Stability of tryptophan during food processing and storage

1. Comparative losses of tryptophan, lysine and methionine in different model systems

BY HENRIK K. NIELSEN*, D. DE WECK, P. A. FINOT, R. LIARDON AND R. F. HURRELL†

Research Department, Nestlé Products Technical Assistance Co. Ltd, CH-1814 La-Tour-de-Peilz, Switzerland

(*Received 8 May 1984 – Accepted 7 September 1984*)

1. The stability of tryptophan was evaluated in several different food model systems using a chemical method (high pressure liquid chromatography after alkaline-hydrolysis) and rat assays. Losses of tryptophan were compared with the losses of lysine and methionine.

2. Whey proteins stored in the presence of oxidizing lipids showed large losses of lysine and extensive methionine oxidation but only minor losses of tryptophan as measured chemically. The observed decrease in bioavailable tryptophan was explained by a lower protein digestibility.

3. Casein treated with hydrogen peroxide to oxidize all methionine to methionine sulphoxide showed a 9% loss in bioavailable tryptophan.

4. When casein was reacted with caffeic acid at pH 7 in the presence of monophenol monooxygenase (tyrosinase; *EC* 1.14.18.1), no chemical loss of tryptophan occurred, although fluorodinitrobenzene-reactive lysine fell by 23%. Tryptophan bioavailability fell 15%, partly due to an 8% reduction in protein digestibility.

5. Alkali-treated casein (0·15 M-sodium hydroxide, 80°, 4 h) did not support rat growth. Chemically-determined tryptophan, available tryptophan and true nitrogen digestibility fell 10, 46 and 23% respectively. Racemization of tryptophan was found to be 10% (D/(D+L)).

6. In whole-milk powder, which had undergone 'early' or 'advanced' Maillard reactions, tryptophan, determined chemically or in rat assays, was virtually unchanged. Extensive lysine losses occurred.

7. It was concluded that losses of tryptophan during food processing and storage are small and of only minor nutritional importance, especially when compared with much larger losses of lysine and the more extensive oxidation of methionine.

Most foods are cooked, industrially-processed or stored before consumption. During such treatments protein can react with other food components or chemical additives (Hurrell, 1980, 1984). These reactions can lead to flavour and colour formation, loss of nutritional value and occasionally to potentially-toxic compounds. Nutritional reasearch has for many years been concentrated on the stability of lysine, especially during reactions with reducing sugars (Mauron, 1981), and, to some extent, methionine (Cuq *et al.* 1978). The stability of protein-bound tryptophan has been very little investigated, primarily due to the lack of adequate methodology, but also because lysine and the sulphur amino acids are more often the first limiting amino acids (Food and Agriculture Organization, 1970). A few reports have indicated, however, that degradation of tryptophan during food processing and storage could be of nutritional importance. O'Brien (1966) and Kanazawa *et al.* (1975) found large tryptophan losses on reaction of protein with oxidizing lipids. Tryptophan has also been shown to be sensitive to oxidation by hydrogen peroxide (Hachimori *et al.* 1964; Anderson *et al.* 1975; Steinhart, 1979), and Dworschak & Hegedüs (1974) have reported severe tryptophan losses in milk powders as a result of Maillard reactions.

In the present work, we have investigated the stability of tryptophan in several different food-model systems representing the major reactions of proteins during processing and

* Present address: Novo Industri A/S, Novo Allé, DK-2880 Bagsvaerd, Denmark.
† For reprints.

storage. We have studied the reactions of protein with (1) oxidizing lipids, these reactions may occur during the storage of cereal and fish products (Harrison *et al.* 1976; Khayat & Schwall, 1983); (2) H_2O_2, which may be used to sterilize processing equipment and packaging materials (Cuq *et al.* 1978); (3) polyphenolic acids; such reactions may occur during the extraction of plant proteins (Hurrell *et al.* 1982); (4) alkali, which may be used to improve protein solubility or other physical characteristics (Tannenbaum *et al.* 1970; Provansal *et al.* 1975); and (5) reducing sugars, Maillard reactions occur during the baking of bread, the production of breakfast cereals and, most importantly, during the processing and storage of milk products (Hurrell, 1980; Hurrell *et al.* 1983). Using both a chemical method (high pressure liquid chromatography (HPLC) after alkaline-hydrolysis) and bioavailability with rats, only minor losses of tryptophan were found. These losses would appear to have little nutritional significance, especially when compared with the much larger losses of lysine and more extensive oxidation of methionine.

EXPERIMENTAL

Preparation of test materials

Reaction with oxidizing lipid. Whey protein, methyl linolenate and water (1:0·5:0·27, by wt) were mixed and stored in sealed tins for 4 weeks at 37° in the presence of 4 mol oxygen/mol lipid. This is referred to as the 'basal conditions'. Three other samples were also prepared, a 'low water activity' sample to which no water was added (this sample contained only the 30 g water/kg already present in the whey protein); an 'O_2 limitation' sample whose O_2 uptake was limited to 1 mol/mol lipid; and a 'high temperature' sample, which was stored at 55°. After storage, the lipid was extracted once with methanol and twice with methanol–hexane (2:1, v/v) and dried. For further experimental details see Nielsen *et al.* (1985*c*).

