Chemical and nutritional changes in stored herring meal

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That the protein of foods can suffer loss of nutritive value as a result of overheating during processing is well known, and Carpenter, Ellinger, Munro & Rolfe (1957) have reported the effects of heating under various conditions on the nutritive value and available lysine of the protein of fish meal. It has been shown also that interaction between proteins and reducing sugars can occur during storage in the 'dry' state, even under such mild conditions as a few days at $37°$ and 70% atmospheric relative humidity (Lea & Hannan, 1950), leading to a reduction in the availability of a number of amino-acids and particularly of lysine (Henry & Kon, 1950).

Recently, a further possible cause of loss in the nutritive value of the protein of foods containing highly unsaturated lipids has been disclosed by the experiments of Tappel (1955) on 'model' systems containing linoleic acid or cod-liver oil, emulsified with protein and an oxidative catalyst (haemoglobin, haemin or cytochrome c). On shaking such a mixture in air at $37°$ for 1–2 days extensive interaction of the protein with the oxidizing fat occurred, leading to the formation of yellow-brown products and, ultimately, of insoluble dark brown copolymers of high oxygen and nitrogen content. The total amino-acids recoverable from the copolymers, even after hydrolysis with concentrated hydrochloric acid, was of the order of 16% less than expected from their nitrogen content, indicating an appreciable destruction of amino-acids. Such changes must be accompanied by a considerable fall in the nutritive value of the protein.

The present investigation has been made to determine how far similar changes may occur during the storage of an actual feeding product. Herring meal has been chosen as the test material, since it is valued as a source of high-quality protein but also contains a considerable quantity of highly unsaturated fat.

The oil in the meal was found to oxidize rapidly but, despite these changes, comparative feeding tests with chicks on meal stored for $2\frac{1}{2}$ months in air at $25°$ and in nitrogen at $-20°$ showed no more serious reduction in the value of the meal as a source of supplementary protein than could be accounted for by the observed very slight (4%) fall in available lysine. After 12 months at $25°$ the loss of available lysine had risen to about 9%.

Almquist (1956) and Meade (1956) have recently reported reductions in the percentage of extractable oil as a result of storage or of 'curing'. The present experiments,

which also showed slight reductions in the percentage of extractable oil, are of interest in indicating the very high degree of oxidation of the oil in the meal that can be tolerated by chicks without obvious adverse effects.

Meade (1956) found that the anti-oxidant, NN'-diphenyl-p-phenylenediamine (DPPD), added at a concentration of 0·05 %, was of value in controlling the spontaneous heating of piles of freshly dried fish scrap during the 'curing' process, before grinding and bagging. In the present work 2:6-di-*tert*.-butyl-4-methylphenol (BHT) at 0·005 % has been found to retard considerably oxidation of the oil in the meal during storage at 25°.

A brief account of this work has already been published (Carpenter, 1958; Lea & Parr, 1958).

EXPERIMENTAL

Preparation and storage of the meals

Preparation. A sample of herring meal was obtained from a commercial plant where the herrings had been cooked with steam and passed through a screw-press to express most of the oil and also the aqueous 'stick' liquor (used to prepare condensed fish solubles). The residual press-cake had then been passed through hot-air driers and ground. The freshly ground meal was transported to the laboratory in a container packed with solid carbon dioxide which minimized chemical change by cooling the meal and displacing oxygen. The fresh meal (no. 1) had a proximate analysis of 69·9 % crude protein (N × 6·25), 10·7 % ether extract, 9·1 % ash and 11·1 % moisture.

Part of this meal was further dried in the laboratory, in vacuo at atmospheric temperature, to reduce its moisture content to 6·2 % (meal no. 2).

Addition of anti-oxidants. Two portions of meal no. 2 were treated respectively with (*a*) 0·005 % BHT with 0·005 % citric acid, in 1 % propylene glycol, and (*b*) 0·005 % 2:5-di-*tert*.-butylhydroquinone (DBHQ) with 0·005 % citric acid, in 1 % propylene glycol; all concentrations were calculated on the dry weight of the meal. The anti-oxidant solutions were sprayed on to the meal while it was being 'tumbled' by rotation in a suitable container. The treated meal was then stored in air at 25° as described below for the untreated material.

Storage. 'Control' portions of meals 1 and 2 were sealed in gas-tight cans, 'gas-packed' in oxygen-free nitrogen and held at −20° until required.

To provide conditions permitting free access of atmospheric oxygen while maintaining the moisture contents unchanged, portions of the meals were packed in cans with a large head space and periodically flushed with air during storage to replace oxygen absorbed and remove carbon dioxide produced.

Air- and nitrogen-packed cans of both meals were stored at 37, 25 and 10°. Smaller samples of the meal, defatted by extraction with chloroform-methanol (1:1, v/v) and readjusted to the original moisture contents on the fat-free basis, were similarly stored.

Measurement of absorption of oxygen. Small portions of the whole meals and of the defatted meals were held in Warburg-type vessels fitted with mercury manometers and containing oxygen; absorption was measured at 37, 25 and 10°. The moisture

content of each meal was maintained at its initial value and any carbon dioxide produced was absorbed by a sodium-hydroxide solution of appropriate concentration in the side bulb.

Examination of the stored meals

Colour. The colours of the fresh and of the stored meals were measured by reflected light in a Lovibond Tintometer with artificial-light attachment and recorded in terms of red and yellow units.

