five grams dry weight of casein are dissolved in 100 ml of 0·1 M sodium phosphate buffer, pH 7 or 8. The nitrogen content of casein is 15·7 g nitrogen per 100 g dry weight (Kjeldahl determination with a Tecator analyzer). Two millilitres of casein solution are placed in pyrex tubes, which are heat-sealed and then heated as indicated above. 0·1 to 0·2 ml of the tube contents are used for protein hydrolysis.

Hydrolysis with methane sulfonic acid (according to Simpson et al., **1976)**

The following samples are placed in 18 mm × 180 mm pyrex tubes: 0·5 ml of a 10 mM tryptophan solution in water, or 0·5 ml of the glycyl-L-tryptophyl-glycine solution, or 0·1 (or 0·2) ml of the casein solution. One millilitre of an 8N methane sulfonic acid solution (containing 0·4% tryptamine) is added. The volume is completed to 2 ml with distilled water. Dissolved gases are evacuated by freezing in liquid nitrogen and slow rewarming under a vacuum of approximately $0{\cdot}7 \times 10^{-4}$ bars. The tube is then heat-sealed under vacuum, and placed in an air oven at 110 ± 1·5 °C for 24, 48 or 72 h.

After opening by heat-shock, 2 ml of 3·5N sodium hydroxide are added to the hydrolyzate. The tube contents are poured into a volumetric flask and completed to 5 ml with distilled water.

Hydrolysis was also carried out with the addition of cysteine (1·25 or 2·5 mM), of the following amino acid mixture: Asp, Thr, Ser, Glu, Pro, Gly, Ala, Val, Cys, Met, Ile, Leu, Tyr, Phe, Lys, His, Arg (0·625 mM each), of glucose (1·25 to 10 mM) or of starch (75 mg/2 ml).

Hydrolysis with sodium hydroxide (according to Hugli & Moore, 1972)

The following samples are placed in 8 mm × 80 mm polypropylene tubes (Bioblock, Strasbourg) themselves inserted in 18 × 180 mm pyrex tubes: 0·5 ml of a 10 mM tryptophan solution in water, or 0·1 (or 0·2) ml of the casein solution. One millilitre of an 8·4N sodium hydroxide solution is added. The volume is completed to 2 ml with distilled water. The pyrex

tube is heat-sealed under nitrogen after thorough evacuation of air, and placed in an air oven at $110 \pm 1{\cdot}5\,°C$ for 24, 48 or 72 h.

After opening by heat shock, 1·4 ml of 6N HCl are added to the hydrolyzate. The tube contents are brought to 5 ml with distilled water.

Hydrolysis was also carried out with the addition of cysteine, of an amino acid mixture, of glucose, or of starch (see above).

High pressure liquid chromatography

HPLC was performed in a Spectra Physics SP 8000 with an RP 18 (7 μm) column, at room temperature. Elution was carried out with a mix of methanol–0·01M sodium acetate buffer, pH 4 (15–85 % v/v, respectively), at a flow rate of $1\,ml\,min^{-1}$. Absorbance was measured at 278 (or 350) nm.

UV absorption spectra

These spectra were measured on the tripeptide solutions, after 1/30 dilution, using a Beckman DB-GT spectrophotometer.

RESULTS AND DISCUSSION

(1) Stability of tryptophan during hydrolysis with methane sulfonic acid

Free tryptophan, heated for various lengths of time, used for protein hydrolysis (4N methane sulfonic acid, 110 °C, under nitrogen) remains relatively stable, as determined by HPLC (Table 1). The addition of cysteine (1·25 or 2·5 mM) or of an amino acid mix (0·625 mM each, see 'Materials and Methods') does not influence the stability of tryptophan

TABLE 1

Tryptophan Recovery after 'Hydrolysis' of Free Tryptophan with Methane Sulfonic Acid. 2·5 mM Free Tryptophan, 4N Methane Sulfonic Acid, 110 °C, under Nitrogen. Each Figure Corresponds to an Independent Hydrolysis

Duration of hydrolysis	24 h	48 h	72 h
Tryptophan recovery (%)	100; 100; 100 100	100; 98 98; 98·5	97; 92 97·5

TABLE 2

Tryptophan Recovery after 'Hydrolysis' of Free Tryptophan with Methane Sulfonic Acid in the Presence of Glucose. 2·5 mM Free Tryptophan, 4N Methane Sulfonic Acid, 110 °C, 24 h, under Nitrogen. Each Figure Corresponds to an Independent Hydrolysis

Glucose (mM)	1·25	2·5	5	10
Tryptophan recovery (%)	95–92	76·5–75	47·2–50	46–43

after 24 h at 110 °C under nitrogen. The addition of glucose (1·25 to 10 mM), however, causes a tryptophan loss up to 55 % of the initial content (Table 2). The addition of starch (75 mg/2 ml) leads to an almost total degradation of tryptophan (99·7 and 99·8 % loss after 24 and 48 h of 'hydrolysis', respectively).

