Fish. Technol. (1981) 18, 71-77

Oxidation of Residual Lipids in Fish Protein Concentrates and its Effect on the Nutritional Quality of the Protein

K. DEVADASAN
Central Institute of Fisheries Technology, Cochin-682 029

The paper reviews the work reported on the changes in the nutritive value of fish protein concentrates (FPC) during storage, with special emphasis on the effects of the interactions between oxidised residual lipids and proteins of the FPC. Theories on the oxidised lipid-protein interactions are reviewed and the nutritional significance of these reactions is discussed.

Fish protein is fast gaining acceptance as the final answer to the problem of protein malnutrition in developing nations. With the alarming growth of population in many of these countries, the demand for high quality protein is increasing very fast. The hitherto under utilised marine protein resources alone can meet this demand. Developed nations, especially the Scandinavian countries, are supplying fish protein concentrates (FPC) to developing nations for fighting protein malnutrition. This non-defatted fish protein concentrate from lean fishes, supplied for human consumption, is often referred to as FPC type B. In India also inspite of the traditional vegetarian food habits among sizeable sections of the population, fish protein is becoming acceptable. When FPC is intended for human consumption its nutritional quality is to be studied more thoroughly. There are various factors affecting the nutritional quality of FPC, among which oxidation of residual lipids during storage of FPC is perhaps the most important. This paper reviews the studies on this important aspect of fish protein utilization.

Fish meal and FPC change in colour and odour during storage. These changes are more pronounced when the storage temperature is high. In tropical climate, FPC thus develops undesirable colour and odour faster than in temperate climates. These changes are known to be related to the oxidation of the polyunsaturated fatty acids present in the residual lipids of FPC.

Laksesvela & Aga (1965) undertook a detailed study on the effect of prolonged storage of FPC on its quality. The study was conducted with winter herring fish meal stored in paper bags, for 12 years. Studies by Carpenter (1960) indicated a regular reduction in fluorodinitrobenzene (FDNB) available lysine during storage of FPC for 12 years. But Ambrose & Synder (1964) observed that the *invitro* pepsin digestibility of protein is not affected by storage. When used as the sole source of protein in diets for chicks, the protein quality as determined by protein efficiency ratio (PER) did not change in 7 years, after which it showed signs of deterioration. But the same meal when used as a supplement to a cereal diet, did not show any deterioration on storage.

Solvent extracted FPC generally shows lesser reduction in protein quality during storage than FPC samples from which fat is not removed completely.

Oxygen availability and temperature of storage are two important factors affecting the protein quality in stored non-defatted FPC. According to March *et al.* (1961) low temperatures do not necessarily prevent loss in protein quality. However many other studies have indicated that low temperature storage, especially in the absence of oxygen has a favourable effect on protein quality. Lea *et al.* (1958) compared the protein quality of a sample of herring meal stored at 25°C in presence of air, with a sample stored at —20°C under nitrogen atmosphere. With progressive storage, FDNB lysine registered a greater decrease in the sample stored at 25°C in presence of air. But a similar sample when solvent extracted to remove fat prior to storage at 25°C, retained the content of FDNB lysine comparable to the control sample. Lea *et al.* (1960) confirmed these observations in a subsequent study. But an interesting observation made was that though FDNB lysine was more in the sample kept at freezing temperatures under N_2 atmosphere, it did not show a corresponding better protein quality in feeding trials. Decline in quality as judged by chemical indices (FDNB lysine, available methionine, *invitro* pepsin digestibility) and PER was observed by Carpenter *et al.* (1963) in FPC stored for one year at 20°C with access to air. *Miller et al.* (1970) studied the effect of storage temperature on the quality of FPC by feeding trials, using chicks. During a two year period of storage, the decline in growth promoting quality was similar for two samples of menhaden meal, one stored at —20°C and the other at 25°C. They observed no change in FDNB lysine content during storage but pepsin digestibility showed a gradual decrease.

