Reactions of proteins with oxidizing lipids

1. Analytical measurements of lipid oxidation and of amino acid losses in a whey protein–methyl linolenate model system

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1. The reactions between protein-bound amino acids and oxidizing lipid were investigated in a whey protein–methyl linolenate ($C_{18:3}$)–water model system. The extent of fat oxidation was followed by measuring oxygen uptake, hydroperoxide formation and hydrocarbon (ethane and pentane) formation.

2. Significant losses occurred with lysine (up to 71%), tryptophan (up to 31%) and histidine (up to 57%). Methionine was extensively oxidized to its sulphoxide but less than 2% was further oxidized to the sulphone. No other amino acids were affected.

3. Increasing storage temperature (20°, 37°, 55°) resulted in an enhancement of fat oxidation reactions and amino acid degradation.

4. Increasing water activity (0·28, 0·65, 0·90) increased losses of lysine and tryptophan but had no influence on the oxidation of methionine, the level of remaining hydroperoxides or O_2 uptake. Hydrocarbons were decreased.

5. Limitation of O_2 uptake to 1 mol/mol lipid instead of excess O_2 (O_2 uptake about 2·5 mol/mol lipid in 4 weeks) significantly reduced the degradation of lysine and tryptophan but had less influence on the oxidation of methionine. The level of remaining hydroperoxides was increased but hydrocarbons were unaffected.

Lipid oxidation occurs when unsaturated fatty acids autoxidize via free radical reactions into hydroperoxides, cyclic peroxides, epoxides, etc. These primary products subsequently degrade into a vast number of secondary products such as saturated or unsaturated aldehydes and ketones, bi- and trifunctional compounds and hydrocarbons (Karel, 1973; Chan & Levett, 1977; Frankel, 1982). Many of the latter compounds give off-flavours and cause rancidity in foodstuffs. Such reactions have been reported in potato flakes (Labuza & Bergquist, 1983; Löliger & Jent, 1983), fish products (Khayat & Schwall, 1983), cereals (Harrison *et al.* 1976), poultry (Dawson & Gartner, 1983) and milk products (Hicks, 1980).

Lipid oxidation products can also react with protein-bound amino acids. The most sensitive amino acids have been reported to be methionine (Braddock & Durgan, 1973; Gamage & Matsushita, 1973), cyst(e)ine (Roubal, 1971), tryptophan (Kanazawa *et al.* 1975), lysine (Horigome & Miura, 1974; Chiba *et al.* 1976), histidine (Desai & Tappel, 1963; Yanagita *et al.* 1973; Matoba *et al.* 1982) and tyrosine (O'Brien, 1966; Roubal & Tappel, 1966). Tryptophan and the sulphur amino acids are assumed to be oxidized by the primary products (Tannenbaum *et al.* 1969; Yong *et al.* 1980; Finley *et al.* 1981) whereas lysine is thought to react with the secondary products (Kanazawa *et al.* 1975). In some studies, almost all amino acids appear to have reacted (Roubal & Tappel, 1966; Horigome & Miura, 1974).

In the present work, the influence of different factors (time, temperature, water activity and oxygen limitation) on lipid oxidation and amino acid losses has been investigated chemically in a whey protein–methyl linolenate–water model system. In the following paper (Nielsen *et al.* 1985) the nutritional importance of the interaction between protein and oxidizing lipid has been evaluated using animal assays.

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EXPERIMENTAL

The study consisted of two storage trials in which whey protein was stored with a mixture of methyl linolenate and water. In the first trial, we investigated the influence of storage time and temperature, water activity (a_w) and O_2 on lipid oxidation and on the losses of lysine, methionine and tryptophan. In the second trial, all amino acids were measured and the levels of lysine and methionine were determined by different analytical procedures. The test materials from the second storage trial were subsequently evaluated in rat assays (Nielsen *et al.* 1985).

Preparation of test materials

Protein. Whey protein was prepared from skimmed milk by a procedure similar to that described by Hilpert *et al.* (1977). The ultrafiltration step was extended so as to give a very low lactose content (2 mg/g).

Lipid. For cost considerations, technical grade methyl linolenate was used (Fluka AG; Buchs, Switzerland). It contained (mmol/mol) 700–800 methyl linolineate ($C_{18:3}$), 200–250 methyl linoleate ($C_{18:2}$) and a small amount of other methyl esters (primarily methyl oleate ($C_{18:1}$)).

Model systems. In order to investigate the influence of time and temperature of storage, a_w and O_2 on fat oxidation and amino acid losses, a series of model systems were prepared and stored for 1, 2 and 4 weeks under the conditions described in Table 1. A given quantity of whey protein (100–300 g) was mixed with half its weight of methyl linolenate in a handmixer (type 394; Moulinex SA, Zurich, Switzerland). Either no water, or some water (0·09 g/g protein or 0·27 g/g protein respectively) was sprayed into the product while mixing. The mixture was then milled (3 × 30 s) in a coffee mill (type A 70; Eisenbach, W. Germany) before being weighed (0·75–3·53 g) into 400-ml enamelled aluminium tins (type AKU 84·5; Cebal, Clichy, France) which were sealed under air and stored. After storage, the mixture was ground, extracted once with 20 ml methanol/g lipid and twice with 20 ml methanol–hexane (2:1, v/v) per g lipid (both reagents from E. Merck, Darmstadt, W. Germany). It was then left to dry in air and stored overnight in an evacuated desiccator containing sodium hydroxide. Finally the mixture was ground again and stored at 4° until analysed.