Extraction of the oil. The meal was dried in a vacuum desiccator and extracted at laboratory temperature with light petroleum (b.p. 40–60°) or with chloroform–methanol (1:1, v/v). With the latter the solvent was removed and the extract taken up in chloroform and filtered, with the addition of 'Celite' no. 545 (Johns Manville Co., London) if necessary, before use. Extraction to completion was used for determination of the oil content of the fresh meal. A rapid procedure using four extractions, each with 100 ml. solvent/50 g meal for 10 min was standardized for routine examination of the stored samples to avoid further changes, particularly in peroxide value and colour, during extraction. Solvents were removed at a temperature below 40° in a rotary vacuum evaporator.

Examination of the oil. The colour of the oil was recorded in terms of its absorption ($E_{1cm}^{1\%}$) in chloroform solution at 400–600 mμ. Iodine and peroxide values and the nitrogen and phosphorus contents of the oil were determined by standard procedures.

Chemical and nutritional tests

Estimation of 'available lysine'. The method was a modification (Bruno & Carpenter, 1957) of the procedure originally applied to fish meals (Carpenter *et al.* 1957), in which the materials are shaken with fluorodinitrobenzene. Those lysine molecules in the protein, in which the ϵ-NH$_2$ group reacts to form the dinitrophenyl compound which is recovered after hydrolysis, are termed 'available lysine'.

Feeding tests. White Leghorn × Rhode Island Red cockerels were reared from hatching to 10 days of age on a good commercial ration, and then allotted according to a statistical design to cages with raised wire floors in groups of three or four. They then received the experimental diets in dry, ground form *ad lib.* for a further 10 days. Their weight gain and food consumption were recorded.

In the first trial the control ration was similar to the lysine-deficient mixture used by Grau & Almquist (1944) but it was fortified with further sources of possible growth factors. It was made up of sesame-seed meal 50, lactose 7·5, grass meal 5, ground oat husk 2·5, arachis oil 2·5, cod-liver oil 0·5, NaH$_2$PO$_4$ 1·4, CaCO$_3$ 1·25, NaCl 0·5, MnSO$_4$.7H$_2$O 0·03, KI 0·01, choline chloride 0·17, oxytetracycline supplement (TM-5, Pfizer Ltd, Folkestone, Kent, contributing 7·5 p.p.m. of oxytetracycline hydrochloride to the diet) 0·07, maize starch (with vitamins) to 100. Vitamins were added to contribute to each 100 g of diet: nicotinic acid 5, calcium pantothenate 3, α-tocopheryl acetate 2, thiamine, riboflavin, pyridoxine and folic acid each 1 and biotin 0·1 mg. The use of this basal mixture for the biological assay of lysine will be described in a separate paper. Tests with the addition of lysine have indicated that a supplement

of 0·15 % L-lysine is at approximately the mid-point of the response curve, and still in the region of linear response.

The rations in the second trial were of the type used for the determination of 'gross protein values' with chicks (Carpenter, Ellinger & Shrimpton, 1955) and were originally designed to represent a practical mixture of cereal proteins, together with vitamin and mineral supplements. They consisted of ground barley 15, fine bran 15, yellow-maize meal 10·5, ground oats 10, ground oat husks 13·3, whey powder 10, dried yeast 3, cod-liver oil 1, $CaHPO_4$ 1·6, NaCl 0·5, $CaCO_3$ 0·4, test herring meal and starch to 100.

In each trial the two test samples were herring meal 1A, i.e. meal 1 that had been stored throughout under nitrogen, at $-20°$, and herring meal 1B prepared by storage of meal 1 at $25°$ in air for $2\frac{1}{2}$ months. To minimize further changes in the samples they were taken from sealed cans at 2-day intervals during the trials and mixed immediately into the diets that were eaten during the succeeding 48 h. In the first trial each sample was added to the basal ration at the expense of starch, so as to contribute 0·145 % 'available lysine', and in the second trial was included in the rations so as to contribute 0·29 % 'available lysine'.

In trial 1, each diet was fed to eight groups each of four chicks, and in trial 2, to six groups each of three chicks. The mean initial weights of the chicks were 105 and 86 g respectively, at the time of randomization.

RESULTS

Water relations of the meal. Moisture contents of the meal determined after equilibration with atmospheres of controlled relative humidity at $37°$ were

r.h. (%)	Moisture content (%)
50	7·3
65	9·6
70	10·5
75	12·3

By extrapolation, the meal adjusted to 6·2 % moisture would be in equilibrium with an atmosphere at 30–40 % r.h.

Oil content of the meal. The fresh meal (11·1 % moisture) yielded 10·7 % light-petroleum extract or 15·2 % purified chloroform–methanol extract. The rapid extraction procedure, with chloroform–methanol, gave 13·7 %, i.e. approximately 90 % of the total lipid extractable with this solvent. Changes in the amount of extractable lipid after storage are given in Table 1.

Colour of the meal. The fresh meal matched in the Lovibond Tintometer showed a colour of 1·2 red, 2·0 yellow, i.e. 3·2 total units. On extraction of the oil the values fell to 0·8, 1·4 and 2·2, respectively. Since plots of the individual colour units or of the totals gave essentially the same picture of changes during storage, only the total colour units have been recorded in Fig. 1.

Rapid darkening of both meals occurred in air at all storage temperatures, the rate being greatest at $37°$ and approximately the same at 25 and $10°$. No darkening occurred

in whole meals stored in nitrogen or in fully extracted meals stored in air. Extraction of most of the lipid removed virtually all of the colour from the meals stored at 10°, but left those stored at the higher temperatures appreciably discoloured.

