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FACTORS AFFECTING THE ACCUMULATION OF METMYOGLOBIN IN PREPACKAGED BEEF.

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Introduction.

The discolouration of prepackaged fresh beef limits its storage life and restricts the development of centralised cutting and prepackaging operations. The meat pigment myoglobin can exist in reduced, oxygenated or oxidised form and the amount of each present is closely related to the colour of the meat. Discolouration is due primarily to the formation of the oxidised pigment metmyoglobin at the surface which imparts a brown colour to the meat and makes it unacceptable to the consumer.

Reflectance spectrophotometry has been used to measure the relative amounts of myoglobin derivatives at the surface of cut meat (Snyder '65, Stewart et al '65). Because the technique is non-destructive it can be used to follow colour changes which occur in intact beef samples and it is eminently suitable for studying the conditions which cause these changes. The measurement of discolouration in prepackaged fresh beef involves finding the amount of metmyoglobin present relative to the other two pigment derivatives.

There is generally considerable variation in the degree of discolouration that occurs on the surface of cut meat. Sampling is therefore very important and must be adequate to ensure that any results are based on measurements which are representative of the whole surface. Another sampling difficulty is associated with the small area of surface actually sampled by the beam of the spectrophotometer. The instrument used in the present work was a Unicam spectrophotometer fitted with an SP890 diffuse reflectance accessory in which the area sampled is a circle 2.25 cm diameter.

Statistical data are required on the variability of individual discolouration measurements in order to predict the number of replicate determinations which will give any specified level of accuracy. The objectives of the present work are to establish confidence limits for reflectance measurements and to use the method to compare discolouration rates of similarly treated meat samples. The data also provide a statistical basis for comparing meat samples under different experimental treatments.

The metabolic activity of large numbers of bacteria $>$ log 7/g causes a rapid reduction in the partial pressure of oxygen which in turn accelerates the rate of metmyoglobin formation (Heiss & Eichner '69). When the partial pressure of oxygen falls below a value of 30 mm Hg the autoxidation rate is accelerated (George & Stratman '52). The optimum oxygen pressure for the formation of metmyoglobin is about 4 mm Hg (Brooks '35). Bacteria also attack myoglobin directly, causing denaturation and making it more susceptible to oxidation. (Fox '68).

In order to study the contribution of intrinsic biochemical factors to the rate of discolouration in meat the effect of bacterial contamination must first be eliminated. This can be accomplished by dipping the complete muscle in boiling water before cutting and by working under aseptic conditions.

Prepackaging consumer cuts from individual muscles emphasises possible intermuscular differences in meat quality. This study investigates intermuscular differences as a factor influencing susceptibility to discolouration of fresh beef.

Another factor of practical importance in discolouration work is the age of meat post-mortem. Vacuum packaging permits refrigerated storage of fresh meat for much longer periods than is normally possible with carcase meat. In addition to the beneficial effects of ageing on tenderness, vacuum storage gives better stock control and greater flexibility in marketing. However it is important that other quality attributes are not impaired. In particular as far as prepackaging is concerned, it is important that the meat should not be more liable to discolour when it is finally exposed to the atmosphere.

Experimental.

Beef was obtained from young heifers, 0 or 2 teeth, which were kept for at least a week after purchase, well fed and rested and finally slaughtered in the Meat Research Department abbatoir under standard conditions. All muscles had normal ultimate pH values within the range 5.4 - 5.8. Three to five days post-mortem one hindquarter from each carcase was deboned and six individual muscles, M. longissimus dorsi, M. psoas major, M. semitendinosus, M. semimembranosus, M. gluteus medius and M. vastus lateralis dissected out. Each muscle was dipped in boiling water and then cut into steaks approximately 1.5 cm thick with a sterile knife. Ten samples were selected at random from these steaks and placed in plastic containers 4.5 cm x 3.5 cm x 1.5 cm. The size of these was chosen to fit the sample holder of the SP890 diffuse reflectance unit.

The samples were held at 0°C for one hour and then overwrapped with PVC meat grade film. After a further two hours at 0°C the spectrum of each piece of meat was recorded on the spectrophotometer.

The samples were returned to the refrigerator until the following day when they were placed in an incubator for $7\frac{1}{2}$ hours at 25°C. Reflectance spectra were recorded again after this time.

Primal cuts containing these six muscles were prepared from the other side of the carcase. These were packed under vacuum in Cryovac bags and aged at 0°C for three weeks. After this time muscles were dissected out, dipped in boiling water and the samples prepared as before for reflectance analysis.

Reflectance measurement.