These initial results with free tryptophan appear to indicate that protein hydrolysis with methane sulfonic acid cannot be used for tryptophan determination when carbohydrates are associated to the protein. Such a situation is often encountered in foods.

When the *tripeptide* glycyl-L-tryptophyl-glycine is hydrolyzed with methane sulfonic acid, tryptophan losses of approximately 4 and 13 % are found after 24 and 72 h, respectively (Table 3). Assuming that tryptophan degradation during hydrolysis follows first order kinetics with respect to tryptophan concentration (see Cuq & Cheftel, 1982), the following regression line can be calculated:

$$\ln [\text{tryptophan}] = -20 \times 10^{-3} \times t + 2{\cdot}24 \qquad (r = 0{\cdot}989)$$

This corresponds to an initial tryptophan concentration of 9·39 mM, very close to the exact value (9·4 mM).

TABLE 3

Tryptophan Recovery from Glycyl-L-Tryptophyl-Glycine and from Casein after Hydrolysis with Methane Sulfonic Acid. 2·35 mM Tripeptide or 0·5 % (w/v) Casein, 4N Methane Sulfonic Acid, 110 °C, under Nitrogen

Duration of hydrolysis	24 h	48 h	72 h
Recovery of tryptophan from glycyl-L-tryptophyl-glycine (%)	96	90	87
Recovery of tryptophan from casein (g/100 g dry weight)	1·32	1·20	1·15

With *casein*, the exact initial tryptophan content is not known. From the values obtained after 24, 48 and 72 h of hydrolysis (Table 3), the following regression line is obtained:

$$\ln [\text{tryptophan}] = -2{\cdot}85 \times 10^{-3} \times t + 0{\cdot}336 \qquad (r = 0{\cdot}976)$$

Extrapolation to time 0 of hydrolysis therefore gives an initial tryptophan concentration of 1·4 g per 100 g of casein dry weight. Internal standards with the addition to casein of various concentrations of free tryptophan show a 10% loss after 24 h of hydrolysis.

When glucose (10 mM) or starch (75 mg/2 ml) is added to casein or to various other proteins before hydrolysis, tryptophan losses are markedly enhanced (Table 4). This confirms that hydrolysis of protein foods with methane sulfonic acid cannot be used for tryptophan determination.

TABLE 4

Tryptophan Recovery from Various Proteins after Hydrolysis with Methane Sulfonic Acid, with or without the Addition of Starch or Glucose. 0·5% (w/v) Protein, 4N Methane Sulfonic Acid, 110°C, 24 h, under Nitrogen, with or without 10 mM Glucose or 3·75% (w/v) Starch

	Determined	*Theoretical*	*With added*	
			Starch	*Glucose*
Tryptophan (g/100 g casein dry weight)	1·33	ND	tr	0·55
Tryptophan (mole per mole of protein)				
Lysozyme	5·4	6	0·03	ND
β Lactoglobulin	1·7	2	tr	0·48
αs_1 Casein	1·85	2	tr	0·64

tr = Traces.
ND = Not determined.

(2) Stability of tryptophan during hydrolysis with sodium hydroxide

Free tryptophan, heated for 24, 48 or 72 h in the conditions used for protein hydrolysis, remains relatively stable, as determined by HPLC (Table 5). Tryptophan losses, however, are higher and more variable than those in methane sulphonic acid (see Table 1). The addition of cystein (1·25 or 2·5 mM), of starch (75 mg/2 ml), or of the amino acid mix (0·625 mM each) does not markedly influence the tryprophan losses, which

TABLE 5

Tryptophan Recovery after 'Hydrolysis' of Free Tryptophan with Sodium Hydroxide. 2·5mM Free Tryptophan, 4·2N Sodium Hydroxide, 110°C, under Nitrogen. Three HPLC Determinations per Assay