A second trial was made in which larger quantities of test materials were stored for 4 weeks under identical conditions to the first trial (Table 1), except that the conditions of 'low temperature' and 'medium a_w' were not included. These larger quantities (1 kg) were mixed in a Hobart mixer and were passed through a sieve of 1·5 mm mesh size before weighing (13·3–62·7 g) into 7·1-litre lacquered tins (Pulverdose 4·5 kg; Nestlé, Vevey, Switzerland) and stored.

Analytical methods

In the first storage trial, duplicate samples were prepared and each analysed once. The sample-to-sample variation was therefore included in the standard errors. Head-space analysis was only run on duplicate samples for the 'basal conditions'. For the second storage trial, single samples only were prepared, but they were analysed in duplicate.

Lipid oxidation. O_2 uptake and hydrocarbon formation were determined by the head-space technique described by Löliger & Jent (1983). Lipid hydroperoxides were determined by their ability to oxidize ferrous to ferric, which forms a red complex with thiocyanide (Stine *et al.* 1954). The reaction was carried out in methanol–hexane (5:1, v/v).

Nitrogen. N was measured by a Kjeldahl procedure using mineralization and distillation units from Büchi Laboratoriums Technik AG, Flawil, Switzerland (Digestor 430, Distillation Unit 322 and Control Unit 342) and titration units from Metrom AG, Herisau, Switzerland

Table 1. *Storage conditions*

(0·5 g methyl linolenate/g whey protein)

	Temperature (°)	Water activity* (a_w)	Approximate moisture content (g/kg)	Oxygen (mol/mol lipid)
Basal conditions	37	0·90, 0·84	170	4
O_2 limitation	37	0·90, 0·84	170	1
Low temperature	20	0·90	170	4
High temperature	55	0·90, 0·84	170	4
Low a_w	37	0·28, 0·33	20	4
Medium a_w	37	0·67	70	4

* First value is a_w in first storage trial, second value is the a_w in second storage trial.

(Dosimat 655, Titrator E 526 and Dosiprint E 533A). A factor of 6·25 was used to convert N to protein.

Tryptophan. Tryptophan was measured by reverse-phase high pressure liquid chromatography with fluorimetric detection after hydrolysis in NaOH under vacuum. 5-Methyltryptophan was added as an internal standard before hydrolysis and its recovery was used to correct for tryptophan losses (Nielsen & Hurrell, 1985).

Total amino acids. These were determined by ion-exchange chromatography on an amino acid analyser (Beckman Multichrom or Beckman 121; Beckman Instruments, Munich, W. Germany, or Biotronik LC 7000; Biotronik Wissenschaftliche Geräte GmbH, Munich, W. Germany) after 24 h acid-hydrolysis under reflux in 6 M-hydrochloric acid with N_2-bubbling.

Total methionine and cyst(e)ine. The protein was first oxidized with performic acid (16 h, 4°). Excess performic acid was destroyed with HBr and total methionine and cyst(e)ine were determined as methionine sulphone and cysteic acid respectively, after 24 h acid-hydrolysis in 6 M-HCl under reflux (Moore, 1963).

Methionine and methionine sulphoxide. Methionine and its sulphoxide were determined separately after alkaline-hydrolysis (Neumann, 1967). The same NaOH-hydrolysates, in which tryptophan was measured, were run on the amino acid analyser. The measured values were corrected using the recoveries of added free methionine (90%) and methionine sulphoxide (83%) and for the 5% of free methionine that was transformed into the sulphoxide during the hydrolysis.

α-Amino adipic acid. This proposed lysine degradation product was measured after acid-hydrolysis or after dimethyl sulphoxide (DMSO)-HCl hydrolysis (Spencer & Wold, 1969). α-Amino adipic acid was purchased from Calbiochem, Los Angeles, CA., USA.

Fluorodinitrobenzene (FDNB)-reactive lysine (direct method). The direct FDNB procedure of Carpenter (1960) as modified by Booth (1971) was used. A factor of 1·09 was used to correct for the loss of dinitrophenol (DNP)-lysine during acid-hydrolysis. FDNB and methoxycarbonyl chloride were purchased from Fluka AG, Buchs, Switzerland and Eastman Kodak Co., Rochester, NY., USA respectively.

FDNB-reactive lysine (by difference). Lysine (rather than DNP-lysine) was determined in the acid-hydrolysates which had been prepared for the direct FDNB method. This value was taken as a measure of the inaccessible or 'bound' lysine which had not reacted with FDNB, but was released as lysine on acid-hydrolysis. Reactive lysine by difference was then calculated as 'total lysine' minus 'bound lysine' (Roach *et al.* 1967).

a_w. A 'Hydroskop DT' from Rotronic AG, Zürich, Switzerland was used to measure a_w.