The influence of the species of fish used for making FPC on the reduction in quality during storage was studied by March *et al.* (1966). In a comparison between herring and menhaden meals they did not observe any significant difference between the FPC samples. In their experiments on chick growth with meals, all samples showed a decline in quality in the first 6–11 months, after which the changes were not significant.

The effect of lipid oxidation during storage of FPC, on its protein quality, has been the subject of many investigations in different laboratories.

Njaa *et al.* (1966) made detailed studies using fish meal prepared from different species of herring. The samples were stored with and without addition of butylated hydroxy toluene (BHT) and the effect of lipid oxidation on the nutritive value was studied by feeding trials on rats. The values were compared with those for freshly prepared fish meals. These studies could not demonstrate a significant reduction in protein quality induced by fat oxidation. However the reduction in protein quality due to lipid oxidation was apparent in meals prepared from certain species like the North Sea herring, though in Scando-Atlantic herring meals, this effect was not striking. The fact that BHT added meals also showed some reduction in protein quality suggests that antioxidants cannot arrest these deteriorative changes completely.

Opstvedt *et al.* (1970) and Opstvedt (1975) have reported the effect of lipid oxidation on the protein quality of fish meal. In these studies ethoxyquin is used (1, 2 dihydro-6-ethoxy, 2, 2, 4 trimethylquinoline) is used as antioxidant to prevent lipid oxidation. Commercially prepared fish meal samples from North Sea herring and mackerel were stored with and without addition of ethoxyquin for a period of 5 years. The changes in protein quality as measured by PER, NPU (net protein utilization) and available methionine and lysine, showed that the antioxidant added sample was 15% better than the controls. The deteriorative changes in the samples were apparent during the first year of storage after which the decrease in quality was negligible. Studies conducted by El-Lakany & March (1974) in Canada on the effect of lipid oxidation on protein quality of fish meal have also lead to some interesting conclusions. They used herring meal for the study which was stored at 20°C with and without addition of ethoxyquin. One sample was stored at —20°C without addition of antioxidant also. After 40 weeks of storage, the samples were compared to evaluate their protein quality. In FDNB reactive lysine, *invitro* pepsin digestibility and the ability to support growth in chicks, there was no significant difference between the sample stored at–20°C without antioxidant and that stored at 20°C with antioxidant. However the samples stored at 20°C without antioxidant was inferior in quality. Studies by March *et al.* (1965) showed that meals containing oxidised fat supported 6–9% lower growth in chicks compared to unoxidised meals, when fed as a supplement to a cereal diet, though the two samples were comparable in their FDNB reactive lysine.

South African workers have reported results of similar studies on pilchard meal. A comparison of pilchard meals stored with and without addition of ethoxyquin did not show any consistant effect of storage time on the protein quality. However the antioxidant added meal was superior in its FDNB reactive lysine, available methionine, protein digestibility as well as NPU when compared to the control sample (Dreosti *et al.* 1969). Wessels (1971) and Wessels & Moodie (1975) found inconsistant and variable differences between antioxidant treated and untreated South African anchovy meals after storage. They tested the samples by feeding chicks. Almquist (1956) did not observe any reduction in pepsin digestibility in anchovy meal after 4 months storage. But storage for another 7 months caused a 2% reduction.

In India Moorjani *et al.* (1965) studied the changes in protein quality of air dried anchovy meal, with normal fat content, during storage at 28–33°C for 2 months. They have reported a 15% reduction in FDNB reactive lysine in these samples compared to controls from which fat was removed by solvent extraction prior to storage. There was no significant difference in the PER values of the samples when tested on rats. But the fat containing sample was found to be inferior in its pepsin digestibility and available essential amino acids.

