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BIOCHEMICAL STUDIES ON THE DISCOLOURATION OF FRESH MEAT

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INTRODUCTION

Maintenance of a bright red colour on the surface of fresh meat is of interest to both the meat industry and the consumer. When exposed to air, myoglobin, the major oxygen binding pigment in post mortem muscle, can either be oxygenated to the bright cherry red oxymyoglobin or oxidised to the brown pigment metmyoglobin (Met Mb). The relative proportions of these pigments will largely depend upon the oxygen tension within the tissue fluid surrounding the myoglobin (1) (2) which may vary according to the oxygen demand of the meat itself when exposed to air. Increasing surface bacteriological contamination during prolonged storage may also have an influence on the state of the pigments (3). Additionally, reducing the oxygen tension in the meat promotes the autoxidation of the pigment giving rise to the marked brown discolouration which is visible at the surface and is associated with poor meat quality.

It has been observed in this laboratory that the oxymyoglobin of different meats demonstrate varying degrees of stability during storage. There have been numerous reports in the literature (4-9) on the stability of myoglobin pigments at the surface of fresh meat but very limited information is available on the mechanisms which control pigment stability (10) (11) and none which fully explain these observed variations between different meat tissues. Lamb muscle has a relatively short display life due to the rapid autoxidation which occurs on the surface and it has been shown recently (12) that this meat possesses an extraordinary high oxygen uptake early post mortem (5 hours). It was also observed that the subsequent decline in biochemical oxygen demand (B.O.D.) during maturation appeared to be related to a corresponding decrease in N.A.D. concentration in the meat. Other workers have demonstrated an increased oxygen utilisation in meat tissue by the addition of N.A.D. or N.A.D.H. (10) (13) but little evidence is available linking the endogenous N.A.D. content of the meat with the respiratory rate post mortem.

In an attempt to elucidate the controlling factors which contribute to the reduction in oxymyoglobin stability, and therefore to meat discolouration, it was thought pertinent to measure and compare the B.O.D. and the N.A.D. contents of three different meats which show contrasting pigment stability during their retail display life.

Furthermore, a system exists in meat which is capable of reducing the metmyoglobin formed from oxymyoglobin (15)(15). This system, referred to as the metmyoglobin reducing activity (M.R.A.) is normally active to a greater or lesser degree, when meat is held under anaerobic conditions. However, in instances where the oxygen tension within the exposed surfaces becomes low, the M.R.A. may possibly function in the conversion of oxidised pigment. It was decided therefore, to include the measurement of M.R.A. in this series of experiments to gain additional information on its role, if any, in the colour stability of fresh meat during storage under controlled aerobic conditions.

METHODS

Muscles and Sampling Procedures

Approximately 200 g samples of lamb, beef and pork muscle were excised from the left M. Semimembranosus of each of eight carcasses at 1 hour post mortem, and stored at 0-4°C. Small samples were removed from these portions of muscle for B.O.D., N.A.D., and M.R.A. estimations at 5,24,48,72 and 144 hours post mortem.

B.O.D. Measurement

Approximately 1g of minced muscle was accurately weighed, incubated in 3ml of isotonic Ringer's solution, the whole adjusted to pH 5.8 and equilibrated in a Warburg flask at 15°C. The oxygen uptake was measured manometrically at 10 minute intervals for the initial samples and at 20 minute intervals in the remaining samples over a 2 hour incubation period (16). The oxygen uptake, expressed as ul/g wet tissue / 2 hours incubation, was used as a measure of the oxygen demand.

N.A.D. Content

Frozen samples, excised at 5,24,48 and 72 hours were extracted in perchloric acid and neutralised by the method described previously (17.) N.A.D. content was estimated by the method of Klingenberg using alcohol dehydrogenase (18). Results are expressed as u moles N.A.D / g wet tissue.

M.R.A. Values

Three samples were selected at random from the eight portions of muscle for each type of meat. M.R.A. values were determined using a modified procedure based on the method of Watts etal, (10).