The sample thickness is such that no light is reflected from the background and under these conditions reflectance has been termed "reflectivity" and has the special symbol R_∞. (Judd & Wyszecki '63). The ratio of the absorption coefficient (K) to the scattering coefficient (S) is related to R_∞ according to the following expression.

$$K/S = \frac{(1 - R_\infty)^2}{2 R_\infty} \qquad \text{(Kubelka \& Munk '31).}$$

This function is used to relate percent reflectance to quantities of meat pigments and tables are available (Judd & Wyszecki 1963) for converting percent reflectance to the K/S value.

The spectrophotometer was arranged to record in absorbance units and these readings were first converted to R_∞ from standard tables relating absorbance to percent transmittance. The K/S value corresponding to each R_∞ was then read from the appropriate table.

Magnesium carbonate was used as a reference standard, representing 100% reflectance.

Absorbances at 572nm and 525nm were recorded for each sample, converted to K/S values and the K/S ratio calculated at these two wavelengths. 525nm is an isobestic point for all three pigment derivatives while 572nm is isobestic for myoglobin and oxymyoglobin.

$(K/S)_{572}/(K/S)_{525}$ is at a maximum when the pigment is present as oxymyoglobin plus reduced myoglobin and when metmyoglobin is absent. With fully oxygenated fresh meat shortly after cutting the pigment is present as the oxymyoglobin derivative. Subsequent discolouration, due to the formation of the oxidised metmyoglobin derivative, results in a reduction of $(K/S)_{572}/(K/S)_{525}$ which reaches a minimum value on total conversion of the pigment to metmyoglobin (Stewart et al '65).

Intermediate ratios between these two extremes are assumed to follow a linear relationship. This assumption has been proved valid for a model system using suspensions of non-fat dried milk powder containing known amounts of myoglobin, but not for intact meat samples. (Snyder & Armstrong '67).

In the present work the difference between the initial $(K/S)_{572}/(K/S)_{525}$

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and the value after $7\frac{1}{2}$ hours at 25°C, which represents the amount of metmyoglobin formed under these conditions, is taken as the susceptibility to discolouration of the piece of meat.

Results.

Ten replicate determinations were done on each muscle and the mean $(K/S)_{572}/(K/S)_{525}$ calculated for each sample. Initial $(K/S)_{572}/(K/S)_{525}$ data for all muscles after 4-6 days and after three weeks ageing are given in Table 1.

Table 1. Initial values of $(K/S)_{572}/(K/S)_{525}$. Average of 10 determinations per muscle.

Muscle	Ageing Time	Animal 1	2	3	4	5
M. longissimus dorsi	4-6 days	1.437	1.417	1.450	1.434	1.405
	3 weeks	1.422	1.400	1.341	1.402	1.411
M. psoas major	4-6 days	1.350	1.407	1.429	1.352	
	3 weeks	1.409	1.405	1.384	1.341	
M. semitendinosus	4-6 days	1.404	1.449	1.405	1.411	1.432
	3 weeks	1.436	1.432	1.378	1.400	1.461
M. semimembranosus	4-6 days	1.378	1.428	1.403	1.346	1.372
	3 weeks	1.354	1.428	1.376	1.355	1.398
M. gluteus medius	4-6 days	1.368	1.374	1.321	1.328	1.319
	3 weeks	1.375	1.357	1.372	1.292	1.338
M. vastus lateralis	4-6 days	1.385	1.389	1.434	1.346	1.377
	3 weeks	1.444	1.357	1.392	1.351	1.375

Table 2. Values of $(K/S)_{572}/(K/S)_{525}$ after $7\frac{1}{2}$ hours at 25°C. Average 10 determinations per muscle.

Muscle	Ageing Time	Animal 1	2	3	4	5
M. longissimus dorsi	4-6 days	1.447	1.243	1.179	1.375	1.347
	3 weeks	1.427	1.305	1.285	1.373	1.245
M. psoas major	4-6 days	.866	1.087	0.912	1.034	
	3 weeks	.916	1.024	0.800	0.888	
M. semitendinosus	4-6 days	1.101	1.220	1.208	1.422	1.315
	3 weeks	1.285	1.232	1.120	1.352	1.276
M. semimembranosus	4-6 days	1.316	1.206	1.120	1.247	1.275
	3 weeks	1.280	1.204	1.149	1.167	1.235
M. gluteus medius	4-6 days	1.190	1.163	1.162	1.198	1.154
	3 weeks	1.183	1.043	1.100	1.083	1.142
M. vastus lateralis	4-6 days	1.325	1.050	1.352	1.235	1.201
	3 weeks	1.276	1.011	1.315	1.190	1.235