Geisler & Contreras (1967) investigated the effect of increased fat in a fish meal sample on its protein quality during storage. They added increasing amounts of anchovy oil (0–15%) to solvent extracted anchovy meal and kept in petridishes for 5 months. With increasing oil content, there was a corresponding decrease in protein digestibility and FDNB reactive lysine. These changes were more pronounced during the first 15 days after which further changes were less apparent.

Thus it is seen that during storage of non-defatted FPC, there is a noticeable loss in its nutritional quality. This deterioration can be retarded by the addition of an antioxidant and by keeping the FPC in an inert atmosphere. Removal of fat by solvent extraction also retards the deteriorative changes. These observations suggest that the loss in nutritional quality of the protein is related to the oxidation of residual lipids in the FPC and the interaction of the protein with oxidised lipids.

A review of the studies and theories on the reaction of protein and oxidised lipids is therefore desirable. Several reviews covering different aspects of these interactions are available (Kaunitz, 1967; Schauenstein, 1967; Tappel, 1973; Karel *et. al.*, 1975; Gardner, 1979).

For studying the protein-oxidised lipids reactions, Tappel (1955) made use of a model system in which emulsions of linoleic acid or cod liver oil were oxidised at 37°C for 24 h in an aqueous suspension of casein and other proteins. The studies suggested a denaturation of proteins by oxidised lipids. Both the protein and lipid fractions were found to lose their characteristic chemical properties. Tappel (1955) therefore suggested the formation of co-polymers between proteins and the oxidation products from lipids. He postulated covalent linkages between the carbonyls from oxidised lipids and the amino groups of proteins, similar to those formed during Mailard reaction between proteins and reducing sugars. Later studies (Venolia & Tappel, 1958) could not however confirm this. But chemical bonds between oxidized lipids and protein were observed by Desai & Tappel (1963). They found that peroxy bonds are the main bonds formed during this reactions.

Narayan & Kumerow (1958) reported results of similar studies. Narayan *et al.* (1964) postulated the formation of a net work of hydrogen bonds holding the oxidised lipids between parallel peptide chains of proteins, yielding an extended beta keratin configuration. Cumulatively the weak hydrogen bonds can give stability to the lipids protein complex. This theory assumes that the ketonic and hydroxy groups are the main reactive groups involved in protein-oxidised-lipid interactions and that the functional amino and sulphydryl groups are not involved in linkages.

Kwon *et al.* (1965) and Crawford *et al.* (1967) observed that proteins interact with aldehydes, products of oxidative degradation of lipids, forming enamine bonds. The bifunctional malonaldehyde can cross link protein chains via schiff base formation. This aldehyde has therefore received special attention in such studies. Chio & Tappel

(1969) studied reactions of malonaldehyde and protein and suggested intra and inter molecular cross binding. Reactions of malonaldehyde and amino acids have been studied by Buttkus (1969) also.

Raubal & Tappel (1966) postulated that protein polymers can be formed without direct participation of lipids and that the lipid fraction only initiates the polymerisation reactions. Lipid fractions of the protein lipid co-polymers could either be embedded in the protein polymer or connected to the protein polymer by non-covalent linkages. The reactions according to this theory, could be represented as

$$LO + PH \longrightarrow LOH + P'$$

(Lipid radical) Protein (Protein radical)

$$P' + P \quad P - P'$$

$$P - P' + P' \longrightarrow P - P - P'$$

These authors suggested formation of disulphide bonds in proteins forming such polymers. But Zirlin & Karel (1969) have suggested that reactions between oxidized lipids and proteins may lead to breakage of the protein polypeptide chain, especially at low water activity.

Thus it can be seen that various types of reactions are possible in the interaction between oxidized lipids and proteins. They can be classified into four general types.

i) Protein polymers without any lipids

ii) Polymers of proteins and oxidized lipids

iii) Co-valently linked polymers of oxidized lipids and proteins

iv) Polymers of oxidized lipids and broken peptide chains

The relative preponderance of each of these in the product may depend on various factors like reaction conditions, nature of the lipid and protein. Of these, the polymers held together by physical bonds require water for their stability and so cannot be expected to be present in systems like FPC type B which has a low moisture content.