Reaction with H_2O_2. A casein solution (30 g/l, pH 8·5, type Hammarsten; E. Merck, Darmstadt, W. Germany) was oxidized with 6·8 g H_2O_2/l (Perhydrol; E. Merck) at 50°. After 1 h, $1{\cdot}56 \times 10^6$ units catalase (*EC* 1.11.1.6; Serva Feinbiochemica, Heidelberg, W. Germany)/l was added and 30 min later the absence of H_2O_2 was confirmed (no oxidation of potassium iodide). The solution was finally cooled and freeze-dried.

Reaction with polyphenols. Casein (50 g/l, type Hammarsten; E. Merck) was reacted with caffeic acid (5 g/l; Sigma Chemical Co., St Louis, Mo, USA) at pH 7 in the presence of 2×10^5 units monophenol monooxygenase (tyrosinase, *EC* 1.14.18.1; Sigma Chemical Co.)/l. The mixture was stirred for 3 h at room temperature with constant O_2 bubbling. The casein–caffeic acid complex was precipitated at pH 4·6, centrifuged, freeze-dried and milled (Hurrell *et al.* 1982).

Alkaline treatment. Casein (50 g/l, type Hammarsten; E. Merck) was solubilized in 0·15 M-sodium hydroxide and incubated at 80° for 4 h. It was then cooled, neutralized to pH 7 with 6 M-hydrochloric acid, freeze-dried and milled.

Maillard reactions. Whole-milk powder was stored in the cans at 50° for 9 weeks or at 60° for 4 weeks so as to develop 'early' and 'advanced' Maillard reactions respectively (Hurrell *et al.* 1983). The same powder was heated at 100° in sealed Pyrex tubes (20 g/tube).

Analytical methods

Tryptophan was measured by HPLC after hydrolysis in NaOH (Nielsen & Hurrell, 1985). Methionine and methionine sulphoxide were determined in the same NaOH-hydrolysates using a Beckman 121 amino acid analyser (Beckman Instruments, Munich, W. Germany) (Nielsen *et al.* 1985*c*). Total methionine and cyst(e)ine were determined as methionine sulphone and cysteic acid respectively after preliminary performic acid oxidation (Moore,

1963). Total amino acids were determined in acid-hydrolysates using a Beckman Multichrom amino acid analyser (Beckman Instruments). Reactive lysine and lactulosyl-lysine in milk powders were determined by the furosine method (Finot *et al.* 1981). Fluorodinitrobenzene (FDNB)-reactive lysine was determined by the procedure of Carpenter (1960) as modified by Booth (1971). In order to estimate the racemization of tryptophan, 44 mg protein was hydrolysed in 20 ml 6 M-HCl containing 14 mg indolyl-3-propionic acid (Sigma Chemical Co.) (Gruen & Nicholls, 1972). Tryptophan was then partly purified by reverse-phase HPLC using a water–methanol gradient. The gas chromatography–mass spectroscopy procedure used to separate the D- and L-isomers was identical to that used by Liardon *et al.* (1981) for other amino acids.

Animal experiments

Available tryptophan. The rat assay was based on the work of Gupta & Elvehjem (1957). Male weanling Sprague Dawley rats (Voss, Tuttlingen, W. Germany) weighing 40–50 g were fed for 2 d on the basal diet limiting in tryptophan. The basal diet contained (g/kg): casein 30, zein 100, gelatin 100, L-lysine hydrochloride 11, L-methionine 4, L-threonine 3, L–valine 3, L-isoleucine 3, L-histidine hydrochloride monohydrate 4, sucrose 250, arachis oil 100, cellulose 50, minerals (USP-XVII as recommended by the Association of Official Analytical Chemists, 1980) 50, vitamins (Peret *et al.* 1973) 12·5 and maize starch to 1000. After 2 d, the rats were allocated to individual screen-bottom cages in a randomized one-way blocked (body-weight and position in the rack) experimental design (six rats per group) and given the test diets. For the standard curve, the basal diet was supplemented with L-tryptophan at 0·16, 0·32, 0·48 and 0·64 g/kg. The whey-protein-based samples, casein-based samples and whole-milk-powder samples were added at levels of 10·5 and 21·0, 17·9 and 35·8, and 16·8 and 33·6 g crude protein (nitrogen × 6·25)/kg respectively at the expense of gelatin, starch and arachis oil so as to keep the diets isonitrogenous and isoenergetic (arachis oil was only adjusted with the whole-milk-powder samples). The test diets were given *ad lib.* for 14 d and weight gain and food intake were measured. Based on the food conversion efficiencies (FCE), available tryptophan was calculated using the slope-ratio method of Finney (1964).

True N digestibility. For casein and whole-milk-powder samples, the following procedure was followed. Male weanling Sprague Dawley rats were given a stock diet (NAFAG no. 850; Gossau, Switzerland) for 2 d and were then randomly allocated to the experimental diets as mentioned previously. The test materials were added as the only protein source at 100 g crude protein/kg, at the expense of starch, to a basal diet which otherwise contained (g/kg): sucrose 250, arachis oil 100, cellulose 50, minerals (USP-XVII) 50, vitamins (Peret *et al.* 1973) 12·5 and maize starch to 1000. For the whole-milk-powder samples, the arachis oil was adjusted to keep the diets isoenergetic. The diets were given *ad lib.* and weight gain and food intake were monitored. From day 3 to day 7, faeces were collected and true N digestibility was calculated using a group given a diet containing 25 g whey protein/kg to estimate endogenous faecal N (corrected for food intake). At this protein level, the animals just maintained their body-weight. As the alkali-treated casein did not support rat growth, the group receiving the alkali-treated casein was, from day 7 to day 14, given a diet containing both the alkali-treated protein (66·7 g crude protein/kg) and untreated casein (100 g crude protein/kg). Faeces were collected from day 11 to day 14 and the true faecal digestibility of the alkali-treated casein was calculated using the 3–7 d values from the group given control casein to correct for the undigested control casein. The endogenous faecal N was also estimated from the 3–7 d values.