Table 1. *Lipid extractable with chloroform–methanol from herring meals stored in air or in nitrogen expressed as a percentage of that obtained from the initial material**

Temperature (°C)	Storage period (months)	Meal 1 (11·1% moisture)		Meal 2 (6·2% moisture)	
		Stored in air	Stored in nitrogen	Stored in air	Stored in nitrogen
37	0·7	100	—	98	—
	2	96	100	96	99
25	2·3	97	—	98	—
	4	97	—	97	—
	5·5	—	—	95†	—
	12	89	104	94	102
10	4	101	100	100	101

  * With the rapid procedure used, meals 1 and 2 gave initial values of 13·7 and 14·4% extractable material, respectively.
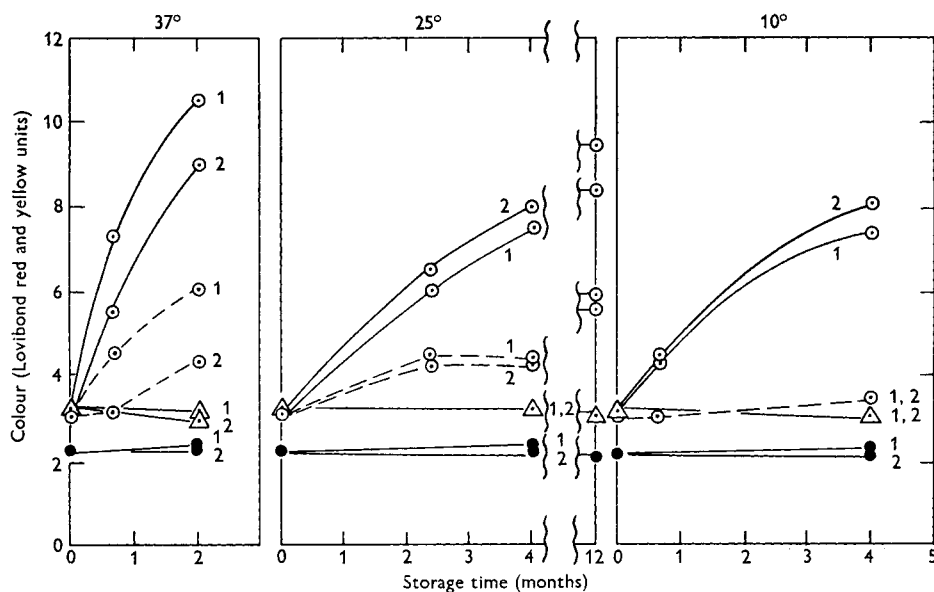 † Extraction with light petroleum, initial value 11·3%.

Fig. 1. Colour changes at three temperatures in herring meals 1 (11·1% moisture) and 2 (6·2% moisture) during storage in air, o—o; and in nitrogen, △—△; in air-stored (whole) meals after extraction of most of the lipid, o- - -o; and in completely defatted meals stored in air, ●—●.

Absorption of oxygen by the meal. Oxygen-absorption measurements were made during the first 20 days of storage in order to supplement the information provided by the chemical determinations, which usually began at this point. To permit direct comparison of the whole with the defatted meals the volumes of oxygen absorbed have all been corrected to the dry, fat-free basis (Fig. 2).

Meals defatted with methanol–chloroform showed only a slight residual capacity for absorbing oxygen. Petroleum-extracted meal oxidized much more rapidly, in keeping with the considerable proportion of the total lipid (about 30 %) which resists extraction by this solvent. The moisture content of the meal markedly influenced the course and rate of the oxidation.
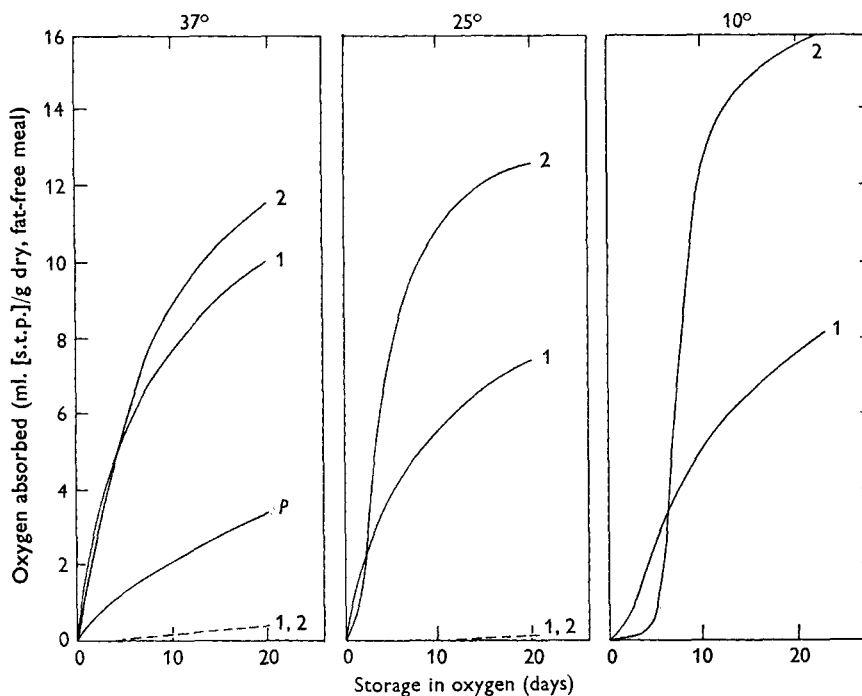

Fig. 2. Absorption of oxygen at three temperatures by whole herring meals 1 (11·1 % moisture) and 2 (6·2 % moisture), ——— ; and by the meals defatted with chloroform-methanol, - - - -. The defatted meals were adjusted to the original moisture contents on the fat-free basis. Curve P, meal defatted with light petroleum.