RESULTS

Lipid oxidation

Preliminary experiments showed that the small quantity of test material (0·75–3·53 g) contained in the 400 ml tin readily-absorbed O_2 from the air of the headspace. The partial pressure of O_2 did not appear to influence the reaction velocity and the transport of O_2 from the air to the powder was not rate-limiting. The results from the first storage trial are shown in Fig. 1(*a–d*).

O_2 *uptake.* After 1 week, there was no O_2 left in the samples in which O_2 uptake was limited to 1 mol/mol lipid (Fig. 1(*a*)). In other samples, between 2 and 2·5 mol O_2 were absorbed per mol lipid in the first week and afterwards little O_2 disappeared. O_2 uptake at 20° was slightly slower than at 37° or 55°.

Hydroperoxides. Temperature had an important effect on the formation and subsequent destruction of hydroperoxides (Fig. 1(*b*)). After 1 week at 20°, the level of hydroperoxides present in the sample was still high although it was reduced progressively on further storage. After 1 week's storage at 37°, the samples contained less hydroperoxides than at 20°. It was subsequently shown that the hydroperoxides in this sample had increased rapidly during the first few days, reached a maximum and then fallen to the 7 d level. No hydroperoxides were left after 1 week at 55°. The effect of O_2 limitation was surprising; when O_2 intake was limited, more hydroperoxides were detected.

Hydrocarbons. Fig. 1(*c*) shows the amount of ethane and pentane found in the head-space analyses. The ratio ethane:pentane was in accordance with the levels of their respective precursors (methyl linolenate and methyl linoleate) present in the model system. Decreasing a_w gave higher hydrocarbon levels. O_2 limitation had no effect, whereas lowering the temperature significantly slowed down the reaction.

Non-extractable lipids. The protein content of the samples reflects the increasing amount of bound lipid which was resistant to extraction. All factors had an influence on the level of non-extractable lipid and hence the protein content (Fig. 1(*d*)). The non-extractable lipid was high at high a_w, excess O_2 and higher temperatures (37° or 55°). Assuming that the changes in protein content are due to incorporation of lipids only, a decrease in protein content (N × 6·25) from 827 g/kg (day 0) to 649 g/kg (55° sample, 4 weeks) means that the amount of lipid bound to the protein in the stored sample was 0·33 g/g protein. During the incubation, 0·57 g lipid was present per g protein (N × 6·25). During the storage period more than half the lipid had thus reacted with the protein or, in other ways, become unextractable.

Amino acid losses

Influence of time and temperature of storage, a_w and O_2. The kinetics of lysine, methionine and tryptophan losses together with the formation of methionine sulphoxide under the different storage conditions are shown in Fig. 1(*e–h*). FDNB-reactive lysine was progressively reduced over the 4-week storage period up to a maximum of 71%. Lysine degradation increased with increasing temperature and a_w and was significantly reduced by limiting the O_2 uptake to 1 mol/mol lipid. Tryptophan was far more stable than lysine and significant degradation occurred only at high a_w, excess O_2 and elevated temperature. A maximum loss of 31% was observed (Fig. 1(*e*)).

Methionine was readily oxidized by the oxidizing lipid and was the least stable of the three amino acids studied. At 55°, it had completely disappeared after 1 week and, under all conditions, more than 60% was oxidized after 2 weeks. Normally the decrease in methionine led to a corresponding increase in methionine sulphoxide.