For whey protein samples a similar procedure was used to determine N digestibility but faeces were collected from day 11 to day 16 (Nielsen *et al.* 1985*a*).

RESULTS

Table 1 shows the tryptophan contents of the control proteins and the treated samples determined in a rat assay. In this animal assay, one point on the standard curve (0·32 g/kg) did not fall on the straight line going through the other points. After the assay, portions of the standard-curve diets were suspended in water, filtered and tryptophan in the filtrate determined by HPLC. No deviation from a linear curve was found and we are not able to explain why the 0·32 g tryptophan/kg group grew better than would be expected. In other assays, linear standard curves have been obtained up to FCE of 0·52. The same relative responses (to within 1%) of the treated samples compared with the control protein were obtained whether the 0·32 g tryptophan/kg group was included in the calculations or not; even leaving out altogether the whole standard curve from the calculations and comparing the response of rats given the treated samples with those given the untreated control protein gave the same relative tryptophan-availability values. In Table 1, the calculations were made with the standard curve, without the 0·32 g tryptophan/kg group, and the discussion is based on the relative values.

Reaction with oxidizing lipid. The chemically-determined tryptophan content of whey protein (Table 2) fell a maximum of 26% on storage with oxidizing methyl linolenate. The reductions in bioavailable tryptophan were much larger (up to 71%) but seemed to be mainly due to the fall in overall protein digestibility. Chemically-determined tryptophan multiplied by true N digestibility agreed well with the rat-assay values.

In all the treated samples, FDNB-reactive lysine and methionine were reduced to a greater extent than chemically-determined tryptophan. Methionine was extensively oxidized to its sulphoxide.

Reaction with H_2O_2. Methionine in this sample was completely oxidized to methionine sulphoxide but no significant decreases were observed in either the bioavailable or the chemically-determined tryptophan (Table 2).

Reaction with caffeic acid. Chemically-determined tryptophan remained unchanged after reaction between casein and caffeic acid at pH 7 in the presence of monophenol monooxygenase (Table 2). Bioavailable tryptophan fell 15% and true N digestibility 8%. Methionine and FDNB-reactive lysine fell 21% and 23% respectively.

Alkaline treatment. Table 3 shows that the treatment of casein with NaOH resulted in losses of serine (36%), lysine (33%) cyst(e)ine (25%), methionine (19%), arginine (15%), threonine (14%) and tryptophan (10%). Among the amino acids essential and semi-essential for man, racemization of cyst(e)ine, threonine, phenylalanine and methionine was larger than the 10% (D/(D+L)) found for tryptophan. This latter value was measured after acid-hydrolysis, which destroyed 60–70% of the tryptophan. It is assumed that the acid-degradation of tryptophan is independent of its enantiomer form.

When this alkali-treated casein was given to rats at 100 g/kg diet as the only protein source, extensive weight losses (Fig. 1) and severe diarrhoea occurred. From day 7 to day 14, the rats were given 66·7 g alkali-treated protein/kg plus 100 g control casein/kg. They then grew at least as well as the control group from day 0 to day 7. Table 4 shows food intake, FCE and N digestibility for the two groups. True N digestibility of the alkali-treated casein was determined twice; between days 3 and 7 using a group given 25 g whey protein/kg to estimate endogenous faecal N, and between days 11 and 14 using the values from the control group days 3–7 to estimate endogenous faecal N plus the undigested control casein. Both values have potential systematic errors. For the first, faeces collection was difficult because of the diarrhoea and, for the second, the digestibility of the control protein could have been reduced due to the presence of the alkali-treated protein. The two values, however, were fairly close and we believe that their mean (0·76) is a reasonable estimate of the true N digestibility of this alkali-treated casein.

Table 1. *Bioavailable tryptophan in test materials as determined in rat assay*

Test material*	Level of supplementation† (g/kg diet)	Food conversion efficiency†	Calculated potency: Absolute value (mg/g crude protein (nitrogen × 6·25)) Mean	Absolute value 95% confidence limits	Relative to control protein (%) Mean	Relative to control protein 95% confidence limits
L-Tryptophan	0, 0·16, 0·32, 0·48, 0·64, —	0·107, 0·152, 0·319, 0·330, 0·431, —	—	—	—	—
Whey protein:						
Untreated	11·9, 23·9	0·248, 0·387	27·7	24·4–31·3	100	—
Treated with oxidizing methyl linolenate:						
Basal conditions	15·4, 30·8	0·178, 0·255	15·6	12·7–18·7	56	45–68
Low water activity	13·1, 26·2	0·253, 0·387	27·9	24·7–31·5	101	88–115
Oxygen limitation	14·0, 28·0	0·194, 0·373	24·5	21·4–27·9	89	77–102
High temperature	15·6, 31·2	0·123, 0·178	8·1	5·0–11·0	29	18–40
Casein:						
Control	19·9, 39·8	0·242, 0·388	16·2	14·3–18·3	100	—
Hydrogen peroxide treated	21·1, 42·2	0·211, 0·371	14·8	13·0–16·8	91	79–105
Caffeic acid treated	21·2, 42·3	0·211, 0·347	13·8	11·9–15·7	85	73–98
Alkali treated	21·7, 43·3	0·142, 0·264	8·8	7·1–10·6	54	43–66
Whole-milk powder:						
Untreated	66·7, 133	0·219, 0·325	13·8	11·8–15·8	100	—
9 weeks at 50° ('early' Maillard)	66·7, 133	0·218, 0·351	15·0	13·1–17·1	109	93–129
4 weeks at 60° ('advanced' Maillard)	66·7, 133	0·218, 0·300	12·7	10·9–14·7	92	77–110

* For details of experimental conditions, see p. 282.