To convert the reading for total reflectance to a K/S ratio the tables published by Judd (19) were consulted. Values quoted were :

100% Metmyoglobin gives a K/S $\frac{572 \text{ nm}}{525 \text{ nm}}$ of 0.88

100% Nitrosomyoglobin gives a K/S $\frac{552 \text{ nm}}{525 \text{ nm}}$ of 1.37

In practice these ratios were found to vary for lamb, beef and pork muscles and Table I shows the observed ratios and the difference in the K/S value from oxidised to reduced forms of the pigments.

TABLE I

Observed K/S Ratios for Lamb, Beef and Pork M. Semimembranosus

	K/S Ratio for 100% Met Mb	K/S Ratio for 100% NO. Mb	Change in Ratio
PORK	0.85	1.34	0.49
LAMB	0.85	1.54	0.69
BEEF	0.85	1.74	0.89

Metmyoglobin reduction was calculated using the data above and the activity has been expressed as the percentage conversion per hour as an average of three samples.

RESULTS

Averaged values for B.O.D. and N.A.D. content for the three types of muscle are presented in Tables II and III together with their standard deviations.

LAMB MUSCLE

The initial B.O.D. value was substantially lower in this experiment than that observed previously with lamb muscle, 150 ul/g/2 hours compared with 260 ul/g/2 hours. Subsequent levels however, were in close agreement with the previous findings, the B.O.D. falling steadily to a plateau value (after 48 hours post mortem) of approximately 80 ul/g/2 hours.

N.A.D. concentrations were generally lower than observed previously dropping from an average initial content of 0.66 u/moles at 5 hours post mortem to half this value at 72 hours post mortem.

TABLE II

B.O.D. Values for Lamb, Beef and Pork M. Semimembranosus between 5 and 144 hours Post Mortem

TIME POST MORTEM (HOURS)	LAMB		BEEF		PORK	
	Average ul/2 Hours	S.D. (±)	Average ul/2 Hours	S.D. (±)	Average ul/2 Hours	S.D (±)
5	150	37	171	32	108	21
24	136	18	85	22	55	19
48	74	11	51	11	34	9
72	85	15	45	13	39	12
144	76	13	25	10	38	18

TABLE III

N.A.D. Contents for Lamb, Beef and Pork M.Semimembranosus between 5 and 72 hours Post Mortem

TIME POST MORTEM (HOURS)	LAMB		BEEF		PORK	
	Average u moles/g	S.D. (±)	Average u moles/g	S.D. (±)	Average u moles/g	S.D. (±)
5	0.66	0.12	0.60	0.09	0.75	0.24
24	0.51	0.11	0.49	0.09	0.45	0.19
48	0.38	0.06	0.33	0.11	0.43	0.15
72	0.33	0.03	0.26	0.05	0.39	0.14

The correlation coefficient calculated between the N.A.D. content and the B.O.D. was 0.577 indicating a very highly significant ($P < 0.001$) correlation.

M.R.A. values showed an unexpected pattern (Figure I) in that the conversion rate was only 43%/hour intially but rose subsequently to 84%/hour by 24 hours post mortem. Thereafter the M.R.A. value fell steadily to reach a conversion rate of 42% / hour at 72 hours, this rate being maintained until the sixth day post mortem.

Beef Muscle

The average B.O.D. value for beef muscle fell steeply from 170 ul/g/2 hours initially to 51 ul/g/2 hours within 48 hours. Oxygen uptake decreased only slightly thereafter resulting in an ultimate value of 25 ul/g/2 hours.

The observed decrease in N.A.D. content between 5 and 48 hours post mortem was almost linear falling from 0.60 u moles/g to 0.33 u mole/g. The rate of fall then markedly decreased resulting in a value of 0.26 u moles/g by 72 hours post mortem.

The correlation coefficient calculated between B.O.D. values and N.A.D. content was 0.888 again demonstrating a very high degree of significance between these two parameters. ($P < 0.001$).

Averaged values for the M.R.A. (Figure I) were observed to fall almost linearly from an initial conversion rate of 110% / hour to 22% / hour at 72 hours post mortem. The rate of conversion then stabilised and continued at a plateau level to give 19% conversion/hour in the final sample.