Control samples without lipid were also prepared and stored at 37° and at 55°. Table 2 shows the amino acids remaining in these samples after 28 d storage. At 55°, small losses
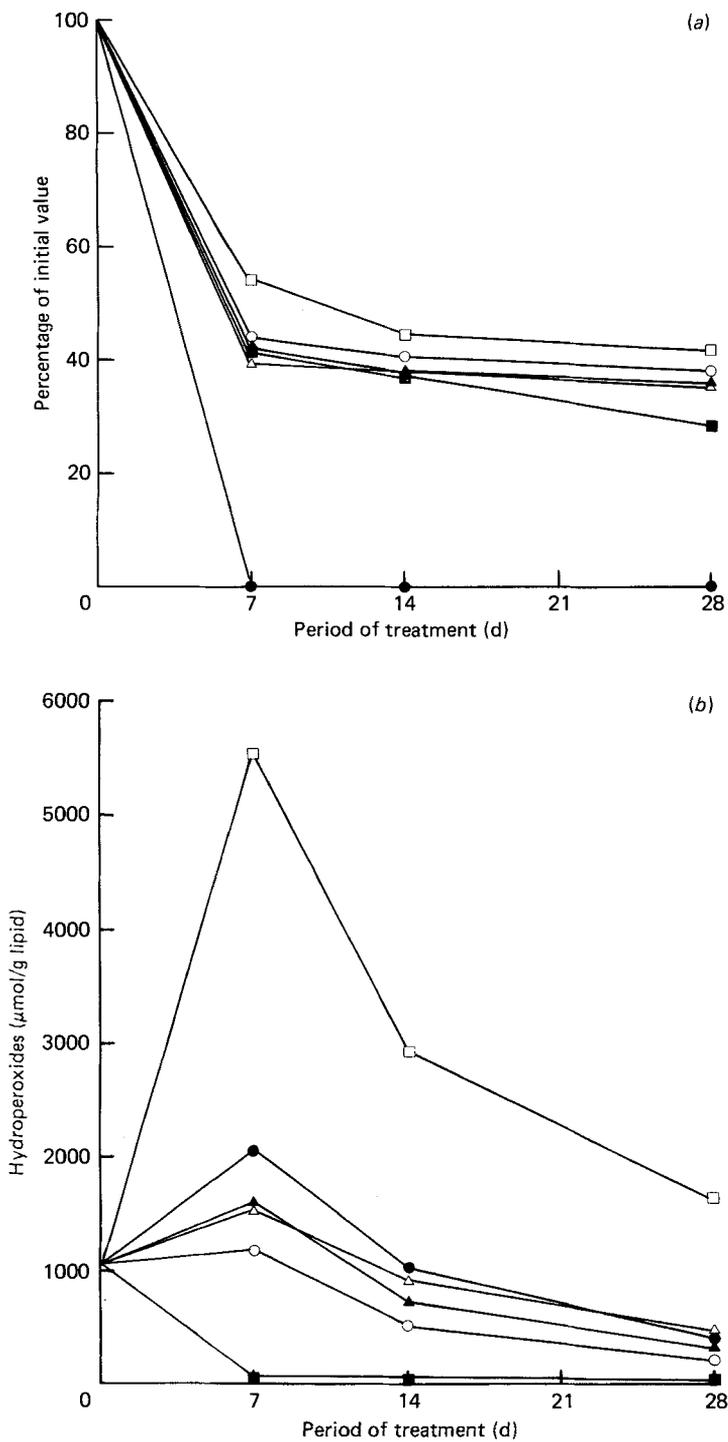
Fig. 1(*a*, *b*). For legend, see p. 68.
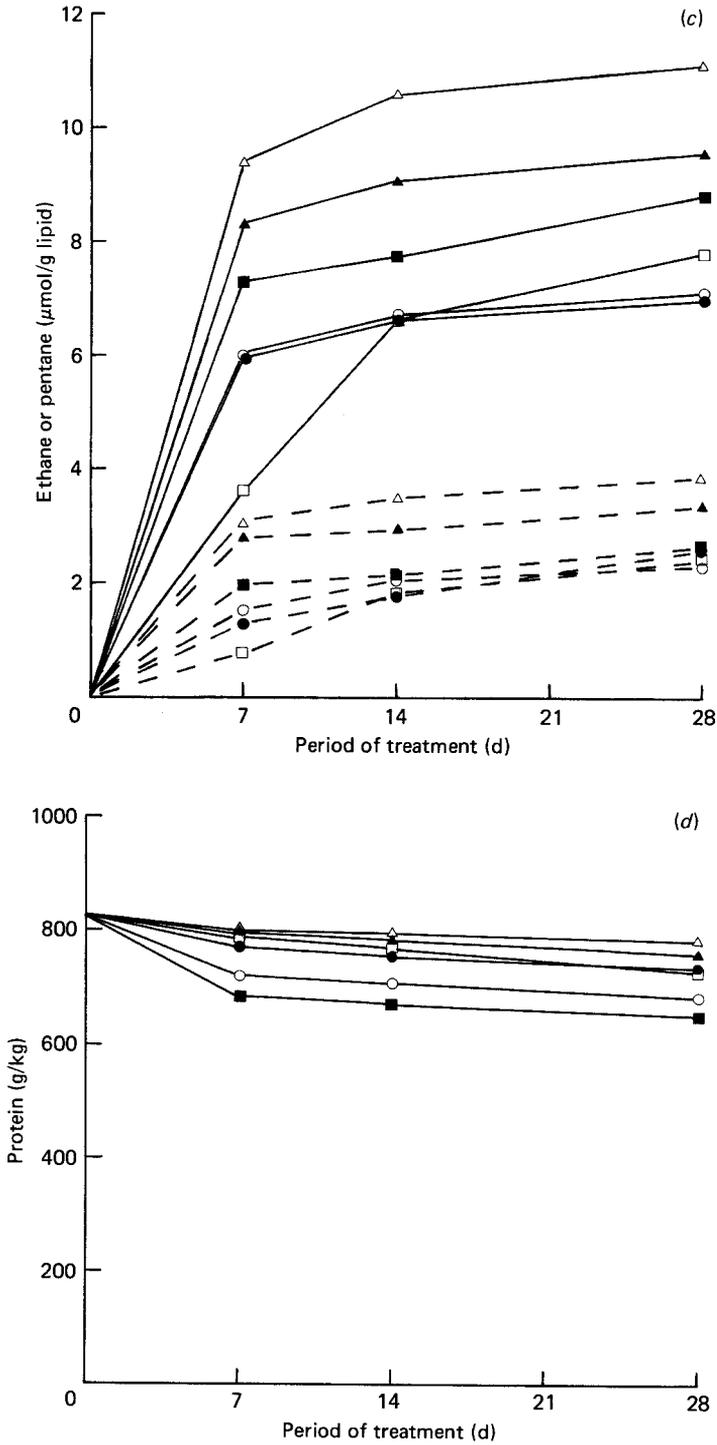
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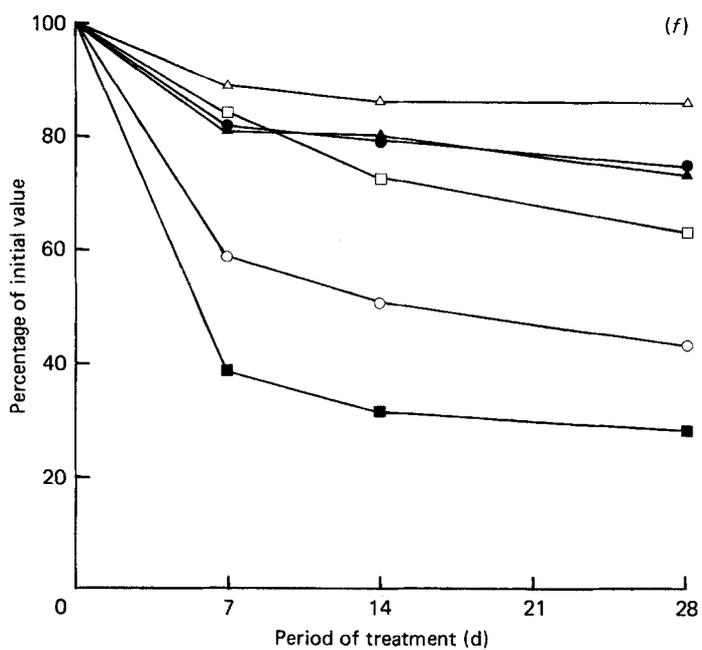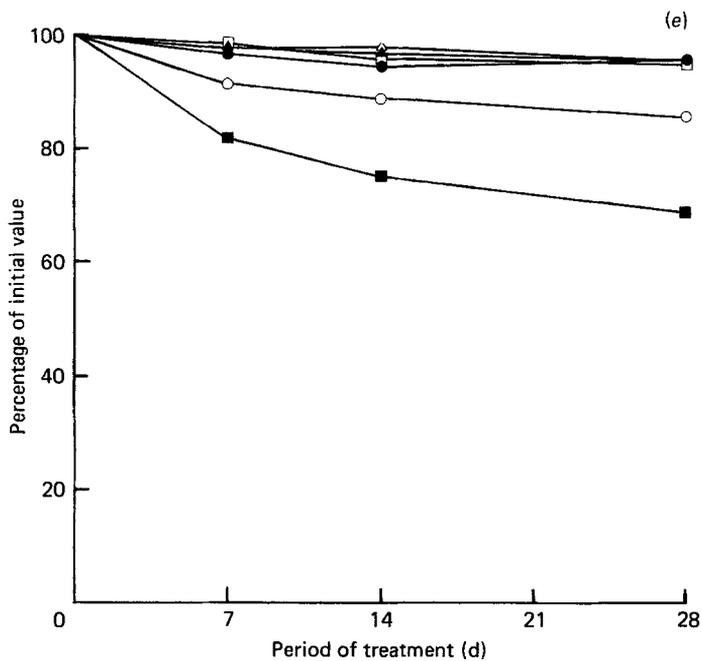
Fig. 1 (*e, f*). For legend, see p. 68.
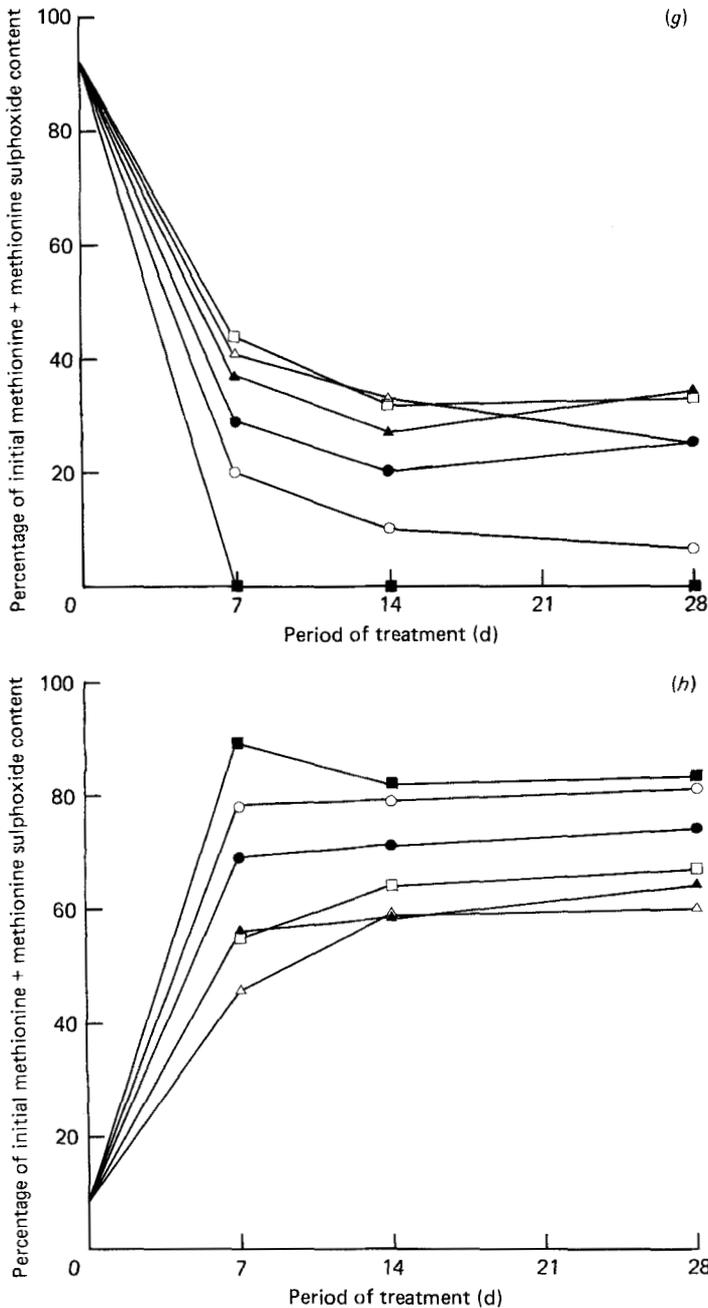
Fig. 1. Kinetics of (a) oxygen, (b) hydroperoxides, (c) ethane (———) and pentane (––––), (d) protein content, (e) tryptophan, (f) fluorodinitrobenzene (FDNB)-reactive lysine, (g) methionine, (h) methionine sulphoxide in a whey protein–methyl linolenate model system stored under different conditions. Basal conditions (○), O$_2$ limitation (●), low water activity (a$_w$) (△), medium a$_w$ (▲), low temperature (□), high temperature (■) (see Table 1 for experimental details). Pooled standard errors for mean of duplicate samples: O$_2$ 0·7% (only basal conditions in duplicate), hydroperoxides 30 μmol/g lipid, ethane 0·30 μmol/g lipid and pentane 0·1 μmol/g lipid (only basal conditions in duplicate), protein content 2 g/kg, tryptophan 1·2%, FDNB-reactive lysine 1·4%, methionine 3·2% and methionine sulphoxide 2·0%. Initial value (mg/g crude protein (N × 6·25)) at day 0 for tryptophan was 23·4, FDNB-reactive lysine was 107, methionine was 22·9 and methionine sulphoxide as methionine equivalent was 2·0.