It is known that polymers held by co-valent linkages are favoured by acidic conditions, whereas alkaline conditions favour formation of physical bonds. Temperature also has an effect on this reaction rate as shown by Venolia & Tappel (1958) and Kwon *et al.* (1965). They showed that an increase in temperature brings about a corresponding increase in the reaction rate between oxidised lipids and proteins. However these reactions could proceed even at a very low temperature (Braddock & Dugan, 1973). Lea *et al.* (1960) also observed an increase in reaction rate with temperature though this effect was less pronounced beyond 37°C. Yanagita *et al.* (1973) have also supported this view. But Buttkus (1967) found some other physical conditions, besides temperature, also equally important. He showed that the reaction between protein and malonaldehyde was almost the same at —20°C and 20°C even though this was considerably higher than that at 0°C. He attributes this effect of freezing to a concentration of reactants and also to a catalytic action of ice crystals. It is reasonable to think that temperature affects the rate and not the type of reaction.

The effect of the lipid on the interaction between proteins and oxidised lipids was studied by Yanagita *et al.* (1973) and Giesler and Contreras (1967). They observed that increased lipid in the protein, increased the interactions though this is disputed by some workers.

These studies were all with dry proteins. In aqueous systems, the rate of reaction between oxidised lipids and proteins varied depending on the nature of the protein as shown by Venolia & Tappel (1958) and Raubal & Tappel (1966).

The picture of the interactions between auto oxidising lipids and proteins is thus a complex one. It is generally believed that the lower aldehydes formed are the most reactive compounds in such a system. The low flavour threshold values of lower aldehydes also makes them very important compounds in these studies (Sessa & Rackis, 1977; Eriksson *et al.* 1976). Hydroperoxides are more reactive with proteins than ketones. In the protein part, the active site can be the free amino group of lysine or guanidyl group of arginine. The free carboyl group of acidic amino acids also may react with hydroperoxides forming ester bonds. —SH group of crysteine, S—S—group of cystine and sulphide group of methionine are also reactive.

Relatively few studies have been reported on the effect of the interactions between oxidised lipids and proteins on the nutritional availability of proteins. Most of the reported results are based on *inviro* studies. Desai & Tappel (1963) studied the effect of these interactions on the rate of hydrolysis of the protein by dilute hydrochloric acid. The native protein was hydrolysed more easily, compared to the protein-oxidized lipid complex. The rate of hydrolysis showed maximum reduction with histidine, serine, proline, argenine and the sulphur amino acid residues.

Raubal & Tappel (1966) indicated that the deciding factor was the nature of the protein. Buttkus (1967) reported the temperature dependance of the reaction. At 100°C the amino acids mostly affected were histidine, arginine, tyrosine and methionine in that order, whereas at frozen temperatures the losses were maximum for lysine, tyrosine, methionine and arginine in that order. Braddock & Dugan (1973) observed considerable loss in total histidine, lysine and methionine when linoleate was oxidised in a solution of myosin at 50°C. Studying the reaction between malonaldehyde and albumin in an aqueous medium. Crawford *et al.* (1967) found out that FDNB reactive lysine registered a regular decrease with increasing malonaldehyde concentration. Pepsin digestibility also decreased when oxidised lipids reacted with proteins (Yanagita *et al.*, 1973; El. Lakany & March, 1974).