FIGURE I

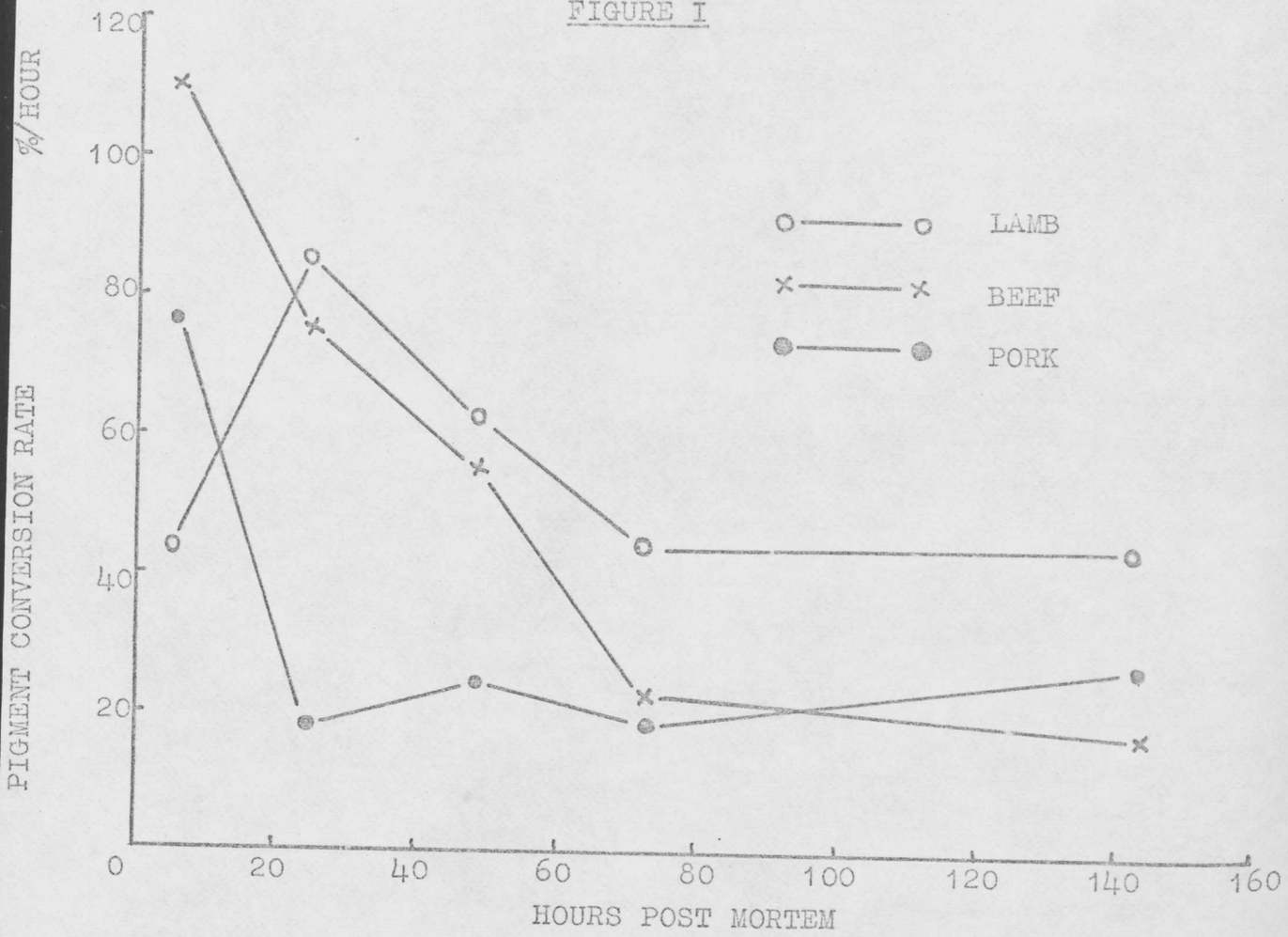
