OXYGEN UPTAKE DURING INITIAL LIPID OXIDATION OF MEAT FIBRES

PRADO-BARRAGAN L.A. and TAYLOR A.J.

Department of Applied Biochemistry & Food Science, University of Nottingham, Loughborough, England

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SUMMARY

Measuring lipid oxidation in whole meat is difficult due to the chemical variability within meat and the problems encountered in lipid oxidation analysis. The use of meat fibre systems for the study of lipid oxidation in meat offers the possibility of an homogeneous system that allows manipulation of physical factors such as temperature, pH and water activity as well as chemical factors such as the presence or absence of pro- and anti- oxidants.

Oxygen uptake during lipid oxidation of meat fibres at Aw 0.96 (fresh meat) and Aw 0.70 (roasted meat) was determined at 4, 25 and 50 °C. At 4 °C, no notable oxidation was observed over a 40 h period whereas at 25 °C and 50 °C, oxidation was observed within 22 and 6 h respectively. Traces showing typical lipid oxidation activity (lag phase followed by oxidation) were obtained from fibres at 25°C. The rate of lipid oxidation was faster at Aw 0.96 than at 0.70 for all temperatures.

Introduction

As a result of lipid oxidation, changes in flavour, colour, texture and nutritive value are bound to occur. Lipid oxidation in meat has become even more important with the increasing marketing of restructured and precooked meat products as well as the utilization of deboned muscle tissues. The basic chemistry of lipid oxidation reactions has been researched and some of the factors that affect the course of the reaction (pH, temperature, catalysts, etc.) have been recognised. Meat model systems using lipid emulsions (Liu & Watts, 1970; Lee *et al.*, 1975; Fisher & Deng, 1977), and microsomal fractions (Rhee & Ziprin, 1987; Kanner *et al.*, 1988) have been used in an attempt to mimic lipid peroxidation in muscle. These models have been used for the study of the general problems of lipid peroxidation but not for simulating lipid peroxidation in muscle foods. However, due to the variability of chemical composition within the muscle, chemical and physical analysis of whole meat gives uncertain results and, as products of lipid oxidation can further react with meat constituents, so these tests tend to underestimate the degree of oxidation (Hoyland & Taylor, 1991). One of the advantages of using meat fibres rather than whole meat, lipid emulsions or microsomal fractions is that meat fibres closely resemble the structure and organization of meat. They also offer the easy homogenization and manipulation of the system which allows the adjustment of physical factors like temperature, pH and water activity (Aw) as well as chemical factors such as presence or absence of pro- and anti- oxidants, so the effect of these factors on meat lipid oxidation can be determined.

Oxygen uptake has been used to follow the mechanism of lipid oxidation in muscle tissue extracts (Lee *et al.*, 1975; Fischer & Deng, 1977; Silberstein & Lillard, 1978) and in freeze-dried muscle foods (Chipault & Hawkins, 1971). Rhee (1978), made specific recommendations for the study of lipid oxidation in tissue homogenates used as a meat model system, and early reports by Martinez & Labuza (1968), Tuomy *et al.*, (1969) and Heidelbaugh & Karel (1970) have indicated the suitability of the oxygen uptake methodology for the study of lipid oxidation on meat that has been freeze-dried despite the fact that protein oxidation may also absorb oxygen as pointed out by Seo (1976).

Materials and Methods.

All laboratory reagents (Analytical grade) were obtained from SIGMA Chemical Co. (Poole, UK). The pork meat used in this study was obtained from a local slaughterhouse, portions of *Longissimus dorsi* (LD) muscle were excised from pork carcass at 24 h postmortem.

Preparation of meat fibres:
Meat samples were trimmed of all visible fat, minced and pigments extracted with ice-cold 0.04 M phosphate buffer at pH 6.8 (Warris, 1979). Meat fibres were prepared as outlined previously (Prado *et al.*, 1994. In Press).