In *invivo* tests, it is generally seen that proteins after reaction with oxidised lipids, take longer time to leave the stomach, that too in a comparatively lesser degraded state. But in PER and in intestinal digestion no adverse effect is observed. Heating of protein in presence of oxidised lipids is found to reduce protein digestibility in general. All amino acids are generally affected by this, but the effect is more pronounced in lysine and methionine. Oxidative changes in methionine is particularly easy. Methionine is oxidised to its sulphoxide, which further gets oxidised to methionine sulphone. But this takes place in acidic conditions only (Njaa, 1962) and so in natural foods this does not easily happen (Cuq *et al.* 1973; Slump & Schreuder, 1973). Cystein, the other sulphur containing amino acid is oxidized to cysteic acid when casein is heated with oxidized lipids (Tannenbaum *et al.* 1969). But this reaction is not very fast at room temperature. Tannenbaum *et al.* (1969) has correlated the oxidation of methionine to the polymerization of protein. Many authors maintain, that methionine sulphone and cysteiec acid cannot serve as sources for the parent amino acids in diets. (Njaa, 1962; Miller and Samuel, 1970). However methionine sulphoxide is reported to have the same biological activity as DL-methionine, though D-methionine sulphoxide is found to have reduced biological activity (Njaa, 1962, Slump & Schreuder, 1973). But Miller & Samuel (1970) and Ellinger & Palmer (1969) report results contrary to this. According to them DL-methionine sulphoxide has only 25% biological activity of DL-methionine. Cuq *et al.* (1973) and Kuzmicky *et al.* (1974) support this view. These differences may be due to different factors. Rats may adapt to utilize methionine sulphoxide or utilization of methionine sulphoxide may improve with age. Again the free methionine sulphoxide and methionine sulphoxide in a protein may not be utilized to the same extent. Recent reports also suggest that presence of oxidized sulphuramino acids in the protein chains makes it resistant to hydrolysis, reducing the utilization of all amino acids (Pieniazek *et al.* 1975). The studies in general have one thing in common. While protein digestibility as such may fall only marginally due to reactions between oxidised lipids and proteins, the loss in availability of individual amino acids is much greater. This is an aspect which has not been satisfactorily explained so far. It can be said that, lysine and the sulphur containing amino acids are affected to a greater extent by these interactions. Histidine and tysosine are also among the amino acids easily damaged by oxidised lipids.

The oxidised lipid-protein interactions is thus a complex problem, which calls for much more detailed study to arrive at clear and definite conclusions.

References

Almquist, H. J. (1956) *Agric. Fd Chem.* **4**, 638

Ambrose, M. & Synder, D. G. (1964) *Fishg, News int.* 3, 210

Braddock, R. J. & Dugan, L. R. Jr. (1973) *J. Am. Oil Chem. Soc.* **50**, 343

Buttkus, H. (1967) *J. Fd Sci.* **32**, 432

Buttkus, H.(1969) *J.Am. Oil Chem. Soc.***46**,88

Carpenter, K. J. (1960) *Biochem. J.* **77**, 604

Carpenter, K. J., Lea, C. H. & Parr, L. J. (1963) *Br. J. Nutr.* **17**, 151

Chio, K. S. & Tappel, A. L. (1969) *Biochem.* **8**, 2827

Crawford, D. L., Yu, T. C. & Sinnhuber, R. O. (1967) *J. Fd Sci.* **32**, 332

Cuq, J. L., Provansal, M., Gillux, F. & Cheftel, C. (1973) *J. Fd Sci.* **38**, 11

Desai, I. D. & Tappel, A. L. (1963) *J. Lipid Res.* **4**, 204

Dreosti, G. M., Merve, R. P. & Post, B. J. (1969) *Fishing Industry's Research Institute (FIRI South Africa) Annual Report* p. 40

El-Lakany, S. & March. B. E. (1974) *J. Sci. Fd Agric.* **25**, 899

Ellinger, G. & Palmer, R. (1969) *Proc. Nutr. Soc.* **28**, 42 A

Eriksson, C. E., Lundgren, B. Vallentin, K. (1976) *Chem. Senses Flavour.* **2**, 3