Table 2. *Peroxide values (μmole/g) of oils extracted from air-stored herring meals*

Meal 1 (11·1% moisture)				Meal 2 (6·2% moisture)							
Days at 37°			Days at 25°	Days at 25°							
0	20	60	71	0	1	2	4	7	11	22	60
18	8	4	5	4	3	7	7	8	9	25	18

Peroxide value of the oil. Peroxide values of chloroform–methanol extracts of the stored meals are given in Table 2. The lower initial value of meal 2 was due to partial degradation of unstable peroxides during drying in vacuo and holding in nitrogen before repacking for the air-storage experiment.

Light-petroleum extracts of fish meal 1 showed a similar behaviour, an initial value of 16 falling to 9 during storage for 20 days at 37°.

Iodine value of the oil. The iodine value of the oil of the air-stored samples fell

rapidly at first at all three temperatures, but the rate of change slowed down more quickly the higher the temperature (Fig. 3). The reason for the slight apparent rise in the iodine value of the oils from the nitrogen-stored samples, particularly at 10°, is not clear; it may have been due to extraction with the oil of non-fatty substances reactive towards iodine monochloride.
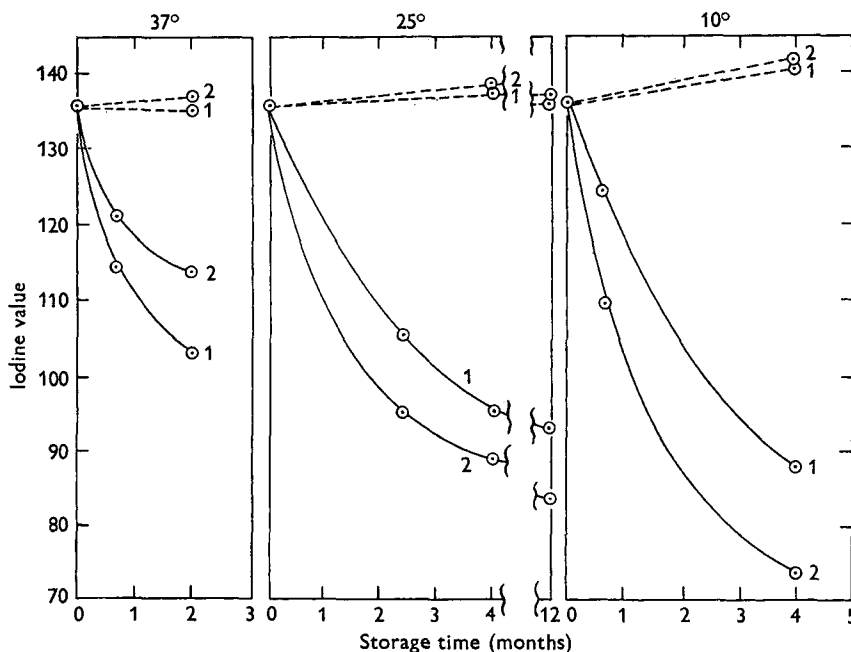

Fig. 3. Changes at three temperatures in the iodine value of oil extracted from herring meals 1 (11·1 % moisture) and 2 (6·2 % moisture) during storage in air, o—o; and in nitrogen, o - - - o.

Colour of the oil. On storage of the meal in air the oil extractable from it was found to have darkened, the degree of change being greater with the low- than with the high-moisture meal and increasing rather than decreasing with reduction of storage temperature (Fig. 4). Oil from meals stored in nitrogen lightened slightly in colour as compared with the fresh (control) meal.

Nitrogen and phosphorus contents of the oil. A light-petroleum extract of fresh meal 1 contained 0·25 % nitrogen and 0·37 % phosphorus; the chloroform–methanol extract contained 0·89 % nitrogen, 0·71 % phosphorus. On the assumption that the phosphorus is present entirely as monoamino-glycerophospholipid, about two-thirds of the nitrogen of the petroleum extract and one-third of the nitrogen of the chloroform–methanol extract could be accounted for as phospholipid, and the two extracts would contain respectively about 10 and 20% of phospholipid, corresponding to about 1 and 3%, respectively, of the meal.

On storage of the meals the nitrogen contents of the extracts increased appreciably, the changes being greater in air than in nitrogen and tending to be greater at the lower storage temperatures (Table 3).

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Nutritive value of the meal

'*Available lysine*'. The results are summarized in Table 4. Consistently lower figures were obtained with samples that had been stored in air than with the 'initial' sample, or with samples that had been stored in nitrogen. The mean figures for 12 months' storage in air at 25° correspond to a decline of 9 % in the available-lysine value of the crude protein in the material; at $2\frac{1}{2}$ months the decline was 4 %. The small difference after storage in nitrogen was within the experimental error of the method.