Duration of hydrolysis	24 h	48 h	72 h
Tryptophan recovery (%)	105, 91 97	91, 86 92	92, 83·5 92

TABLE 6

Tryptophan Recovery after 'Hydrolysis' of Free Tryptophan with Sodium Hydroxide in the Presence of Glucose. 2·5mM Free Tryptophan, 4·2N Sodium Hydroxide, 110°C, 24 h under Nitrogen. Two HPLC Determinations Per Assay

Glucose (mM)	1·25	2·5	5	10
Tryptophan (%)	96; 98	92; 94	83; 92	92; 88

TABLE 7

Tryptophan Recovery from Various Proteins after Hydrolysis with Sodium Hydroxide, with or without Addition of Starch or Glucose. 0·5% (w/v) Protein, 4·2N Sodium Hydroxide, 110°C, under Nitrogen, with or without 10mM Glucose or 3·75% (w/v) Starch

Protein	*Duration of hydrolysis* 24 h	48 h	72 h
Tryptophan (g/100 g casein dry weight)			
Casein	1·36–1·26 1·35–1·29	1·35–1·30	1·10–1·17
Casein + starch	1·27–1·31	1·11–1·24	1·16–1·19
Casein + glucose	1·19–1·25	0·96–1·05	ND
Tryptophan (mole per mole of protein)			
αs_1 Casein	1·4	1·7	1·65
Lysozyme	5·65	5·82	6
β Lactoglobulin	1·8	2·2	ND

ND = Not determined.

are equal to 6, 8, 9 and 9%, respectively, after 24 h of 'hydrolysis'. The addition of glucose (1·2 to 10 mM) prior to hydrolysis also has little influence (Table 6).

Various protein solutions, with or without starch or glucose added, were submitted to hydrolysis with 4·2N sodium hydroxide at 110 °C. The results (Table 7) indicate that the addition of starch to casein does not improve tryptophan recovery, contrary to the findings of Hugli & Moore (1972). However, the presence of starch or glucose does not markedly increase the tryptophan loss.

Hydrolysis of carbohydrate-containing protein foods with sodium hydroxide therefore appears to be applicable for the determination of tryptophan.

(3) Tryptophan degradation during heat treatments of glycyl-L-tryptophyl-glycine

(*3.1.*) *Direct HPLC analysis, and absorption spectra of the reaction products*

A 9·4 mM solution of the tripeptide was heated at 125 °C for various lengths of time in the presence of air or oxygen or under nitrogen. Some of the HPL chromatographs of the heated solutions are shown on Fig. 1. A marked decrease in the concentration of the tripeptide occurs after 3 h at 125 °C under oxygen and at pH 8, together with the formation of free tryptophan, of glycyl-L-tryptophan, of L-tryptophyl-glycine and of three unidentified derivatives with elution times of 9, 16 and 17 min. The concentrations of tripeptide and its products of hydrolysis as a function of time are given in Table 8. The sum of the molar concentrations of free tryptophan and the two dipeptides is inferior at all times to the initial tripeptide molarity. This points to the occurrence of tryptophan degradation. This degradation is much more marked after 48 h; it is also more important in the presence of oxygen than in the presence of air. A similar behaviour was noted for the thermal degradation of free tryptophan (Cuq & Cheftel, 1982).

The absorption spectra of the various heated samples (Fig. 2) indicate that their 278 nm absorbance is only slightly smaller than that of the initial tripeptide. This probably means that the indole ring is not modified during the heat treatments. The appearance of an absorbance maximum at 400 nm probably corresponds to complex condensation reactions.