Table 2. *Amino acids (mg/g crude protein (nitrogen × 6·25)) in control samples (without lipid) stored at 37° and 55° for 28 d*

		28 d storage at:		Pooled
	Day 0	37°	55°	SEM*
Tryptophan	23·4	23·4	22·6	0·3
FDNB-reactive lysine	107	104	102	1·0
Methionine	22·9	24·3	21·7	0·8
Methionine sulphoxide as methionine equivalents	2·0	0·2	2·7	0·5

FDNB, fluorodinitrobenzene.
* Pooled standard error of mean for all values given in Fig. 1 and Table 2.

of both tryptophan (3%) and lysine (5%) were found whereas, at 37°, no significant losses were observed. Methionine appeared to be slightly oxidized to methionine sulphoxide during the sample preparations at day 0 and during the storage of the control at 55°.

Total amino acids. Table 3 shows the total amino acids remaining in the model systems after the second 4-week storage trial. Most amino acids were unaffected but important losses occurred for lysine, histidine and tryptophan. Degradation was highest at high a_w, excess O_2 and high temperature. The variations in leucine, aspartic acid and glutamic acid were due to the large standard errors.

Lysine degradation. Table 4 shows the lysine content of the five samples from the second storage trial as measured in different ways. The total lysine values of the stored samples show that not all the original lysine molecules were released during acid-hydrolysis. Lysine values measured by the direct FDNB-method were lower than the total values. This indicates that some lysine residues had reacted with fat-oxidation products and could no longer react with FDNB. During acid-hydrolysis these lysine residues regenerated lysine, which was measured by ion-exchange chromatography of the FDNB-treated protein hydrolysates. When these bound-lysine values were subtracted from the total lysine values, FDNB-reactive lysine 'by difference' was obtained. The values 'by difference' were in good agreement with the 'direct' values.

Using ion-exchange chromatography, α-amino adipic acid could not be detected in the acid hydrolysates (with or without DMSO) of the 'basal conditions' sample.

