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# Effect of Haemoglobin and Ferritin on Lipid Oxidation in Raw and Cooked Muscle Systems

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*ABSTRACT*

*The effect of haemoglobin (Hb) concentration and ferritin on lipid oxidation in raw and heated water-washed muscle systems was investigated. Lipid oxidation catalyzed by Hb increased with increasing concentration of Hb up to ~3 mg/g in raw muscle systems and thereafter remained constant as the level of Hb was increased to 10 mg/g. In heated systems, the pro-oxidative effect increased as the concentration of Hb increased to 10 mg/g. In raw ferritin systems, oxidation was not observed. Cooked systems, however, showed significant lipid oxidation. Ascorbic acid exerted a pro-oxidant effect in the ferritin system, while nitrite (100 mg/kg) inhibited both Hb- and ferritin-induced oxidation.*

## INTRODUCTION

Earlier investigations in this laboratory (Tichivangana & Morrissey, 1984, 1985) indicate that myoglobin, haemoglobin and inorganic ions function as pro-oxidants in both raw and cooked muscle systems. However, non-haem iron is considered to be the major pro-oxidant. The ability of haem pigments to act as catalysts of lipid oxidation in muscle systems is still not certain. Tappel (1962), Watts (1962), Wills (1966) and Liu (1970*a*,*b*) conclude that haemoglobin, myoglobin and the cytochromes are very active catalysts of

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lipid oxidation. On the other hand, Sato & Hegarty (1971) and Love & Pearson (1974) presented data suggesting that haem iron *per se* had no catalytic effect. The recent findings of Tichivangana & Morrissey (1984, 1985) suggest that haem pigments exert very little catalytic activity in raw muscle systems, but heated muscle systems were very susceptible to lipid oxidation.

The activity of haem iron depends very much on the concentration of catalyst used. It has been reported that when haem is present at high concentrations (low ratio of lipid to haem) it behaves as an anti-oxidant (Kendrick & Watts, 1969; Hirano & Olcott, 1971). The mechanism of this effect has not been elucidated. The former workers reported optimum linoleate-to-haem ratios of 100 for haem, 250 for metmyoglobin and 500 for methaemoglobin. At haem concentrations of two to four times the optimum catalytic amount, complete inhibition occurred.

It is unlikely that much free iron is present in muscle. Undoubtedly, the major portion of body iron is present as haem complexes in haemoglobin, myoglobin and several haem-containing enzymes. There are also several iron-containing non-haem enzymes present in tissue, such as succinic dehydrogenase and xanthine oxidase. Varying amounts of iron–protein complexes which function in the storage and transport of non-haem iron are also present. These complexes include an insoluble (haemosiderin) fraction and soluble ferritin (Hazell, 1982; Bogunjoko *et al.*, 1983). Tichivangana & Morrissey (1985) speculate that iron may be released from ferritin and haemosiderin on heating, thereby rendering muscle systems more susceptible to oxidation than the unheated control.

The objective of the present study was to determine the effect of haem concentration on the oxidative stability of raw and cooked fish muscle systems. The effect of heat, ascorbic acid and nitrite on the possible catalytic activity of ferritin is also considered.

## MATERIALS AND METHODS

### Fish supply

Freshly caught mackerel were purchased from the local fish market.

### Preparation of water-washed muscle fibres (WF)

Muscle was minced and extracted with deionised water as described by Tichivangana & Morrissey (1984).

### Preparation and treatment of model systems

A sample (~100 g) of the WF preparation was mixed thoroughly with the appropriate pro-oxidant.

### Reagents

Horse heart haemoglobin and horse spleen ferritin were purchased from Sigma Chemical Co. Ltd., Poole, Dorset, Great Britain. All other chemicals were 'AnalaR' grade purchased from British Drug Houses, Poole, Dorset, Great Britain.

### Method of cooking

Samples were sealed in 30 × 18 cm retortable bags (Seaward Laboratories, London) and then placed in a hot water bath to cook the samples to an internal temperature of 70°C for 30 min. Following cooking, the samples were cooled and stored at 4°C for 48 h prior to assessing lipid oxidation.

### Assessment of lipid oxidation

Lipid oxidation was assessed by the 2-thiobarbituric acid (TBA) method of Ke *et al.* (1977). Sulfanilamide was added to all samples containing nitrite to eliminate residual nitrite, by formation of a diazonium compound, which interferes with colour development in the TBA test (Zipser & Watts, 1962). The TBA values were expressed as milligrams of malonaldehyde per kilogram of tissue.