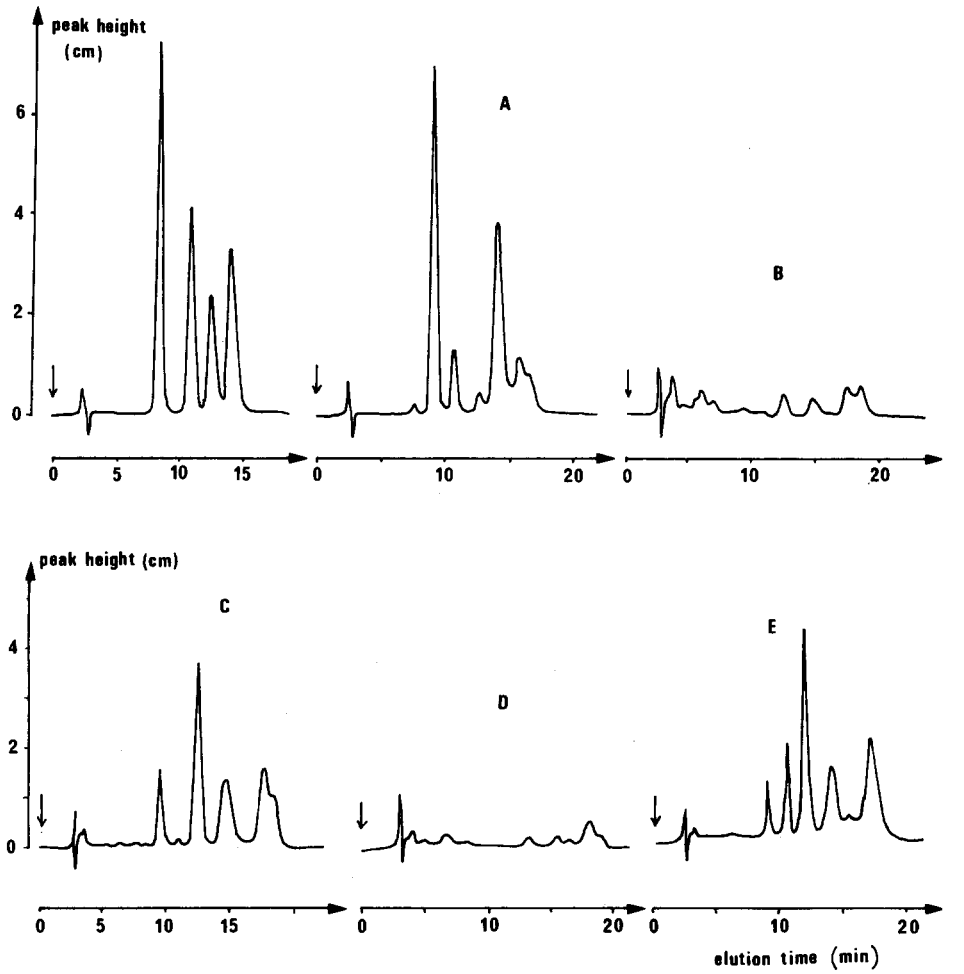

Fig. 1. HPL chromatographs of heat-treated solutions of glycyl-L-tryptophyl-glycine. SP 8000 analyzer; RP 18 column; eluant: methanol – 0·01 M sodium acetate buffer, pH 4 (15–85 % v/v); flow rate: 1 ml min^{-1}; absorbance at 278 nm; 10 μl samples. Standard: trp 0·55 mM; trp–gly 0·44 mM; gly-trp 0·38 mM; gly–trp–gly 0·47 mM. A: gly–trp–gly 9·4 mM heated at 125 °C for 3 h under O_2 (dilution 1/5). B: same as A but heated for 48 h. C: same as B but heated under N_2. D: gly–trp–gly 4·7 mM heated at 125 °C for 24 h under air in the presence of 10 mM glucose (dilution 1/5). E: gly–trp–gly 9·4 mM heated at 125 °C for 24 h under air (dilution 1/5).

TABLE 8

Concentrations of Tryptophan (trp), Tryptophyl-Glycine (TG), Glycyl-Tryptophyl (GT) and Glycyl-Tryptophyl-Glycine (GTG) after Various Heat Treatments at 125°C of a 9·4mM GTG Solution, pH 8. Determinations by HPLC

Concentrations	*Conditions of heat treatment at 125°C*									
	0 h	*3 h*		*9 h*		*24 h*		*48 h*		
		Air	O_2	*Air*	O_2	*Air*	O_2	*Air*	O_2	N_2
Trp (mM)	0	0·07	0·05	0·32	0·23	0·61	0·22	0·09	tr	0·57
TG (mM)	0	0·79	0·28	1·74	1·5	0·97	0·76	tr	tr	0·1
GT (mM)	0	0·49	0·37	1·05	0·9	3·37	2·3	1·38	0·36	3·04
GTG (mM)	9·4	4·1	3·8	1·9	1·65	1·45	1·15	1	0·3	1·4