† Multiple values given for each test material represent the different groups of rats receiving different levels of that test material.

Table 2. *Comparison of tryptophan, lysine, methionine and methionine sulphoxide values (mg/g crude protein (nitrogen × 6·25)) and N digestibility*

(Values in parentheses represent the values of the treated samples as percentages of the corresponding untreated control except for methionine and methionine sulphoxide, which are given as percentages of the sum of methionine and methionine sulphoxide in the corresponding untreated control)

Test material*	Bioavailable tryptophan	N digestibility	Tryptophan by HPLC	Tryptophan by HPLC multiplied by N digestibility	Reactive lysine†	Methionine	Methionine sulphoxide in methionine equivalents
Whey protein:							
Untreated	27·7	0·991	22·8	22·5	105	21·5 (99)	0·2 (1)
Treated with oxidizing methyl linolenate:							
Basal conditions	15·6 (56)	0·701 (71)	19·5 (86)	13·7 (60)	42 (40)	1·2 (6)	20·1 (93)
Low water activity	27·9 (101)	0·969 (98)	22·9 (100)	22·1 (98)	89 (85)	5·4 (25)	16·3 (75)
Oxygen limitation	24·5 (89)	0·905 (91)	21·9 (96)	19·8 (88)	78 (74)	4·0 (19)	18·1 (83)
High temperature	8·1 (29)	0·471 (48)	16·9 (74)	8·0 (35)	31 (30)	0·0 (0)	20·1 (93)
Casein:							
Untreated	16·2	0·987	13·4	13·2	86	28·3 (96)	1·3 (4)
Hydrogen peroxide treated	14·8 (91)	0·977 (99)	13·1 (98)	12·8 (97)	83 (96)	0·0 (0)	27·8 (94)
Caffeic acid treated	13·8 (85)	0·905 (92)	13·6 (101)	12·3 (93)	66 (77)	22·4 (79)	5·3 (18)
Alkali treated	8·8 (54)	0·758 (77)	12·0 (90)	9·1 (69)	65 (75)	23·4 (85)	2·7 (9)
Whole-milk powder:							
Untreated	13·8	0·923	14·3	13·2	86 (82)	25·5 (94)	1·7 (6)
9 weeks at 50°	15·0 (109)	0·907 (98)	14·5 (101)	13·2 (100)	68 (79)	27·1 (100)	1·5 (6)
4 weeks at 60°	12·7 (92)	0·692 (75)	14·5 (101)	10·0 (76)	17 (20)	24·9 (92)	3·7 (14)
SE of analytical value	1·5	0·036	0·2	2	1	0·6	0·5

HPLC, high pressure liquid chromatography.

* For details of experimental conditions, see p. 282.

† Reactive lysine of the whey protein and casein samples were determined by the flourodinitrobenzene-technique; for the milk powders, the furosine method was used.

Table 3. *Total amino acids and racemization of control and alkali-treated (0·15 M-sodium hydroxide, 4 h, 80°) casein*

Amino acid	Total (mg/g crude protein (nitrogen × 6·25))			Racemization (D/(D+L))		
	Control	Alkaline treatment	SEM*	Control	Alkaline treatment	SEM†
Tryptophan	13·4	12·1	0·1	0·013‡	0·111‡	0·008
Lysine	91·0	60·8	0·7	0·005	0·072	0·009
Methionine	31·4	25·3	0·5	0·000	0·175	0·000
Cystine	4·4	3·3	0·1	0·000§	0·31§	—
Leucine	120·3	124·5	4·3	0·001	0·043	0·001
Isoleucine	58·5	53·5	0·7	0·000	0·021	0·003
Valine	71·5	70·3	2·0	0·000	0·013	0·001
Phenylalanine	55·8	55·1	0·6	0·000	0·191	0·001
Tyrosine	60·3	60·7	0·6	0·014‡	0·103‡	0·010
Threonine	45·4	39·0	0·1	0·000	0·244	0·001
Serine	65·5	41·6	0·4	0·000	0·398	0·009
Histidine	31·1	29·5	0·2	—	—	—
Arginine	41·1	34·8	0·2	—	—	—
Proline	112·2	114·2	0·4	0·001	0·005	0·000
Alanine	33·2	33·2	0·3	0·002	0·102	0·003
Glycine	20·4	23·0	0·1	—	—	—
Aspartic acid	77·0	78·1	0·6	0·002	0·259	0·006
Glutamic acid	268·1	280·7	9·4	0·000	0·172	0·002

* Pooled standard error of mean of duplicates.
† SEM of duplicates for the alkali-treated casein. SEM for control protein was less than 0·002.
‡ The values for tryptophan and tyrosine were not corrected for the razemization, which occurred during the acid-hydrolysis.
§ Single determination.