Feeding tests. No bird died during the tests, nor did we notice any abnormalities in appearance or behaviour. The mean results for each treatment are set out in Table 5.
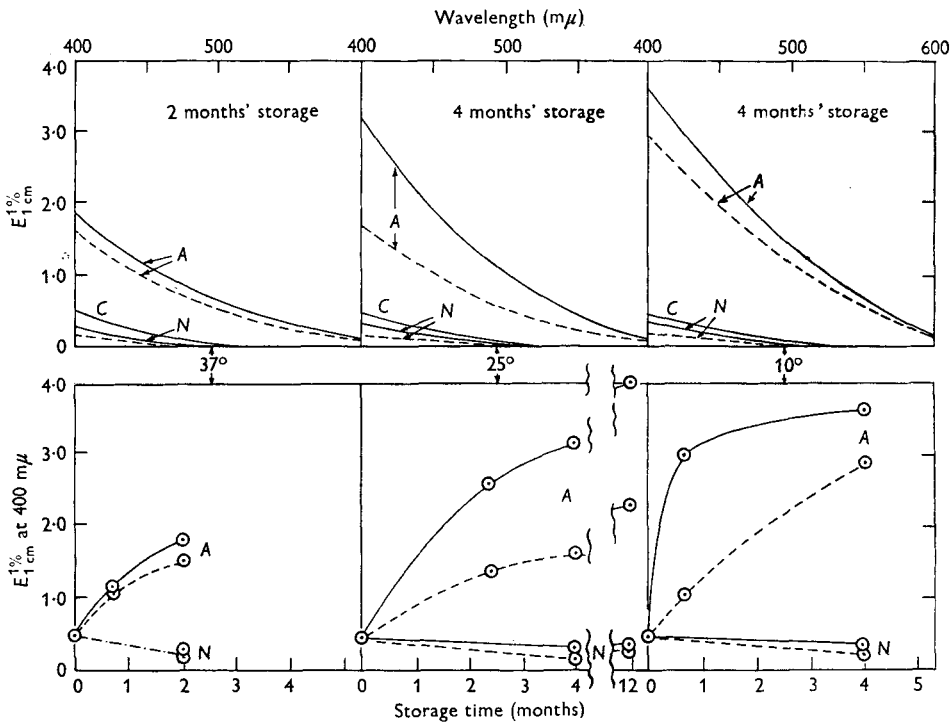

Fig. 4. Changes in the colour in chloroform solution of oil extracted from herring meals 1, - - - -, and 2, ———, stored in air (*A*) and in nitrogen (*N*) for the time shown at three temperatures. Curve *C*, control (unstored) meal.

Table 3. *Changes in the nitrogen content of the chloroform–methanol extracts of herring meals stored in air or in nitrogen*

			Nitrogen content (%)		
Meal	Stored in	Initial	After 2 months at 37°	After 4 months at 25°	After 4 months at 10°
1	Air	0·9	1·2	1·5	1·6
2	Air	0·9	1·1	1·4	1·6
1	Nitrogen	0·9	1·2	1·2	1·2
2	Nitrogen	0·9	1·2	1·2	1·1

Trial 1 formed part of a larger experiment, and an estimate of the standard error of the weight gain with 30 degrees of freedom was obtained by pooling all the available data within this trial.

In each trial the two meals were given so as to contribute the same quantity of available lysine (estimated chemically), and it is concluded that the results showed no significant difference in response to the two meals when given in that way. In other words the feeding trials showed no evidence of there being any more severe damage to the protein in the meal after storage at 25° in air for $2\frac{1}{2}$ months than that indicated by an approximately 4% drop in available lysine.

There was no indication of any decline in palatability of the rations containing the stored meals.

Table 4. *Available lysine content (g/16 g nitrogen) of herring meals stored at 25° in nitrogen or in air*

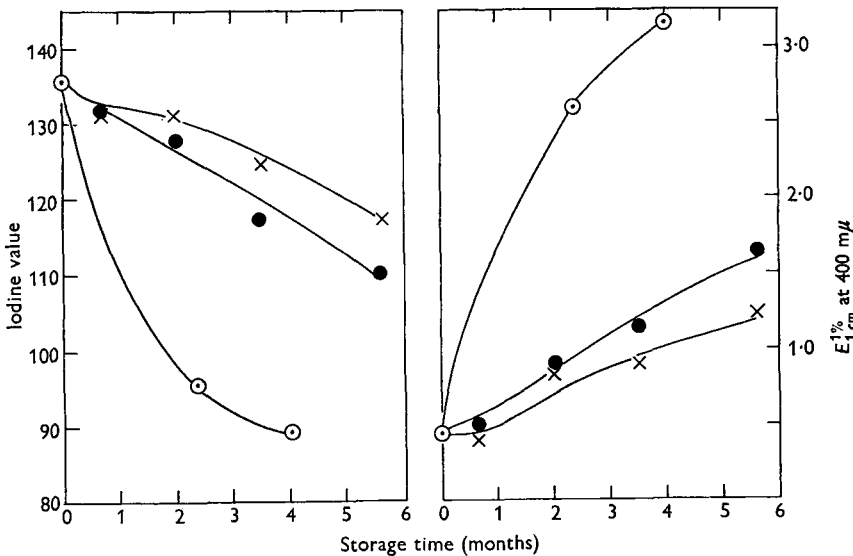| Storage	Meal 1 (11·1% moisture)	Meal 2 (6·2% moisture)
None	7·20	7·20
Whole meals		
In nitrogen for 12 months	7·10	7·05
In air for:		
$2\frac{1}{2}$ months	6·91	—
12 months	6·53	6·59
Defatted meals		
In nitrogen for 12 months	7·26	7·26
In air for 12 months	7·16	7·37

Fig. 5. Effect of anti-oxidants on the iodine value and colour of oil extracted from herring meal 2 stored in air at 25°. ×, meal with 0·005 % 2:6-di-*tert.*-butyl-4-methylphenol and 0·005 % citric acid; ●, meal with 0·005 % 2:5-di-*tert.*-butylhydroquinone and 0·005% citric acid; ○, untreated meal.