## RESULTS AND DISCUSSION

### Effect of haem concentration

Figure 1 shows the effect of haem concentration on the oxidative stability of raw and cooked WF systems after 48 h storage at 4°C. Haemoglobin exerted increasing catalytic activity up to a concentration of ~3 mg/g in the raw WF system. Increasing the concentration of haemoglobin beyond this level resulted in little further increase in TBA values. In fact, a slight but insignificant reduction in TBA value was observed at haem concentrations of 8 and 10 mg/g. This finding does suggest that there is an optimum ratio of haem to lipid for maximum oxidation. It is, however, likely that the ratio of haem pigments to unsaturated fatty acids in most raw meat systems should

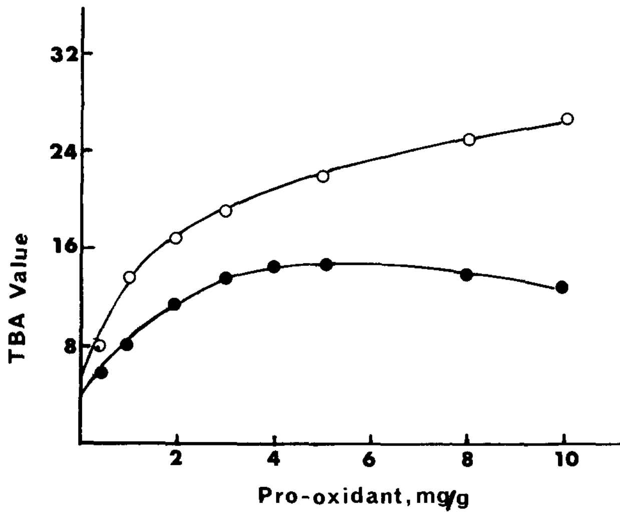


Fig. 1. Effect of haemoglobin on lipid oxidation in raw (○) and heated (●) water-extracted muscle fibres (WF) from fish stored at 4°C for 48 h.

favour catalysis rather than an anti-oxidant effect. The heated haemoglobin systems were very susceptible to lipid oxidation. These findings are similar to those previously reported for cooked fish/myoglobin systems stored for 48 h (Tichivangana & Morrissey, 1984). Increasing levels of haemoglobin, up to 10 mg/g, resulted in progressive increase in TBA values. Thus, when high levels of haemoglobin were used, differences in behaviour were observed for the raw and heated systems. An explanation for these differences may not be difficult to find. Undoubtedly, heating alters the structure of the muscle system through denaturation of lipoproteins in the microsomes and mitochondria and presumably renders the constituent fatty acids accessible for oxidation and changes the ratio of haem to lipid. However, it is far more likely that the differences in behaviour between the raw and heated systems may be associated with changes in the nature and concentration of the catalysts present. It is well recognised that denaturation of haem pigments by heat leads to a release of free iron (Igene *et al.*, 1979; Morrissey & Tichivangana, 1985) and increased catalytic activity. Free iron is highly effective as a pro-oxidant even at levels of ~1 mg/kg (Tichivangana & Morrissey, 1984). Therefore, changes in the nature and concentration of catalyst may account for the differences between the two systems.

## Effects of ferritin on lipid oxidation

Ferritin was added to water extracted muscle systems at a concentration of 2 mg/g. The level employed was similar to that reported by Hazell (1982) and

**TABLE 1**
Influence of Ferritin and Haemoglobin on TBA Values of Raw and Heated, Water-Extracted Fish Muscle (WF) Stored at 4°C for 48 h

| *System* | *Mean TBA value*[a] | |
|---|---|---|
| | *Raw* | *Heated* |
| WF alone | 2·4 | 3·5 |
| WF + 2 mg/g ferritin | 3·1 | 7·3 |
| WF + 3 mg/g haemoglobin | 9·4 | 16·4 |

[a] Mean TBA value of four replicates performed in duplicate.

Bogunjoko *et al.* (1983) for muscle systems. The data in Table 1 show that ferritin exerted very little catalytic activity in raw systems compared to the WF control. This finding is in agreement with that of Wills (1966). In contrast to the raw systems, the heated ferritin systems were very susceptible to lipid oxidation. Haemoglobin (3 mg/g) exerted a significant pro-oxidant effect in raw muscle which is in line with earlier studies (Tichivangana & Morrissey, 1985) and Fig. 1. However, it is also much more effective in heated systems due to the release of free iron (Morrissey & Tichivangana, 1985). The results clearly indicate that intact ferritin is not a catalyst of lipid oxidation in meat. However, cooking probably denatures the ferritin molecule, as it does for myoglobin and haemoglobin, releasing $Fe^{2+}$ which then catalyzes lipid oxidation.