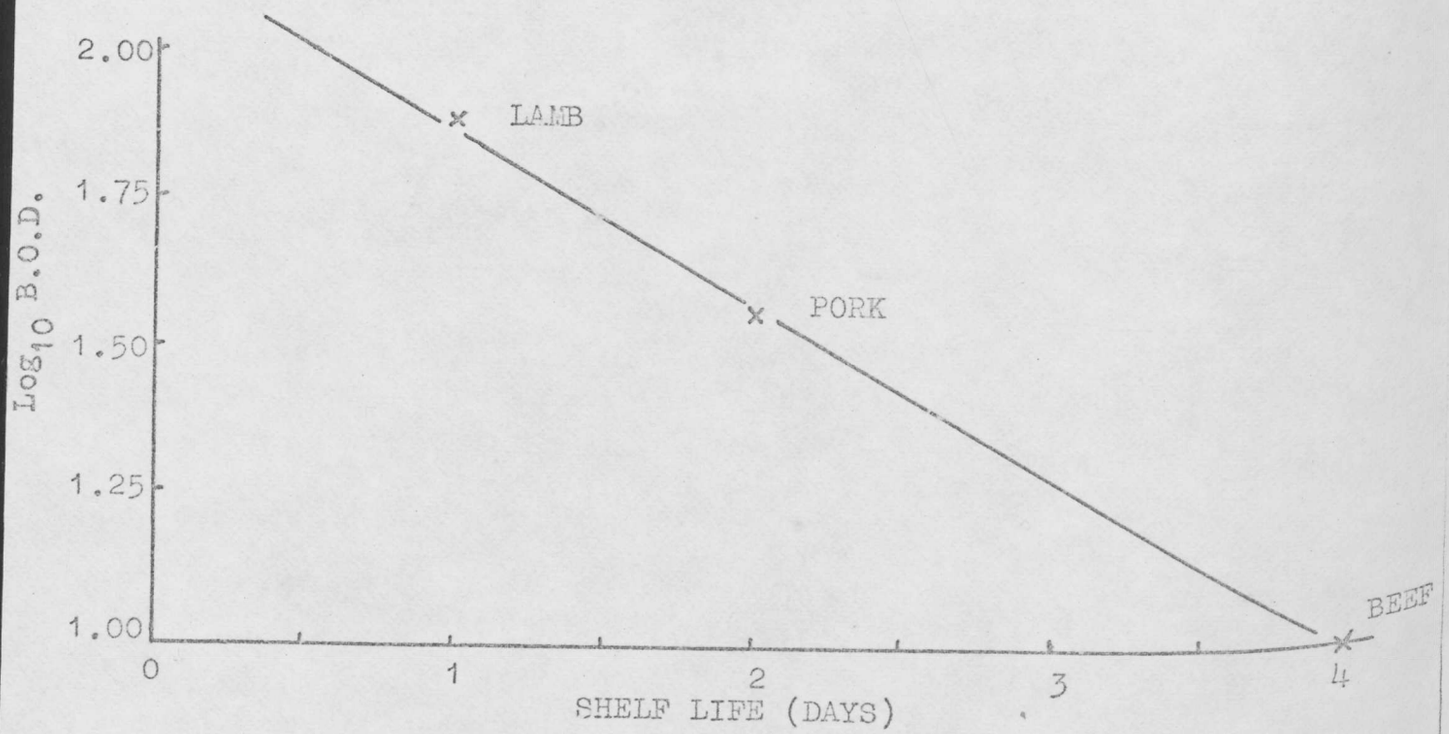