Gardner, H. W. J. (1979) *J. Agric. Fd Chem.* **27** (2), 220

Geisler, H. W. & Contreras, E. (1967) *Fishg. News int.* **6**, 38

Karel, M., Schaich, K. & Roy, R. B. (1975) *J. Agric. Fd Chem.* **23**, 159

Kwon, T. W., Menzel, D. B. & Olcott, H. S. (1965) *J. Fd Sci.* **30**, 808

Kuzmicky, D. D., Kohler, G. V., Walker, H. G. Jr. & Mackey, B. E. (1974) *Poult. Sci.* **53**, 45

Kaunitz, B. H. (1967) *Fd Technol.* **21**, 278

Laksesvela, B. & Aga, A. T. (1965) *J. Sci. Fd Agric.* **16**, 743

Lea, C. H., Parr, L. J. & Carpenter, K. J. (1958) *Br. J. Nutr.* **12**, 297

Lea C. H., Parr, L. J. & Carpenter, K. J. (1960) *Br. J. Nutri.* **14**, 91

March, B. E., Biely, J., Goudie, C., Claggott, F. & Tarr H. L. A. (1961) *J. Am. Oil Chem. Soc.* **38**, 80

March, B. E., Biely, J., Tarr, H. L. A. & Claggott, F. (1965) *Poult. Sci.* **44**, 679

March, B. E., Biely, J. Goudie, C. & Tarr, H. L. A. (1966) *J. Fish. Res. Bd Can.* **23**, 395

Miller, D., Kifer, R. R. & Ambrose, M. E. (1970) *Poult. Sci.* **49**, 1005

Miller, D. & Samuel, P. D. (1970) *J. Sci. Fd Agric.* **21**, 616

Moorjani, M. N., Lahiri, N. L., Balakrishnan Nair, R., Upadhye, A. N. & Venkat Rao, S. (1965) *Fd Technol.* **19**, 110

Narayan, K. A. & Kumerow, F. A. (1958) *J. Am. Oil Chem. Soc.* **35**, 52

Narayan, K. A., Sugai, M. & Kumerow, F. A. (1964) *J. Am. Oil. Chem. Soc.* **41**, 254

Njaa, L. R. (1962) *Br. J. Nutr.* **16**, 571

Njaa, L. R., Utne, F. & Braekkan, O. R. (1966) *Fiskeri, dir. Skr. Ser. Technol. Unders.* **5**, 1

Opstvedt, J. Olsen, S. & Urdahl, N. (1970) *Acta. Agric. Scand.* **20**, 175

Opstvedt, J. (1975) *Acta Agric. Scand.* **25**, 53

Pienizek, D. Radowska, M. & Kunachowicz, H. (1975) *Br. J. Nutr.* **34**, 163

Raubal, W. T., Tappel, A. L. (1966) *Arch. Biochem. Biopnhys.* 113

Schauenstein, E. (1967) *J. Lipid Res.* **8**, 417

Sessa, D. J., Rackis, J. J. (1977) *J. Am. Oil Chem. Soc.* **54**, 468

Slump, P., Schreuder, H. A. W. (1973) *J. Sci. Fd Agric.* **24**, 657

Tannenbaum, S. R., Barth, H. & Rowx, J. P. L. (1969) *J. Agric. Fd Chem.* **17**, 1353

Tappel, A. L. (1955) *Arch. Biochem. Biophys.* **54**, 266

Tappel, A. L. (1973) *Proc. Fed. Am. Soc. Exp. Biol.* **32**, 1870

Venolia, A. W. & Tappel, A. L. (1958) *J. Am. Oil Chem. Soc.* **35**, 135

Wessels, J. P. H. & Moodie, T. (1975) *J. Sci. Fd Agric.* **26**, 625

Wessels, J. P. H. (1971) *Agronomalia.* **3**, 125

Yanagita, T., Sugano, M., Cho, S. & Wada, M. (1973) *J. Agric. Chem. Soc. Japan* **47**, 99

Zirlin, A. & Karel, M. (1969) *J. Food Sci.* **34**, 160