Table 5. *Mean food intake and weight gain of chicks in two feeding trials with unstored and stored herring meal*

Meal	Trial no. 1				Trial no. 2			
	No. of chicks	Herring meal in diet (%)	Mean weight of food eaten (g/chick)	Mean body-weight gain* (g)	No. of chicks	Herring meal in diet (%)	Mean weight of food eaten (g/chick)	Mean body-weight gain† (g)
Control	32	None	179	56	—	—	—	—
1 A (unstored)†	32	2·86	212	92	18	5·74	275	103
1 B (stored for 2½ months in air at 25°)‡	32	2·98	209	93	18	6·00	275	103

* Each of the three means has a standard error of ±2·5 g (based on 30 degrees of freedom).

† Each of the two means has a standard error of ±4·8 g (based on 10 degrees of freedom).

‡ 4 % more of meal 1 B was given than of 1 A, so as to supply equal quantities of 'available lysine', as determined chemically.

Effect of anti-oxidants

Fig. 5 shows that BHT and DBHQ (both with citric acid) considerably retarded oxidation of the oil of air-stored fish meal 2 at 25° as measured by the iodine value and colour of the chloroform–methanol extract, BHT being the more effective of the two. Improvements in the colour of the meal itself were less marked, averaging only 0·5–1 Lovibond unit as compared with the untreated meal.

DISCUSSION

Oil content of the meal. The markedly greater amount of lipid extractable with chloroform–methanol (15·2%) than with light petroleum (10·7%) and the much larger amount of lipid phosphorus extracted with the more polar solvent both indicate that a considerable proportion of the lipid of fish meal is 'bound' to the protein. Further small quantities of lipid would probably have been recoverable by solvent-extraction after digestion of the meal with acid (Stansby, 1947).

Absorption of oxygen by the meal. The meals absorbed oxygen extremely rapidly and extensively, levels of up to 10–20 ml./g being reached in the first 1–2 weeks after manufacture. Obviously, exclusion of air by compressing or tight packing might be a practicable means of controlling oxidative deterioration.

At all three storage temperatures the drier meal oxidized to the greater extent, although it showed an induction period—more marked the lower the temperature—which was not present with the moister meal (Fig. 2). The falling away in rate of oxidation at the higher storage temperatures is consistent with a more rapid accumulation of oxidation inhibitors, probably as a result of browning-type reactions, under these conditions. Had the lower moisture content been achieved by a greater temperature–time treatment, as it would have been for a commercially prepared meal, instead of by the vacuum drying at low temperature used in this experiment, the difference in stability between the two meals would probably have been greater. Banks (1950) has shown that raising the drying temperature markedly increases the stability of the oil in dehydrated herring.

In the present experiments the defatted meals stored under the same conditions of temperature and water-vapour pressure showed only a very slow residual absorption of oxygen, part of which may still have been due to traces of unextracted oil. From two-thirds to three-quarters of the oxygen absorption was due to 'free' (extractable by light petroleum) lipid and most of the remainder to 'bound' lipid, in contrast to the findings of Tappel (1956) on freeze-dried beef in which less than 10% of the oxygen absorbed was attributed to ether-extractable lipid, less than 50% to 'bound' lipid and 50–100% to oxidation of the protein. It is possible that the type of protein oxidation observed by Tappel during the storage of freeze-dried beef had already taken place very largely during the high-temperature drying of the fish meal.

Chemical changes in the oil. Though peroxides are known to be the first-formed products in the autoxidation of fats they are labile substances liable to undergo decomposition and further reaction with fatty and non-fatty constituents of the meal. It is

not surprising, therefore, that at 37° peroxides failed to accumulate at all, though at 25° they showed some signs of accumulating, only to fall away again later (Table 2). At 10°, peroxide values would undoubtedly have risen higher, as observed by Banks (1950) with dehydrated herring, but the values obtained would always depend on the margin between the rates of formation and decomposition which, in turn, would be influenced by such factors as freedom of access of oxygen and moisture content. For this reason, it was thought that the disappearance of double bonds, as measured by the reduction in iodine value (Fig. 3), offered a more useful overall measure of the degree of oxidation and oxidative polymerization of the oil. Oils extracted from the longer-stored meals had become solid, indicating extensive polymerization.

Colour. The measurement of colour changes in the meal (Fig. 1) and in the oil (Fig. 4), and particularly the colour-stability of whole meals in the absence of oxygen and of extracted meals in the presence of oxygen (Fig. 1), indicate that most of the discoloration of the stored fish meal must have been associated with oxidation of the lipid. Combination of nitrogenous substances with oxidizing fats is well known as a cause of browning (Davies & Gill, 1936), and some increase in the nitrogen content of the oils extracted from the meals did occur. Discoloration of fish oils, however, can also occur as a result of oxidation, without the participation of nitrogenous substances, perhaps through the production of semi-aldehydes of dicarboxylic acids (Nonaka, 1956).