Methionine oxidation. Alkaline-hydrolysis was used to determine separately methionine and methionine sulphoxide in the test material from the second storage trial. The two compounds were measured in the same NaOH-hydrolysates as were prepared for tryptophan. The results are shown in Table 5. The recoveries of free methionine and methionine sulphoxide added to casein or to a lipid oxidation sample were on average 90 (SE 3)% and 83 (SE 2)% respectively (four determinations). Of the added free methionine, 5% was oxidized to methionine sulphoxide. In the sample stored at high temperature (55°), no methionine was detected after alkaline-hydrolysis, i.e. methionine sulphoxide was not reduced to methionine during the hydrolysis. Based on these findings, the values for methionine and methionine sulphoxide were corrected.

In the samples incubated with methyl linolenate, methionine was extensively oxidized to its sulphoxide. Even in the low a_w sample, in which virtually no changes in total amino acids had occurred (see Table 3), 75% of the methionine was oxidized.

No changes in total methionine, measured as methionine sulphone after performic acid oxidation and acid-hydrolysis, were seen (Table 5). In addition, during the standard 6 M-HCl hydrolysis with N_2-bubbling, methionine sulphoxide appeared to be quantitatively reduced to methionine.

Table 3. *Total amino acids (mg/g crude protein (nitrogen × 6·25)) of whey protein control and stored whey protein–methyl linolenate model systems from second storage trial*

Amino acid	Control protein	Basal conditions*	Low water activity	Oxygen limitation	High temperature	SEM†
Tryptophan	22·8	19·5	22·9	21·9	16·9	0·3
Lysine	111·7	63·4	103·3	97·7	54·8	1·9
Methionine	24·9	25·0	25·4	25·9	25·8	0·7
Cystine	30·9	30·5	31·1	31·6	31·4	1·1
Leucine	153·9	156·7	163·3	141·6	158·9	9·0
Isoleucine	62·8	62·4	61·7	68·6	65·5	1·5
Valine	58·2	57·9	60·1	63·3	61·3	1·8
Phenylalanine	40·0	41·1	42·1	43·4	42·8	1·3
Tyrosine	39·5	38·8	40·0	39·5	37·9	1·0
Threonine	60·5	62·3	62·1	62·3	59·6	1·9
Serine	58·5	61·1	58·7	58·0	59·3	2·3
Histidine	22·7	14·0	21·9	18·4	12·9	0·1
Arginine	32·2	31·3	32·3	33·2	29·5	0·3
Proline	60·3	57·8	59·1	57·7	57·7	1·6
Alanine	50·5	50·1	49·6	53·4	50·6	0·8
Glycine	21·4	22·2	21·1	23·4	21·4	0·3
Aspartic acid	137·9	132·4	148·7	155·5	133·7	9·1
Glutamic acid	214·3	233·5	231·3	259·5	208·1	6·7
Crude protein (g/kg)	880	682	801	748	673	2

* For storage conditions, see Table 1.
† Pooled standard error of mean of duplicate analyses.

Table 4. *Lysine contents (mg/g crude protein (nitrogen × 6·25)) in samples from second storage trial*

			FDNB-reactive	
	Total* (A)	Bound† (B)	By difference (A − B)	Direct
Control protein	112	0	112	105
Basal conditions	63	23	40	42
Low water activity	103	9	94	89
Oxygen limitation	98	11	87	78
High temperature	55	30	25	31
Pooled SEM	2	1	2	1

FDNB, fluorodinitrobenzene.
* Acid-hydrolysis (6 M-hydrochloric acid).
† Lysine regenerated on acid-hydrolysis of the FDNB-treated proteins.

Regarding the sum of methionine and methionine sulphoxide, virtually constant values were obtained except for the 'high temperature' sample which was slightly lower. No loss in total methionine after performic acid oxidation was observed for this sample, and when an acid-hydrolysate was run on the amino acid analyser, a very small methionine sulphone peak was seen. It corresponded to 0·3 mg/g crude protein and could only account for a fraction of the observed decrease in the sum of methionine and methionine sulphoxide after alkaline-hydrolysis (21·7 → 20·1 mg/g crude protein).

Table 5. *Methionine content (mg/g crude protein (nitrogen × 6·25)) in samples from second storage trial*

	Methionine			Methionine sulphoxide in methionine equivalents (alkaline-hydrolysis) (B)	Methionine + methionine sulphoxide (alkaline-hydrolysis) (A + B)
	Acid-hydrolysis	Performic acid + acid-hydrolysis	Alkaline-hydrolysis (A)		
Control protein	21·6	24·9	21·5	0·2	21·7
Basal conditions	23·6	25·0	1·2	20·1	21·3
Low water activity	23·0	25·4	5·4	16·3	21·7
Oxygen limitation	nd	25·9	4·0	18·1	22·1
High temperature	22·6	25·8	0·0	20·1	20·1
Pooled SEM	0·9	0·7	0·3	0·4	0·9

nd, Not determined.

DISCUSSION

We have investigated the reactions of protein-bound amino acids with oxidizing lipids by storing a mixture of whey protein, methyl linolenate and water in sealed cans under a large excess of air. The extent of fat oxidation was followed by measuring O_2 uptake, the level of hydroperoxides (primary fat-oxidation products) and the hydrocarbons ethane and pentane, which are two of the many secondary fat-oxidation products. Pentane formation in stored food products has been shown to correlate with the development of oxidative rancidity (Löliger & Jent, 1983).