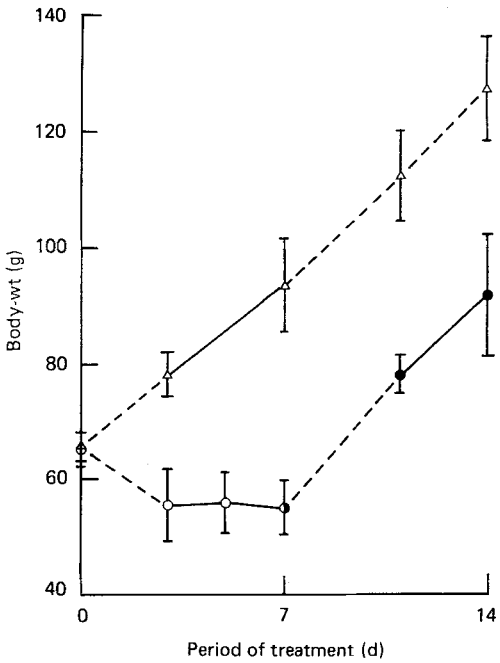

Fig. 1. Body-weights of rats given a diet containing 100 g control casein/kg for 14 d (△) and of rats given 100 g alkali-treated casein/kg from day 0 to day 7 (○) followed by 66·7 g alkali-treated casein/kg plus 100 g control casein/kg from day 7 to day 14 (●).(——) Periods where digestibility was determined. Values are means with their standard errors represented by vertical bars.

Table 4. *Food intake and food conversion efficiency (FCE) of rats given 100 g control casein/kg diet for 14 d or 100 g alkali-treated casein/kg diet for 7 d followed by 66·7 g alkali-treated casein plus 100 g control casein/kg diet from days 7 to 14*

(Mean values with their standard errors for six rats per group)

Period of treatment (d)	Control casein						Alkali-treated casein					
	Food intake (g/d)		FCE (g/g)		True nitrogen digestibility		Food intake (g/d)		FCE (g/g)		True N digestibility	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0–3	10·6	1·0	0·40	0·06	—	—	3·8	0·7	−0·90	0·42	—	—
3–7	11·5	2·2	0·33	0·10	0·987	0·015	4·2	0·8	−0·02	0·24	0·778	0·044
7–11	12·8	1·7	0·38	0·06	—	—	9·2	1·2	0·64	0·08	—	—
11–14	14·6	1·2	0·33	0·05	—	—	10·3	3·3	0·39	0·09	0·737	0·041

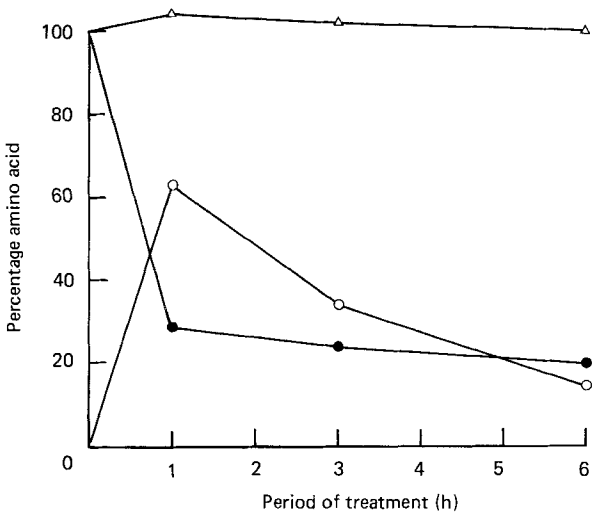

Fig. 2. Percentage of tryptophan (△), reactive lysine (●) and lysine as lactulosyl-lysine (○) remaining in whole-milk powder heated at 100°. Initial tryptophan content was 14·2 mg/g crude protein (nitrogen × 6·25) and initial lysine, 90·0 mg/g crude protein. Pooled SEM were: tryptophan 1%, reactive lysine 1%, lysine as lactulosyl-lysine 1%.

Bioavailable tryptophan fell 46% (Table 2), which is more than the chemical value even when multiplied by true N digestibility.

Maillard reactions. Tryptophan, determined both chemically and in the rat assay (Table 2), was not significantly changed in whole-milk powder which was stored for 9 weeks at 50° or 4 weeks at 60°. The sample stored at 50° still retained its natural colour and was characteristic of a product which had undergone 'early' Maillard damage. The sample at 60° became dark in colour and was characteristic of a product which had undergone 'advanced' Maillard damage (Hurrell *et al.* 1983). Reactive lysine values for these materials, determined by the furosine technique, fell to 79% and 20% of their original level in the 'early' and 'advanced' Maillard samples respectively.

Fig. 2 shows the reactive lysine, the lysine combined as lactulosyl-lysine and the tryptophan content of a whole-milk powder stored at 100°. After 1 h, 63% of the lysine

units were found as lactulosyl-lysine, which was subsequently degraded as the product turned brown. Tryptophan, measured by HPLC after alkaline-hydrolysis, was virtually unchanged

DISCUSSION

We have investigated the loss of tryptophan in model systems designed to represent the major reactions of food proteins during processing and storage. In whey protein reacted with oxidizing methyl linolenate, extensive losses of bioavailable tryptophan were observed. These losses appeared to be mainly due to a general reduction in protein digestibility although some losses in chemically-determined tryptophan also occurred, particularly in the 'high temperature' sample. It would appear that the oxidation products of methyl linolenate can react with tryptophan residues. It must be emphasized, however, that the reactions that had occurred in our model system were much more extensive than would normally be found in stored foodstuffs. The limitation of the O_2 uptake to 1 mol/mol lipid (corresponding to 400 ml air/g lipid) significantly reduced the loss of tryptophan and, in stored foodstuffs where O_2 uptake is usually far less, tryptophan losses would presumably be insignificant. The oxidation of methionine to its sulphoxide and the losses of lysine and protein digestibility are far greater. We have discussed our lipid oxidation model system in greater detail elsewhere (Nielsen *et al.* 1985*a*, *c*).