The fact that reactions between protein and sugar apparently made no major contribution to browning of the meal was probably due to removal of most of the reactive soluble carbohydrate in the aqueous liquor, before the meal was dried.

Possible binding of lipid by the protein. According to the results for oxygen absorption and iodine value most of the oxidation and polymerization of the oil took place relatively early in the storage period; after 4 months further change was relatively slow. Only about 0·5 % lipid, however, became unextractable by fat solvents during this period. On continuing storage to 12 months at 25°, lipid extractable from the drier meal fell to 0·8 % and from the moister meal to 1·5 % below the initial values. Since the extractable lipid of the nitrogen-stored samples increased by 0·3 and 0·6 %, respectively, during this period these amounts should perhaps be added to the losses of the air-stored samples to give the total quantities of oil bound or otherwise rendered insoluble owing to oxidation during storage. The method used does not permit distinction between chemical binding of oxidized oil to the protein and oxidative degradation of the oil to products no longer extractable by fat solvents.

In the experiments of Almquist (1956) initial values of 6–7 % for ether-extractable lipid fell, usually to 3–4 %, during storage for 4 months in sacks, with a relatively slight or no further change to 11 months. Meade (1956) reported that meals protected from overheating during curing by the use of the anti-oxidant DPPD invariably contained 1–2 % more extractable oil than untreated control meal which had been allowed to become overheated. In Tappel's (1955) experiments with model systems approximately 1 g of insoluble copolymer containing 26–84 % of lipid was produced from 8 g of lipid in 1 day at 37°.

The results in Fig. 3 and Table 1 suggest that, whereas oxidation of the lipid probably proceeds further at lower storage temperatures, coupling of the oxidized oil to the

protein (or its destruction) might be greater at the higher temperatures. Loss of extractable oil was, in fact, only detected at the two higher storage temperatures (Table 1), whereas the iodine values changed less rapidly at these temperatures than at 10° (Fig. 3).

Nutritive value of the meal. In previous work (Carpenter *et al.* 1957) losses of available lysine resulting from short periods of heating at 105° were as great in nitrogen as in air. In the present storage experiments, on the other hand, losses were appreciable in air and insignificant in nitrogen: in defatted meal there was no loss, even in air. Together, these results show that inactivation of the lysine was in some way associated with oxidation of the oil.

The most likely mechanisms whereby the nutritive value of the protein of fish meal could be reduced as a result of oxidation of the lipid would be by reaction of aldehydes, peroxides, free radicals or other products of fat oxidation with amino and other reactive groups of the protein side-chains, with destruction of the amino-acids or formation of linkages resistant to enzymic digestion. The chemical determinations of available lysine indicated that some binding of lysine-free amino groups did in fact occur in the stored meals, but that after $2\frac{1}{2}$ months at 25° its extent was still very slight, corresponding to the loss of only about 4 % of the available lysine of the meal: after 12 months at 25° the loss was 9 %. Miller (1956) also has reported that stored fish meals retain their feeding value for at least 3 months.

Methionine is known to be oxidized nearly quantitatively to methionine sulphoxide by hydrogen peroxide at 50–60°, and a similar destruction of methionine, which is often a nutritionally limiting amino-acid in fish meal, in the presence of peroxidizing fat is obviously possible. The second feeding experiment on meal stored for $2\frac{1}{2}$ months at 25°, however, showed no evidence of loss of nutritive value beyond the 4 % loss of available lysine already referred to.

Though destruction both of lysine and of methionine with cystine, therefore, remain possible causes of nutritional damage as a result of oxidation of the oil of stored fish meal, no evidence of a serious occurrence of either has been found during the short period ($2\frac{1}{2}$ months at 25°) of storage of the meal used in the feeding tests, despite extensive oxidation of the oil during this period. The feeding trials were carried out after only $2\frac{1}{2}$ months because chemical examination indicated that at this stage the period of most rapid oxidation and polymerization was already over (Fig. 3): the small residual amounts of the meals stored for 12 months were insufficient for feeding tests. Since chemical analysis at 12 months showed an appreciably larger (9 %) loss of available lysine than at $2\frac{1}{2}$ months (4 %) further nutritional and chemical experiments will be necessary to provide information on these changes and the conditions under which they occur.

It is noteworthy that Meade (1956) found no destruction of lysine, methionine or cystine (assayed after acid hydrolysis) during the 'curing' of dried fish scrap, but did find an appreciably reduced digestibility of the protein of the cured product towards pepsin, which could be prevented by the use of 0·05 % DPPD. Grau & Williams (1955), on the other hand, had previously concluded that low qualities of meals could not be ascribed to heating resulting from oxidation of the oil after drying.

Toxicity of the oil. The destructive effects of autoxidizing lipids on readily oxidizable constituents of food, such as vitamins E, A and C, and on carotenoids and other colouring and flavouring substances is well known. In addition, there is some evidence of toxicity when oxidized and polymerized oils are given at high levels, oils containing major proportions of polyunsaturated fatty acids being more liable to develop objectionable changes of this type on heating or on storage than edible oils and fats of lower unsaturation.

Johnson, Sakuragi & Kummerow (1956) found that the growth-depressing action of thermally oxidized edible oils was related to the decrease in iodine value: maize oil oxidized at 200° until its iodine value had decreased by 16 or 18 units causing severe growth depression, diarrhoea and deterioration in appearance when given to rats at the 20 % level in a synthetic diet.