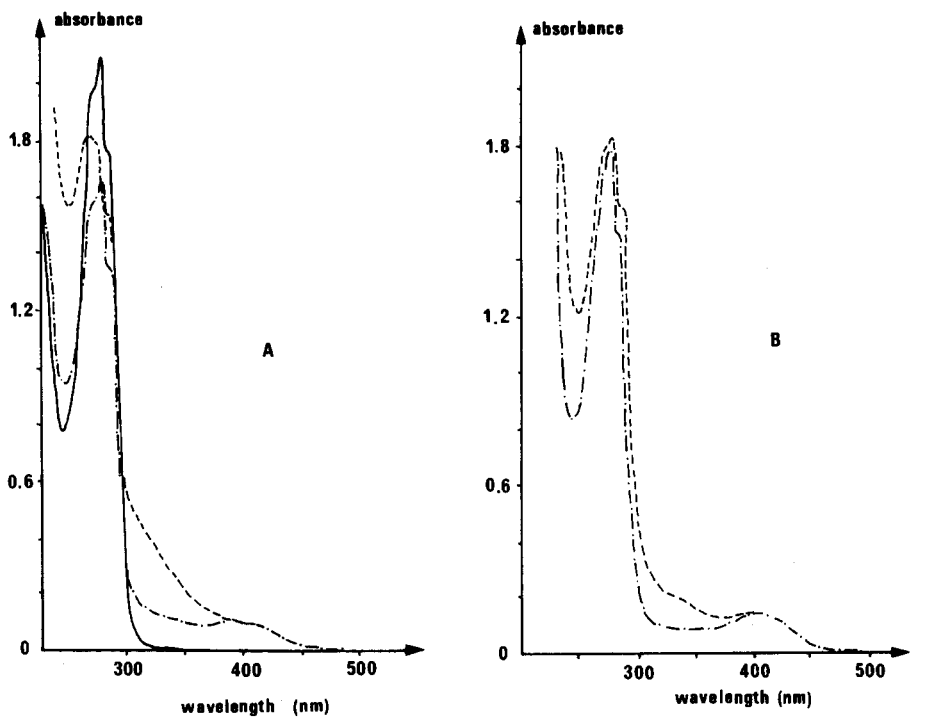

Fig. 2. Absorption spectra of heat-treated solutions of glycyl-L-tryptophyl-glycine. 9·4 mM tripeptide heated at 125°C. A: —— standard tripeptide; —·—·— heated for 9 h under O_2; ———— heated for 24 h under O_2. B: —·—·— heated for 9 h under air; ———— heated for 24 h under air. (dilution 1/30).

(3.2) HPLC analysis after hydrolysis with methane sulfonic acid

The various heated samples of tripeptide are hydrolyzed with methane sulfonic acid and analyzed by HPLC. The results are given in Table 9. Tryptophan losses are important. After 48 h at 125 °C, they reach 72 % in the presence of air, 86 % in the presence of oxygen and 32 % under nitrogen. From the findings reported above, it is, however, possible that these losses correspond to the degradation of free tryptophan released from the tripeptide during the heat treatment.

TABLE 9

Tryptophan Content after Various Heat Treatments at 125 °C of a 9·4mM Glycyl-L-Tryptophyl-Glycine Solution, pH 8. Tryptophan Determination after Hydrolysis with Methane Sulfonic Acid, and HPLC

	Conditions of heat treatments at 125 °C								
	3 h		*9 h*		*24 h*		*48 h*		
	Air	O_2	*Air*	O_2	*Air*	O_2	*Air*	O_2	N_2
Tryptophan content (mM)	8·54	7·69	7·91	7·31	5·82	4·13	2·65	1·28	6·43
Tryptophan loss (%)	9·2	18·1	15·9	22·2	32·1	56·1	71·8	86·4	31·6

The following regression lines can be obtained from the data in Table 9:

$$(125\,°C, \text{air}): \ln[\text{tryptophan}] = -26 \times 10^{-3} \times t + 2{\cdot}27 \quad (r = 0{\cdot}991)$$

$$(125\,°C, \text{oxygen}): \ln[\text{tryptophan}] = -40 \times 10^{-3} \times t + 2{\cdot}27 \quad (r = 0{\cdot}992)$$

They are not very different from those relative to the heat treatment of free tryptophan (Cuq & Cheftel, 1982). The heat lability of peptide-bound tryptophan under nitrogen is, however, surprising in view of the heat stability of free tryptophan under nitrogen.