Although pure ferritin is ineffective as a catalyst, the iron can also be released by low levels of ascorbic acid (Wills, 1966) to become an effective catalyst for lipid oxidation. The data in Table 2 demonstrate this effect in raw muscle systems and also show that the effect of ascorbic acid at the $10^{-2}$ M level is similar to that of heat on the catalytic activity of ferritin.

**TABLE 2**
Influence of Ascorbic Acid (AA) on TBA Values of Raw and Heated, Water-Washed Fish Muscle Fibres (WF) Catalyzed by Ferritin, Stored at 4°C for 48 h

| *System* | *TBA values*[a] | |
|---|---|---|
| | *Raw* | *Heated* |
| WF alone | 2·3 | 3·5 |
| WF + 2 mg/g ferritin | 3·2 | 7·2 |
| WF + 2 mg/g ferritin + $10^{-3}$ M AA | 5·2 | 8·6 |
| WF + 2 mg/g ferritin + $10^{-2}$ M AA | 13·7 | 14·1 |

[a] Mean TBA values of four replicates performed in duplicate.

Thus, we propose that low levels of free iron are also released from ferritin in muscle during cooking and contribute to the overall susceptibility of the system to oxidation. Ascorbic acid at low levels ($10^{-2}$ M) has been shown to catalyze lipid oxidation in meat systems (Tims & Watts, 1958; Sato & Hegarty, 1971). We suggest that part of its effect in meat systems may be due to release of iron from ferritin and perhaps also from myoglobin and residual haemoglobin present in the muscle.

## Effect of nitrite on oxidation catalyzed by ferritin

Nitrite has potent antioxidative effects and eliminates the problem of 'warmed-over' flavour in cooked cured meats (Fooladi *et al.*, 1979) and has anti-oxidative properties in heated WF systems catalyzed by myoglobin and free iron (Morrissey & Tichivangana, 1985).

This phase of the study was designed to investigate the effect of $NaNO_2$ on heated WF systems containing ferritin. The effect of haemoglobin was included for comparative purposes. The data in Table 3 clearly demonstrate that nitrite treatment inhibits lipid oxidation in all heated systems.

The significance of the present findings is two-fold. First, free iron (Tichivangana & Morrissey, 1985), myoglobin and haemoglobin (Tichivangana & Morrissey, 1984) and ferritin (present results) are catalysts for lipid oxidation in heated muscle systems. Secondly, nitrite is now known to inhibit lipid oxidation catalyzed by four of the main components of muscle–ferritin, haemoglobin, myoglobin and free iron. Morrissey & Tichivangana (1985) suggested that nitrite functioned as an antioxidant (i) by the formation of nitric oxide haem complex which had antioxidant properties *per se*, (ii) by the formation of a stable, nitrosylhaemochrome, which blocks the catalytic activity of haem iron and also prevents release of

**TABLE 3**
Effect of Sodium Nitrite on Lipid Oxidation Catalyzed by 2 mg/g Ferritin or 3 mg/g Haemoglobin in Heated Water-Washed Muscle Fibres (WF), Stored at 4°C for 48 h

| *System* | *TBA values*[a] | |
|---|---|---|
| | *No nitrite* | *(100 mg/kg) nitrite* |
| WF alone | 3·6 | 1·7 |
| WF + ferritin | 7·4 | 3·2 |
| WF + haemoglobin | 14·8 | 5·9 |

[a] Mean TBA values of four replicates performed in duplicate.

haem iron as non-haem iron which is a highly effective catalyst and (iii) nitrite appears to 'chelate' non-haem iron, forming a stable, inactive complex. One can only speculate about the function of nitrite where ferritin is the catalyst in heated systems. However, since iron appears to be released from ferritin by heat, it is probable that nitrite may 'chelate' the free iron rather than form a stable complex with the intact ferritin molecule. However, nitrite can also react with other constituents of muscle, such as non-haem proteins, low molecular weight peptides and amino acids (Lin *et al.*, 1974; Lin & Olcott, 1975; Kanner, 1979; Kanner *et al.*, 1984) to form catalytically inactive species. These nitroso-compounds may be important in controlling lipid oxidation in meat systems.