In a second oxidative model system (H_2O_2 treatment) tryptophan was virtually unchanged even though methionine was completely oxidized to its sulphoxide. Methionine sulphoxide has been shown to be highly available as a source of methionine (Nielsen *et al.* 1985*a*). As would be expected, reactive lysine was unaffected and, under our experimental conditions (6·8 g H_2O_2/l, 30 g protein/l, 50°, 1 h), treatment with H_2O_2 would appear to have had little influence on the nutritional quality of the protein, although the influence on cyst(e)ine remains to be investigated.

Steinhart & Kirchgessner (1980), on the other hand, have reported extensive losses of tryptophan in H_2O_2-oxidized soya-bean protein, both measured chemically and in a rat assay. This is probably due to the more severe conditions used by these authors (50 g H_2O_2/l, 250 g protein/l, 60°, 2 h) followed by overnight drying at 55° instead of freeze-drying. The concentrations of H_2O_2 which have been proposed for industrial use (Roundy, 1958; Rasekh *et al.* 1972) are about ten times lower than those used in our study.

Recently it has been shown that free tryptophan can be extensively oxidized under similar experimental conditions to those used in the present study (de Weck & Finot, 1983) illustrating that care should be taken when extrapolating results from experiments on free amino acids to food proteins.

The third oxidative system investigated was the enzymic browning of polyphenolic acids. In the presence of O_2 and a polyphenol oxidase, chlorogenic acid, caffeic acid and other related *o*-diphenols can be oxidized to *o*-semiquinone radicals or *o*-quinone molecules. These compounds are highly reactive and normally polymerize to give brown products of high molecular weight although they also can react with lysine (Hurrell *et al.* 1982) and cysteine residues (Pierpoint, 1971) and can oxidize methionine to its sulphoxide (Hurrell & Finot, 1984). Synge (1975) proposed that tryptophan might also be oxidized during these reactions but there is little evidence from our results to support this suggestion. In our casein–caffeic acid system, tryptophan measured chemically was unchanged and the 15% fall in bioavailable tryptophan was not greatly different from the 8% fall in N digestibility. The FDNB-reactive lysine fell by 23% and the relative stability of tryptophan was the same as that previously reported by Hurrell *et al.* (1981) and Hurrell & Finot (1984) for an identical preparation. Using rat assays these authors reported losses of lysine, methionine and tryptophan of 19, 13 and 8% respectively.

Considering the influence of alkaline treatments, our alkali-treated casein had no growth-supporting properties (Fig. 1). Tovar (1981) similarly observed extensive weight losses and diarrhoea when 310 g NaOH-treated zein/kg, 30 g casein/kg and 30 g of an amino acid mixture/kg were given to rats. Other authors have also found that alkali-treatment decreased protein quality, but weight losses have not been observed (Karayiannis *et al.* 1979; Possompes *et al.* 1983) even though the conditions used by these authors were similar to our own. When untreated casein was added to the diets of our rats, they recovered rapidly and the FCE increased initially to a higher level than that observed in the control group (Table 4). The alkali-treated casein therefore did not appear to have any toxic or growth-inhibiting effects in a short-term assay.

The reasons for the poor performances of the rats receiving the alkali-treated casein are not fully understood. Lower food intake must play a major role, together with the effect of diarrhoea, reduced protein digestibility, destruction of certain essential amino acids and the poor utilization of certain essential D-amino acids which were formed during the treatment (Hayashi & Kameda, 1980; Liardon & Hurrell, 1983). The rat appears to be capable of completely utilizing only D-methionine and D-tryptophan for growth; D-histidine, D-phenylalanine and D-tyrosine are partially utilized; D-valine and D-leucine poorly utilized; and the D-isomers of lysine, isoleucine, threonine and cystine are not used at all (Berg, 1959; Ohara *et al.* 1980). The chemically-determined tryptophan value was relatively stable, which is in agreement with the use of alkaline-hydrolysis for the determination of tryptophan in foods (Nielsen & Hurrell, 1985). The racemization of tryptophan was moderate, approximately that of tyrosine. It was higher than that for leucine, isoleucine, valine and lysine but lower than that for methionine, cystine, phenylalanine and threonine. The non-essential amino acids serine and aspartic acid racemized the most readily. Even though only one experimental condition was investigated, the result indicates that extensive racemization of tryptophan is unlikely to occur during the alkaline treatment of food proteins.

The loss of available tryptophan in the rat assay (Table 1) was greater than would be expected from the chemical value and the true N digestibility (Table 2). As D-tryptophan is fully utilized by the rat (Berg, 1959), tryptophan racemization should have no influence on its bioavailability. It is possible, however, that the racemization of tryptophan or neighbouring amino acids in the protein chain lead to a specific decrease in the enzymic release of tryptophan (Tovar, 1981). Unlike its use in the rat, D-tryptophan is not utilized by man and many domestic animals (Wilkening & Schweigert, 1947; Berg, 1959; Baker *et al.* 1971; Ohara *et al.* 1980). The importance of losses in bioavailable tryptophan during alkaline treatments, however, would appear to be minor when compared with the other possible detrimental effects of these treatments on protein quality.