From the regression lines given above, it can be calculated that the sterilization at 125 °C for 15 min of a 9·4 mM solution of tripeptide at pH 8 in the presence of air (or even of oxygen) would lead to a negligible loss in tryptophan.

In the presence of glucose, the heat degradation of the tripeptide is more extensive. From the comparison of Fig. 1D and E, it appears that the tripeptide, the two dipeptides and free tryptophan react with glucose, probably through Maillard reactions involving their α amino group.

It was hoped that the use of glycyl-L-tryptophyl-glycine would help to investigate the reactivity of the indole ring of peptide-bound tryptophan towards reducing carbohydrates such as glucose. This tripeptide, however, proved to be a bad model, since it readily hydrolyzes when submitted to heat treatments. So far, the studies reported above indicate that, in the absence of reducing carbohydrates, domestic or industrial processing of food proteins would not cause any loss in tryptophan.

(4) Thermal degradation of the tryptophan residues of casein

In a first experiment a 5% (w/v) casein solution, pH 8, was heated at 125°C for up to 48 h in the presence of air, oxygen or nitrogen. The protein was then hydrolyzed with methane sulfonic acid and tryptophan was determined after HPLC. The results are given in Table 10. Under oxygen the tryptophan loss after 48 h reaches 80%, while it is only 36 and 14%, under air and nitrogen, respectively.

TABLE 10

Tryptophan Content after Various Heat Treatments at 125°C of a 5% (w/v) Casein Solution, pH 8. Tryptophan-Determination after Hydrolysis with Methane Sulfonic Acid and HPLC

	Conditions of heat treatments at 125°C								
	3 h		*9 h*		*24 h*		*48 h*		
	Air	O_2	*Air*	O_2	*Air*	O_2	*Air*	O_2	N_2
Tryptophan content (g per 100 g casein dry weight)	1·2	1·12	1·12	1·04	1·09	0·65	0·85	0·27	1·13

It can be calculated that the sterilization of this same casein solution at 125°C for 30 min, in the presence of air, would bring a 1% loss in tryptophan.

In a second experiment, a 4% (w/v) casein solution, pH 7, with or without 1% (or 2%) glucose added, was heated at 125°C for up to 48 h in the presence of air, oxygen or nitrogen. The protein was then hydrolyzed with sodium hydroxide. The concentrations of the remaining tryptophan are given in Table 11. As in the first experiment, it can be seen that protein-bound tryptophan can be thermally degraded, especially in the

TABLE 11

Tryptophan Content after Various Heat Treatments at 125°C of a 4% (w/v) Casein Solution, pH 7. Tryptophan Determination after Hydrolysis with Sodium Hydroxide and HPLC

	Conditions of heat treatment at 125°C								
	3 h		*9 h*		*24 h*			*48 h*	
	Air	O_2	*Air*	O_2	*Air*	O_2	N_2	*Air*	O_2
Tryptophan content (g per 100 g casein dry weight)									
Casein	1·10	0·98	1·08	N.D.	0·76	0·35	1·05	0·63	0·05
Casein + glucose (1% w/v)	1·04	0·95	1·02	0·73	0·82	0·46	1·18	0·63	0·1
Casein + glucose (2% w/v)	1·09	0·84	0·99	0·69	0·63	ND	0·79	0·67	0·04

ND = Not determined.

presence of oxygen. Tryptophan degradation is not significantly influenced by the presence of glucose. This appears to indicate that the indole ring of tryptophan does not react with reducing carbohydrates, in contrast to the suggestion of some authors (Dworschak & Orsi, 1977; Orsi & Dworschak, 1978).

CONCLUSION

The present results, obtained with diluted model systems, suggest that only severe heat treatments in the presence of oxygen cause a significant degradation of protein-bound tryptophan. Moderate heat treatments, such as domestic and industrial cooking, or sterilization, could, however, decrease the nutritional availability of tryptophan if they induced the formation of protein cross-links and/or the isomerization of L- into D-tryptophan residues. The protein digestibility would be reduced in both cases and D-tryptophan has little nutritional value.

Although the indole ring of tryptophan appears not to react with reducing carbohydrates, it should not be forgotten that it is degraded by strong oxidizing agents, and that some catalysts present in foods may enhance its sensitivity to oxygen.

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