## REFERENCES

Bogunjoko, F. E., Neale, R. J. & Ledford, D. A. (1983). Availability of iron from chicken meat and liver given to rats. *Br. J. Nutr.*, **50**, 511–20.

Fooladi, M. H., Pearson, A. M., Coleman, T. H. & Markel, R. A. (1979). The role of nitrite in preventing development of warmed-over flavour. *Food Chem.*, **4**, 283–92.

Hazell, T. (1982). Iron and zinc compounds in the muscle of meats, of beef, lamb, pork and chicken. *J. Sci. Food Agric.*, **33**, 1049–56.

Hirano, Y. & Olcott, H. S. (1971). Effect of heme compounds on lipid oxidation. *J. Am. Chem. Soc.*, **48**, 523–4.

Igene, J. O., King, J. A., Pearson, A. M. & Gray, J. I. (1979). Influence of heme pigments, nitrite and non-heme iron on development of warmed-over flavour (WOF) in cooked meat. *J. Agric. Food Chem.*, **27**, 838–42.

Kanner, J. (1979). *S*-nitrosocysteine (RSNO) an effective anti-oxidant in cured meats. *J. Am. Oil Chem. Soc.*, **56**, 75–6.

Kanner, J., Harel, S., Shagalovich, J. & Berman, S. (1984). Anti-oxidative effect of nitrite in cured meat products: Nitric oxide–iron complexes of low molecular weight. *J. Agric. Food Chem.*, **32**, 512–15.

Ke, P. J., Ackman, R. G., Linke, B. A. & Nash, D. M. (1977). Differential lipid oxidation in various parts of frozen mackerel. *J. Food Technol.*, **12**, 37–47.

Kendrick, J. & Watts, B. M. (1969). Acceleration and inhibition of lipid oxidation by heme compounds. *Lipids*, **4**, 434–58.

Lin, J. S. & Olcott, H. S. (1975). Ethoxyquin nitroxide. *J. Agric. Food Chem.*, **23**, 798–800.

Lin, J. S., Smith, V. & Olcott, H. S. (1974). Loss of free-radical signal during induction period of unsaturated lipid containing nitroxide anti-oxidants. *J. Agric. Food Chem.*, **22**, 682–3.

Love, J. D. & Pearson, A. M. (1974). Metmyoglobin and non-haem iron as prooxidants in cooked meat. *J. Agric. Food Chem.*, **22**, 1032–4.

Liu, H. (1970*a*). Catalysts of lipid peroxidation in meats. 1. Linoleate peroxidation catalysed by metMb or Fe(11)-EDTA. *J. Food Sci.*, **35**, 590–2.

Liu, H. (1970*b*). Catalysts of lipid peroxidation in meats. 2. Linoleate oxidation catalysed by tissue homogenates. *J. Food Sci.*, **35**, 593–5.

Morrissey, P. A. & Tichivangana, J. Z. (1985). The antioxidant activities of nitrite and nitrosylmyoglobin in cured meats. *Meat Sci.*, **14**, 175–90.

Tappel, A. L. (1962). Hematin compounds and lipoxidase as biocatalysts. In: *Symposium on food: Lipids and their oxidation.* (W. W. Schultz, E. A. Day & R. O. Sinnhuber (Eds)), AVI Publ. Co., Westport, Conn., USA, 122–38.

Tichivangana, J. Z. & Morrissey, P. A. (1984). Factors influencing lipid oxidation in heated fish muscle systems. *Ir. J. Food Sci. & Technol.*, **8**, 45–57.

Tichivangana, J. Z. & Morrissey, P. A. (1985). Metmyoglobin and inorganic metals as prooxidants in raw and cooked muscle systems. *Meat Science*, **15**, 107–16.

Tims, M. J. & Watts, B. M. (1958). Protection of cooked meats with phosphates. *Food Technol.*, **12**, 240–3.

Sato, K. & Hegarty, G. R. (1971). Warmed-over flavour in cooked meats. *J. Food Sci.*, **36**, 1098–102.

Watts, B. M. (1962). Meat products. In: *Symposium on foods: Lipids and their oxidation.* (H. W. Schultz, E. A. Dayu and R. O. Sinnhuber (Eds)), AVI Publ. Co., Westport, Conn, USA, 202–14.

Wills, E. D. (1966). Mechanism of lipid peroxide formation in animal tissue. *J. Biochem.*, **99**, 667–76.

Zipser, M. W. & Watts, B. M. (1962). A modified 2-thiobarbituric acid (TBA) method for determination of malonaldehyde in cured meats. *Food Technol.*, **16**(7), 102–4.