The Maillard reactions between protein and reducing sugars are probably the most important reactions which occur in food proteins during processing and storage. Milk products are especially prone to such reactions because of their high lactose content. Our milk powders stored at 50° for 9 weeks or 60° for 4 weeks to develop 'early' and 'advanced' Maillard reactions showed no significant loss of tryptophan even though reactive lysine was reduced by 21% and 80% respectively. Tryptophan availability from the 'advanced' Maillard sample fell only 8%, despite a 25% fall in true N digestibility. This could perhaps be explained by a low release and absorption of lysine and lysine-peptides. Finot & Magnenat (1981), for instance, reported that about half the lysine in casein, which had undergone 'advanced' Maillard reactions with glucose, was excreted in the faeces of rats. In addition, in vitro enzymic release of lysine after Maillard reactions has been found to be especially low (Mauron *et al.* 1955; Scarbieri *et al.* 1973).

In contrast to our findings, Dworschak & Hegedüs (1974) have reported extensive

tryptophan losses in milk powders heated at 100°. After 4 h heating, they reported losses of 74 and 97% for milk powders containing 24·5 and 57 g/kg moisture respectively. When we heated milk powder (30 g moisture/kg) at 100°, no tryptophan losses were observed after 6 h (Fig. 2). This difference is probably due to the different methods used for the determination of tryptophan. Dworschak & Hegedüs (1974) used a colorimetric reaction with *p*-dimethylaminobenzaldehyde whereas, in the present work, HPLC after NaOH-hydrolysis under vacuum has been used. The rat-assay values support our analytical findings as do the results of Gupta & Elvehjem (1957) with roller-dried and spray-dried milk powders. In a subsequent publication (Nielsen *et al.* 1985*b*), the use of different methods for the determination of tryptophan in treated and untreated food proteins is being discussed.

When studying the reactions of tryptophan in aqueous systems both Dworschak & Oersi (1977) and Finot *et al.* (1982) (using free tryptophan and α-*N*-acetyl-tryptophan respectively) have obtained results which indicate that 'advanced' Maillard reaction products can react with the indole ring of tryptophan. Although their reaction conditions were very different from our own, it is also possible that the tryptophan present in these smaller compounds reacts more readily with Maillard reaction products than the tryptophan residues contained in intact food proteins.

One process which we did not investigate is the acid treatment of food proteins. This process is used primarily for the production of taste and flavour enhancers and has been reported to degrade tryptophan extensively (Prendergast, 1974). Such products, however, are only minor dietary components and lack of tryptophan would appear to be of little nutritional significance. If acid-hydrolysates of food proteins are used for dietetic purposes, free tryptophan should be added.

In conclusion, the tryptophan residues in food proteins are relatively stable during processing and storage. They are not easily oxidized and are relatively resistant to reactions with oxidizing lipids, alkali, quinones and reducing sugars. Methionine residues are far more readily oxidized and lysine is far more reactive with other food components. Compared with the potential losses of lysine during food processing and storage, any losses of tryptophan are of only minor nutritional significance.

The authors wish to thank Miss A. Klein, Miss S. Ledermann and Mr J. Vuichoud for technical assistance, and Professor J. Mauron for valuable discussions.

REFERENCES

Anderson, G. H., Li, G. S. K., Jones, J. D. & Bender, F. (1975). *Journal of Nutrition* **105**, 317–325.
Association of Official Analytical Chemists (1980). *Official Methods of Analysis of the Association of Official Analytical Chemists*, p. 774 [W. Horwitz, editor]. Washington DC: AOAC.
Baker, D. H., Allen, H. K., Boomgaardt, J., Graber, G. & Norton, H. W. (1971). *Journal of Animal Science* **33**, 42–46.
Berg. C. P. (1959). In *Protein and Amino Acid Nutrition*, pp. 57–96 [A. A. Albanese, editor]. New York: Academic Press.
Booth, V. H. (1971). *Journal of the Science of Food and Agriculture* **22**, 658–666.
Carpenter, K. J. (1960). *Biochemical Journal* **77**, 604–610.
Cuq, J.-L., Besancon, P., Chartier, L. & Cheftel, C. (1978). *Food Chemistry* **3**, 85–101.
de Weck, D. & Finot, P. A. (1983). *International Journal for Vitamin and Nutrition Research* **53**, 234.
Dworschak, E. & Hegedüs, M. (1974). *Acta Alimentaria* **6**, 337–347.
Dworschak, E. & Oersi, F. (1977). *Acta Alimentaria* **6**, 59–71.
Finney. D. J. (1964). *Statistical Methods in Biological Assay*, 2nd ed. New York: Hafner Publishing Co.
Finot, P. A., Deutsch, R. & Bujard, E. (1981). In *Progress in Food and Nutrition Science*, vol. 5, *Maillard Reactions in Foods*, pp. 345–355 [C. Eriksson, editor]. Oxford: Pergamon Press.
Finot, P. A. & Magnenat, E. (1981). In *Progress in Food and Nutrition Science* vol. 5, *Maillard Reactions in Foods*, pp. 193–207 [C. Eriksson, editor]. Oxford: Pergamon Press.