In the present experiments the oils in the air-stored fish meals showed decreases in iodine value of up to 62 units (45 % of the initial value), as compared, for example, with the average drop of 1 % found in vegetable oils after use for continuous deep-fat frying of potato chips (Melnick, 1957).

On the other hand, Witting, Nishida, Johnson & Kummerow (1957), who give no chemical data, found that the toxicity of oxidized and oxidatively polymerized fish oil produced by blowing air through it for 3 days at room temperature was less than that of oil thermally polymerized without oxidation, in which the molecules are linked mainly through carbon rather than through oxygen. Both oils greatly depressed the growth of rats when given at the 10 % level, but the harmful effect of the oxidized oil could be more easily reduced by increased intake of pyridoxine, riboflavin or protein. This level of feeding, however, is about ten times greater than that at which the oxidized fish oil in the meal was fed to the chicks in the present experiments.

Anti-oxidants. The use of an anti-oxidant such as BHT with citric acid, probably with advantage at a higher level than the 0·005 % used in our test, offers a prospect of inexpensively retarding the oxidation of the oil in the fish meal. Whether this would be entirely advantageous is perhaps doubtful in view of the known adverse effects of highly unsaturated fish oils in the diet of, e.g., pigs (Lea, 1936) in producing soft body fats of enhanced susceptibility to oxidation and to the development of fishy flavours on storage. The thermal polymerization of fish oils to produce oils of lower unsaturation and greater flavour-stability for human consumption, which was practised to some extent after World War 2, has, however, been discontinued, presumably in part at least on nutritional grounds.

A further point which merits consideration is the possibility that other undesirable effects might result from meal containing badly oxidized oil. One aspect, which is being explored, is the possibility that meal in which the oil has oxidized might taint the flesh or eggs more seriously than fresh meal, in a manner analogous to that reported by Callow (1939) in which the yellow colour and fishy flavour of matured hams from pigs receiving cod-liver oil was worse with low-grade than with high-grade oil.

Since this paper was prepared, our attention has been directed to an interesting report by Stansby, Brown, Venolia, Tappel, Olcott & Einsett (1957) on oxidative deterioration in fish and fishery products. Concerned mainly with the oxidation of fish

oils in the tissue, in 'model' emulsion systems, in freeze-dried fish and in the extracted state, and using somewhat different methods of approach, these investigations appear to be largely complementary to those reported here.

SUMMARY

1. Herring meal, prepared by hot-air drying of the press-cake, was stored at two moisture contents (11·1 and 6·2%) and three temperatures (37, 25 and 10°) in air and in nitrogen.

2. Chemical examination showed a very rapid oxidation of the oil of the meals stored in air, particularly during the first few weeks. Oxidation was more extensive at the lower moisture content and at the lower storage temperatures, leading to darkening of the meals and to darkening, reduction in iodine value and solidification (polymerization) of the oils extracted from them. These changes did not occur in whole meals stored in nitrogen or in defatted meals stored in air.

3. Despite extensive oxidation of the oil of the air-stored samples the amount of lipid extractable by solvents decreased by not more than 4% of the initial value during 4 months' storage. After 12 months the low-moisture meal had lost 6–8% and the high-moisture meal 11–15% of its extractable lipid, corresponding approximately to 1 and 2% of the meal.

4. The 'available lysine' in the meal was reduced by only 4% during $2\frac{1}{2}$ months' storage in air at 25°, and feeding tests with chicks failed to indicate any more serious reduction in the value of the meal as a source of supplementary protein. After 12 months the decrease in available lysine was 9%, but no decrease occurred in whole meals stored in nitrogen or in defatted meals stored in air.

5. The anti-oxidant 2, 6-di-*tert.*–butyl-4-methylphenol (BHT) added to the meal at a concentration of 0·005% (together with 0·005% citric acid) considerably retarded oxidation of the oil.

6. The advantages and possible disadvantages of stabilizing the oil of fish meal against oxidation are discussed.

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Riboflavin metabolism of cows and goats and rate of biosynthesis of riboflavin by the lactating goat

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In previous experiments from this laboratory Edwards & Darroch (1956) showed that lactating goats could be kept in positive nitrogen balance while excreting in their milk more lysine than was in their food. They therefore concluded that the goat must have synthesized lysine by the aid of alimentary symbionts (Owen, 1954; Edwards, 1955) and that the amount of lysine synthesized was at least as great as the excess of the lysine output in milk and urine over the dietary lysine.

The diet used in these experiments was not only deficient in lysine, but was also very deficient in the water-soluble vitamins of the B-group. It was therefore a suitable diet for studying the synthesis of these vitamins, and in the experiments about to be described the synthesis of riboflavin by the lactating goat was measured.

Preliminary experiments showed that chemical methods published hitherto for determining riboflavin in urine were not specific. The partition of lyochromes in the urine of cows and goats was therefore studied and a chemical method specific for riboflavin was worked out.

<div align="center">EXPERIMENTAL</div>

Estimation of nitrogen

In the food, milk, urine and faeces nitrogen was estimated by the Kjeldahl method with a mixture of K_2SO_4, $CuSO_4$ and Se as catalyst.

Estimation of riboflavin and its derivatives

Analytical procedure

Riboflavin was estimated fluorimetrically and the fluorimetric results were checked by the microbiological method of Snell & Strong (1939*a*) in the medium of Barton-