Preparation of samples for oxygen uptake:
Solutions of Aw 0.96 and Aw 0.70 were prepared by mixing appropriate amounts of deionised water and propylene glycol (Ledward, 1981). Dry meat fibres (0.05 g ml^{-1}) were added to each solution and the Aw was verified in a Hygrometer (Rotronic-Hygroskop DT, Rotronic AG, Zürich, Switzerland). Oxygen uptake during the oxidation of meat fibres system (5 ml) was measured using an Oxygen Electrode (Read-Out-meter, Rank Brothers, Cambridge, England) coupled to a chart recorder (Phillips PM8251). The recorder chart speed was set at 0.5 mm min^{-1} and sensitivity at 50 V. The electrode was calibrated to "0%" with a sodium dithionite solution and to "100%" with the oxygen saturated propylene glycol solutions. Temperature (4, 25 and 50 °C) was controlled with a recirculating water bath coupled to the oxygen electrode vessel. The equipment was checked for stability over a 2 h period before the assay using standards (5 ml) of suspensions of carboxymethylcellulose and α-cellulose (0.05 g ml^{-1}). Every sample was performed in triplicate and the electrode membrane was replaced after each assay.

Results and Discussion:

Oxygen uptake of meat fibres was determined at Aw 0.96 (fresh meat) and Aw 0.70 (roasted meat), for the three temperatures (4, 25, 50 °C). The temperatures used were selected as they fall within the working range of the oxygen electrode. After equilibration to the desired Aw and temperature, the sample was placed into the oxygen electrode vessel. It was assumed that the amount of oxygen absorbed by the meat fibres was due solely to the oxidation of lipids.

Figure 1 shows the oxygen uptake of the meat fibres at Aw 0.96 incubated at 4, 25 and 50 °C. To avoid confusion, error bars were not included in the figures but typically the percentage coefficient of variation was 5 to 17 % . The differences in the initial oxygen concentration between samples may be explained by the effect of temperature and water content on the oxygen solubility. Higher water content and low temperature tend to increase oxygen solubility (Morris, 1974). For samples at 4 °C an initial oxygen uptake during the first 3 h of incubation was observed but after this period, no notable oxygen uptake occurred. However, at this temperature, an increase in viscosity of the system was observed, which may have been due to extracted protein and thus may have affected the sensitivity of the oxygen electrode. For samples at 25 °C the overall oxidation appeared to take place in two stages. An initial rapid but small absorption of oxygen was followed by a period of oxidation at low rate. At some later time a second phase of oxygen absorption occurred and the shape of the curve at this stage indicated the autocatalytic character typical of fat deterioration. The intermediate period between these two phases of oxygen absorption may vary considerable as was observed in samples at 50 °C, where the autocatalytic stage seemed to overlap the initiation and propagation periods. The variation in time between the phases may depend largely on the amount and susceptibility to oxidation of the sample as well as the presence or absence of catalytic factors, such as temperature in this case. For the meat fibres hydrated to Aw 0.70 (Fig. 2), a steady oxygen uptake throughout the time of incubation was observed at 25 and 50 °C, but the total exhaustion of oxygen was not accomplished even after 40 h of incubation in contrast to samples at Aw 0.96. For samples at 4 °C no notable oxygen uptake was observed within the observation time (40 h).

The different rates of oxidation between samples at the different Aw's studied, may be the result of the predominant effect of water content at any given Aw, as water affects the rate of lipid oxidation in at least three important ways: a) An antioxidant effect due to binding of hydroperoxides, which decrease their reactivity; b) An antioxidant effect due to hydration of metal catalysts, which reduces their catalytic action and c) A pro-oxidant effect due to an increase in the mobility of reactants. Thus there exists a critical water activity below which, continued increase of Aw is increasingly antioxidant, and above which, continued increase of Aw is increasingly pro-oxidant (Heidelbaugh & Karel, 1970). Therefore the knowledge of a system's critical Aw is essential for determining the predominant effect of water content on rates of oxidation. Also to be considered, is that the increased water content causes the swelling of solid matrices exposing new catalytic surfaces, as well as decreasing the viscosity of the system promoting a faster diffusion of oxygen through the catalysts (Labuza & Chou, 1974).

To explain the possible reason for the accelerated rate of oxygen uptake in the early hours of incubation on samples at Aw 0.95, two different inert systems consisting of fibres of α-cellulose and

carboxymethylcellulose, were treated in the same way as the meat fibres at Aw 0.96 and 4 °C. To obtain comparative units, results were taken from the read-out of the oxygen electrode .