Finot, P. A., Magnenat, E., Guignard, G. & Hurrell, R. F. (1982). *International Journal for Vitamin and Nutrition Research* **52**, 226.
Food and Agriculture Organization. (1970). *Amino Acid Content of Foods and Biological Data on Proteins*. Rome: FAO.
Gruen, L. C. & Nicholls, P. W. (1972). *Analytical Biochemistry* **44**, 519–522.
Gupta, J. D. & Elvehjem, C. A. (1957). *Journal of Nutrition* **62**, 313–324.
Hachimori, Y., Horinishi, H., Kurihara, K. & Shibata, K. (1964). *Biochimica Biophysica Acta* **93**, 346–360.
Harrison, B. N., Pla, G. W., Clark, G. A. & Fritz, J. C. (1976). *Cereal Chemistry* **53**, 78–84.
Hayashi, R. & Kameda, I. (1980). *Agricultural and Biological Chemistry* **44**, 891–895.
Hurrell, R. F. (1980). In *Food and Health-Science and Technology*, pp. 369–388, [G. G. Birch and K. J. Parker, editors]. London: Applied Science Publishers.
Hurrell, R. F. (1984). In *Developments in Food Proteins*, vol. 3, pp. 213–244 [B. J. F. Hudson, editor]. London: Applied Science Publishers.
Hurrell, R. F. & Finot, P. A. (1984). In *Nutritional and Metabolic Aspects of Food Safety, Advances in Experimental Medicine and Biology*, vol. 177, pp. 423–425. [M. Friedman, editor]. New York: Plenum Press.
Hurrell, R. F., Finot, P. A. & Cuq, J. L. (1982). *British Journal of Nutrition* **47**, 191–211.
Hurrell, R. F., Finot, P. A. & Ford, J. E. (1983). *British Journal of Nutrition* **49**, 343–354.
Hurrell, R. F., Finot, P. A., Jaussan, V. & Cuq, J. L. (1981). *XX International Congress of Nutrition*, San Diego, CA., USA, Abstr. 260.
Kanazawa, K., Danno, G. & Natake, M. (1975). *Journal of Nutritional Science and Vitaminology* **21**, 373–382.
Karayiannis, N. I., MacGregor, J. T. & Bjeldanes, L. F. (1979). *Food and Cosmetics Toxicology* **17**, 591–604.
Khayat, A. & Schwall, D. (1983). *Food Technology* **37**, 130–140.
Liardon, R. & Hurrell, R. F. (1983). *Journal of Agricultural and Food Chemistry* **31**, 432–437.
Liardon, R., Ledermann, S. & Ott, U. (1981). *Journal of Chromatography* **203**, 385–395.
Mauron, J. (1981). In *Progress in Food and Nutrition Science*, vol. 5, *Maillard Reactions in Food*, pp. 5–35 [C. Eriksson, editor]. Oxford: Pergamon Press.
Mauron, J., Mottu, F., Bujard, E. & Egli, R. H. (1955). *Archives of Biochemistry and Biophysics* **59**, 433–451.
Moore, S. (1963). *Journal of Biological Chemistry* **238**, 235–237.
Nielsen, H. K., Finot, P. A. & Hurrell, R. F. (1985*a*). *British Journal of Nutrition* **53**, 75–86.
Nielsen, H. K. & Hurrell, R. F. (1985). *Journal of the Science of Food and Agriculture* (In the Press).
Nielsen, H. K., Klein, A. & Hurrell, R. F. (1985*b*). *British Journal of Nutrition* **53**, 293–300.
Nielsen, H. K., Löliger, J. & Hurrell, R. F. (1985*c*). *British Journal of Nutrition* **53**, 61–73.
O'Brien, P. J. (1966). *Biochemical Journal* **101**, 12P–13P.
Ohara, I., Otsuka, S.-I., Yugari, Y. & Ariyoshi, S. (1980). *Journal of Nutrition* **110**, 634–640.
Peret, J., Macaire, I. & Chanez, M. (1973). *Journal of Nutrition* **103**, 866–874.
Pierpoint, W. S. (1971). *Rothamsted Experimental Station Report for 1970*, part 2, 199–218.
Possompes, B., Cuq, J. L., Guenoun, D. & Besancon, P. (1983). *Food Chemistry* **11**, 15–26.
Prendergast, (1974). *Food Trade Review* **44**, 14–21.
Provansal, M. M. P., Cuq, J. L. & Cheftel, C. (1975). *Journal of Agricultural and Food Chemistry* **23**, 938–943.
Rasekh, J., Stillings, B. R. & Sidwell, V. (1972). *Journal of Food Science* **37**, 423–425.
Roundy, Z. P. (1958). *Journal of Dairy Science* **41**, 1460–1465.
Scarbieri, V. C., Amaya, J., Tanaka, M. & Chichester, C. O. (1973). *Journal of Nutrition* **103**, 1731–1738.
Steinhart, H. (1979). *Landwirtschaftliche Forschung* **32**, 63–73.
Steinhart, H. & Kirchgessner, M. (1980). *Proceedings of 3rd EAAP Symposium on Protein Metabolism and Nutrition* pp. 312–317, European Association for Animal Production Publication no. 27. Rome: EAAP.
Synge, R. L. M. (1975). *Qualitas Plantarum – Plant Foods for Human Nutrition* **24**, 337–350.
Tannenbaum, S. R., Ahern, M. & Bates, R. P. (1970). *Food Technology* **24**, 96–99.
Tovar, L. R. (1981). The effect of treatment with alkali on the nutritional characteristics of protein. PhD Thesis, University of California, Berkeley.
Wilkening, M. C. & Schweigert, B. S. (1947). *Journal of Biological Chemistry* **171**, 209–212.

Printed in Great Britain