FIGURE II



Pork Muscle

There was a swift decline in B.O.D. from an initial value of 108 ul/g/2 hours to the relatively low value of 34 ul/g/2 hours by 48 hours post mortem. Oxygen uptake values were maintained at this level during the subsequent 96 hours storage.

A high N.A.D. concentration was observed initially (0.75 u moles/g) but was followed by a marked fall within the subsequent 19 hours to 0.45 u moles/g. which corresponded with the concentrations observed in the lamb and beef muscles at this time. Between 24 and 72 hours post mortem the N.A.D. content fell more slowly, reaching a final value of 0.39 u mole/g. It may be noted that the standard deviations for pork muscle were very much higher for each occasion of analysis than for beef or lamb muscle.

Calculation of the correlation coefficient between B.O.D. and N.A.D. content gave a value of 0.471 ($p=0.01$) indicating a less significant correlation between these two parameters in pork muscle than that observed in beef and lamb muscle.

From the M.R.A. values presented in Figure I it can be seen that the initial conversion rate of 68%/hour was rapidly reduced to a quarter of this value within the subsequent 19 hours, and thereafter remained constant up to 72 hours post mortem.

DISCUSSION

The pattern of B.O.D. fall was different in all three types of meat. Pork muscle fell from a low initial value to a plateau level of around 40 ul/g/2 hours within 24 hours post mortem while beef muscle, although higher initially, declined steadily, the ultimate value amounting to less then 30 ul/g/2 hours. Lamb muscle on the other hand, after falling steadily within the initial 48 hours, maintained a much higher plateau level of 80 ul/g/2 hours, corresponding to twice the ultimate value of pork muscle, and treble that observed for beef muscle after six days' storage. The initial B.O.D. value of lamb muscle at 5 hours post mortem was low when compared with the previous observations (12) possibly due to the adjustment of the Warburg flask contents to pH 5.8 on this occasion. However, ultimate values, subsequent to 48 hours, were in good agreement with the previous findings.

Both beef and lamb muscles showed a decline in N.A.D. content with time post mortem which was very highly correlated with the fall in B.O.D., confirming earlier observations with lamb muscle (12). Furthermore, the concentrations of N.A.D. in the beef and lamb muscles were very similar during the period investigated. In contrast N.A.D. values for the pork muscles, although generally higher in concentration, produced a poorer correlation with the corresponding B.O.D. In the pork muscles the increase in B.O.D. for a given increase in N.A.D. content was far less than that observed for the beef and lamb muscles.

It is worthwhile at this juncture to consider the maturation period given to the three meats prior to their retail sale.

Pork and lamb carcasses are generally butchered within 3-4 days of slaughter whereas beef is given a much longer period for maturation of at least 8 days. Thus, relevant B.O.D. and N.A.D. values for linking these parameters to colour stability during retail sale should be related to the age of the meat at time of retail sale. During controlled retail display, the maintenance of the bright oxygenated form of myoglobin for these three meats, before metmyoglobin formation becomes excessive, is in the order lamb (1 day), pork (2 days) and beef (4 days). Comparing the corresponding B.O.D. values at the time of retail sale (extrapolated value for beef at 192 hours was 10 ul/g/2 hours) with these commercially observed colour stability periods, a loose relationship does appear to exist between retail display life and the corresponding B.O.D. value represented by the formula:-

$$\text{Retail display life (Days)} = K \log_{10} \text{B.O.D. value.}$$

and is shown clearly in graphical form in Figure II. It would appear therefore, that length of storage of the meat carcass, prior to retail distribution, could have a major influence on the display life of the retail commodity.

The pattern of change in M.R.A. values post mortem differed for lamb, beef and pork but mirrored the corresponding declines in B.O.D. with storage time. Reasons for the very low initial value observed for lamb muscle are, as yet, unexplained and require confirmation. The ultimate value for pork muscle was rapidly attained within 24 hours whereas for beef and lamb muscles a constant value was only reached after a further 48 hours storage. In addition the beef and pork muscles were observed to have relatively lower ultimate values than the lamb muscles i.e. 20% per hour and 40% per hour respecitively. It would have been more in line with the observed relatively short display life of lamb if the M.R.A. had fallen swiftly to a very low level, since this would have been more related to the rapid formation of metmyoglobin observed during retail display. In contrast, it appears that lamb muscle has a more active system for the regeneration of myoglobin than either beef or pork muscle, and therfore, negates the hypothesis that rapid metmyoglobin formation is a function of lower M.R.A. Furthermore, it is clear that the systems which control the activity of metmyoglobin reduction are not directly related to the proportions of the oxidised and oxygenated pigments at the surface of fresh meat when subjected to aerobic conditions which supports the observations of other workers in this field (10)(11). Therefore, although pork and beef muscle attain a lower M.R.A. during maturation they also possess a low B.O.D. which appears to play a more decisive role in delaying discolouration. Improvement in the colour stability of the surface pigments in lamb muscle would seem to rest on lowering the B.O.D. as rapidly as possible post mortem. This may be possible to achieve by holding the carcass at an elevated temperature during the onset of rigor thereby inactivating or exhausting the enzymes and/or metabolites normally associated with the respiratory pathway. This is endorsed by measurements of the N.A.D. concentrations in muscles cooled at different rates post mortem where it was observed that the N.A.D. content was markedly reduced by slow rates of cooling, (20).

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