The oxygen content was measured in the system of water-propylene glycol-O_2 system (Aw 0.96) for 3 h before the addition of the α-cellulose, carboxymethylcellulose or meat fibres to check for stability. In all cases (Fig. 3), a rapid increase in the oxygen content at the time that the fibres were added was observed, but after two hours, the oxygen content dropped below the level recorded before the fibres addition. This effect may be explained by the fact that before the fibres were added, the oxygen electrode was equilibrated to a water-propylene glycol-O_2 system and this equilibrium was changed to a water-propylene glycol-fibres-O_2 system after the fibres addition. The addition of fibres affects both the initial oxygen concentration of the system by introducing O_2-bound-fibres which creates an oxygen supersaturated system and also it is reducing the free water content due to the fibres water adsorption, which affects the oxygen diffusion. Therefore after the addition of the fibres, the system is taken to a second equilibration stage, which in turn displays an increased initial oxygen uptake in the early stage of the assay. A higher increase of oxygen content was observed for the meat fibres, probably due to a bigger oxygen adhesion to meat fibres than to the cellulose fibres. No changes in viscosity were observed for the inert fibres, which also suggest the effect of temperature on meat protein extraction and subsequent changes in viscosity.

The participation of the propylene glycol-bound water may have an effect on lipid oxidation, as it is assumed that the water bound to propylene glycol is likely to reduce the water's antioxidant effect, but at high water activities (0.90 or above) water continues acting as a pro-oxidant by increasing the mobility of reactants (Heidelbaugh & Karel, 1970). It must also be mentioned that the rate of oxidation may be closely related to oxygen concentration, however the nature of the oxygen electrode is a limiting factor for the long term studies of lipid oxidation.

Conclusion.

Using meat fibres it was possible to measure the effect of water activity on lipid oxidation at different temperatures. The results also show homogeneity between replicates demonstrating that meat fibres are less variable than meat itself.

Oxygen uptake as a function of lipid oxidation was found appropriate for the study of the early stages of oxidation on meat fibres. The rate of oxygen uptake depends on both the Aw and temperature of the system. The rate of oxidation was negligible at 4 °C up to 40 h of incubation at both of the Aw's studied. However at 25 and 50 °C, an increased rate of lipid oxidation with major effect at 50 °C was observed. The stability of the oxygen electrode at temperatures above 50 °C is poor, thus limiting the application of the test.

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Bibliography.

Chipault, J. R. & Hawkings, J. M. (1971). J. Agric. Food Chem. **19(3):**495
Fischer, J. & Deng, J. C. (1977). J. Food Sci. **42:**610
Heidelbaugh, N. D. & Karel , M. (1970). J. Am. Oil Chem. Soc. **47:** 539
Hoyland, D. V. & Taylor, A. J. (1991). Food Chem. **40:**271
Kanner, J., Shegalovich, I., Harel, S., & Hazan, B. (1988). J. Food Chem. **36(3):**409
Labuza, T. P. & Chou, H. E. (1974). J. Food Sci. **39:** 112
Ledward, D. (1981). Intermediated Moisture Meats. In: "Developments in Meat Science-2." Lawrie, R. Ed. Applied Science Publishers. London. Chap. 6
Lee, Y., Hargus, G., Kirkpatrick, J., Berner, D. & Forsythe, R. (1975). J. Food Sci. **40:** 964
Liu, H. P. & Watts, B. M. (1970). J. Food Sci. **35:**590
Martinez, F. & Labuza, T. P. (1968). J. Food Sci. **33:** 241
Morris, J. G. (1974). A Biologist's Physical Chemistry. 2nd Ed. Williams Clowes & Sons. Limited. London. Chap. 3.
Prado, *et al.*, (1994). In Press.
Rhee, K. S. (1978). Food Sci. **43:** 6
Rhee, K. S. & Ziprin, A. (1987). J. Food Bioch. **11(1):**1

Seo, S. W. (1976) . J. Food Sci. **41:** 594
Silberstein, D. A. & Lillard, D. D. (1978). Food Sci. **43:**746
Tuomy, J. M., Hinnergardt, L. C. & Helmer, R. L. (1969). J. Agric. Food Chem. **17:**1360
Warris, P. (1979). J. Food Tech. **14:**75