As storage time progressed, the polyunsaturated lipid oxidized and the oxidation products reacted with the amino acids lysine, methionine, tryptophan and histidine. The reactivity of tyrosine, serine, arginine, proline, alanine and valine reported by other workers (Desai & Tappel, 1963; Roubal & Tappel, 1966; Horigome & Miura, 1974) could not be confirmed. From a chemical point of view, those amino acids with purely aliphatic side-chains would be assumed not to react. As already found by Tannenbaum *et al.* (1969), Karel *et al.* (1975) and Cuq *et al.* (1978), methionine was readily oxidized to its sulphoxide, with little further oxidation to the sulphone. It was not possible to determine whether cyst(e)ine was oxidized since we were not able to measure the different oxidation products. Ion-exchange chromatograms of acid-hydrolysates did not show any cysteic acid peak, but this, of course, does not exclude formation of the intermediate oxidation products as reported by Finley *et al.* (1981).

Storage temperature, a_w and O_2 level all influenced the extent of fat oxidation and the consequent amino acid losses. Increasing storage temperature, as would be expected, enhanced all reactions. Increasing a_w increased the degradation of lysine and tryptophan and decreased hydrocarbon formation but had little influence on the O_2 uptake, the level of remaining hydroperoxides, or on the oxidation of methionine. Similar results have been reported for lysine (Yanagita *et al.* 1973, Matoba *et al.* 1982) and for methionine (Cuq *et al.* 1978). It would appear therefore that methionine oxidation is mechanistically different from the degradation of tryptophan and lysine and that the loss of tryptophan may not result from oxidative degradation, as often assumed (Yong *et al.* 1980), but from reactions of the indole ring with the secondary products of fat oxidation.

O_2 limitation also markedly reduced lysine and tryptophan degradation but had little influence on the oxidation of methionine. Rather surprisingly, more hydroperoxides were formed when O_2 was limited than when it was in excess, indicating that O_2 might enhance

hydroperoxide decomposition. As with the samples stored at different a_w, the levels of ethane and pentane did not correlate with the extent of lysine damage despite the fact that these hydrocarbons are markers for the secondary lipid oxidation products. This is perhaps not unexpected since the hydrocarbons form only a small part of the many different lipid degradation products and they themselves would not be expected to react with lysine. The maximum level of ethane (10 μmol/g lipid) detected in our model systems represents only 1/300th of the methyl linolenate original present, most if not all of which had been oxidized. Lysine degradation would presumably correlate better with the formation of aldehydes and ketones. Only by more thorough examination of the fat oxidation products would a clearer interpretation of this aspect be possible.

The mechanism of lysine degradation is not precisely known. In our stored model systems large losses of lysine were observed and, from Table 4, it would appear that those lysine residues in the stored samples which failed to react with FDNB comprised residues which both did and did not regenerate lysine on acid-hydrolysis. Good agreement was observed between the FDNB-reactive lysine values determined by the 'direct' and by the 'difference' methods, indicating that the bound lysine residues regenerated the same amount of lysine on acid-hydrolysis before and after FDNB treatment. This is in contrast to the behaviour of lactulosyl-lysine after early Maillard reactions (Hurrell et al. 1983) and bound lysine after caffeic acid reactions (Hurrell et al. 1982) but similar to the behaviour of glutamyl-lysine or aspartyl-lysine (Hurrell et al. 1976).

Karel et al. (1975) reported that α-amino adipic acid could be formed from lysine during lipid oxidation. An acid-hydrolysate of the sample stored under the 'basal conditions', however, did not contain this compound. It has also been suggested that the position of the double bond of the Schiff's base (the primary condensation product between lysine and an aldehyde) could change to the other side of the N (Höltemand, 1966) so that acid-hydrolysis would liberate α-amino adipic acid semialdehyde. In order to verify this, an acid-hydrolysis was made in the presence of DMSO, which would be expected to have oxidized the aldehyde to α-amino adipic acid; the ion-exchange chromatogram, however, showed no such peak.

Tryptophan was less degraded than were lysine and methionine. Only at higher temperatures, high moisture content and no O_2 limitation was more than 10% of tryptophan lost. This is in contrast to the results of Kanazawa et al. (1975), who reported large tryptophan losses. Their results could partly be explained by the use of an acid-hydrolysis procedure for the determination of tryptophan. O'Brien (1966), however, used alkaline-hydrolysis and also found in an aqueous system that tryptophan was more sensitive to losses than lysine. It is possible that their aqueous model system favoured oxidative reactions.

In conclusion, the above-mentioned results confirm previous findings that the reactions between proteins and oxidizing lipids can lead to a considerable loss of the essential amino acids lysine and methionine (by oxidation to the sulphoxide). They do not support claims that the extent of tryptophan degradation is equally large. Loss of tryptophan in the present study, although significant, was much less than that of either lysine or methionine. Animal assays are needed to evaluate the nutritional consequences of these reactions and such assays are described in the following publication (Nielsen et al. 1985).

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