The pooled standard deviation for initial $(K/S)_{572}/(K/S)_{525}$ data in Table 1. is o.034 and in general the muscle standard deviation is of this order. However, individual muscles occasionally show much greater variability. 15% of muscle samples of 10 replicate determinations have standard deviations greater than 0.050 and 2% have standard deviations greater than 0.080. $7\frac{1}{2}$ hour $(K/S)_{572}/(K/S)_{525}$ data in Table 2 are more variable. The pooled standard deviation in this case is 0.045. Again occasional muscles show greater variability. Here 15% of samples have standard deviations greater than 0.060 and 2% greater than 0.090. On the basis of the pooled standard deviation for data of initial type, and $7\frac{1}{2}$ hour type, respectively the number of determinations necessary to obtain specified accuracies at 95% confidence limits are given in Table 3.

Table 3. Sample size (number of determinations).

Initial $(K/S)_{572}/(K/S)_{525}$	$7\frac{1}{2}$ hour $(K/S)_{572}/(K/S)_{525}$	Accuracy 95% confidence limits.
σ = 0.034	σ = 0.045	
44	78	± 0.01
7	13	± 0.02
5	9	± 0.03

The number of determinations actually recorded in the present work is 10. The standard error of the mean for initial $(K/S)_{572}/(K/S)_{525}$ data is 0.011. Similarly for $7\frac{1}{2}$ hour $(K/S)_{572}/(K/S)_{525}$ data the standard error of the mean is 0.014. Corresponding coefficients of variation are 2.4% and 3.8% respectively. It should again be stressed that single muscles may show considerably more variation than is indicated by the mean standard error. A correspondingly larger number of determinations would be required for these samples to obtain the accuracies shown in Table 3.

The mean initial $(K/S)_{572}/(K/S)_{525}$ for the six individual muscles are given in Table 4. Differences between individual muscles are numerically small but an analysis of variance of the data (representing 6 muscles from 5 animals and at 2 ageing times) shows that this intermuscular difference is significant.

Table 4. $(K/S)_{572}/(K/S)_{525}$ Initial Muscle means.

M. long. dorsi	M. psoas.	M. semiten.	M. semimem.	M. gluteus	M. vastus
1.405	1.387	1.422	1.384	1.350	1.384

Standard error of mean 0.006

The greatest $(K/S)_{572}/(K/S)_{525}$ is obtained for M. semitendinosus and the least for M. gluteus medius. The difference between them is approximately 0.07.

Discolouration data are presented in Table 5 as the difference between the initial $(K/S)_{572}/(K/S)_{525}$ and the corresponding ratio for each muscle after $7\frac{1}{2}$ hours at 25°C. The difference between initial and $7\frac{1}{2}$ hour ratios represents the change in pigment composition that has taken place in the muscle, specifically the accumulation of metmyoglobin relative to the other two derivatives that has occurred. The conversion of the pigment to the metmyoglobin derivative is a measure of the susceptibility of a muscle to discolour under the experimental conditions.

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Table 5. Discolouration @ 25°C $\left[(K/S)_{572}/(K/S)_{525}\right]_0 - \left[(K/S)_{572}/(K/S)_{525}\right]_{7\frac{1}{2}}$

Muscle	Ageing Time	1	2	3	4	5
M. longissimus dorsi	4-6 days	-0.010	0.174	0.171	0.059	0.058
	3 weeks	-0.005	0.095	0.056	0.029	0.166
M. psoas major	4-6 days	0.464	0.320	0.517	0.318	
	3 weeks	0.493	0.381	0.584	0.453	
M. semitendinosus	4-6 days	0.303	0.229	0.197	-0.011	0.117
	3 weeks	0.151	0.200	0.258	0.048	0.185
M. semimembranosus	4-6 days	0.062	0.222	0.283	0.099	0.097
	3 weeks	0.074	0.224	0.227	0.188	0.163
M. gluteus medius	4-6 days	0.178	0.211	0.159	0.130	0.165
	3 weeks	0.192	0.314	0.272	0.209	0.196
M. vastus lat.	4-6 days	0.060	0.339	0.082	0.111	0.176
	3 weeks	0.168	0.346	0.077	0.161	0.140

With the exception of three measurements (table 5) the muscle samples are discoloured after $7\frac{1}{2}$ hours at 25°C. and have a lower $(K/S)_{572}/(K/S)_{525}$ ratio. Samples of M. psoas major are generally visibly brown after this treatment whereas it is sometimes difficult to see any change in M. longissimus dorsi.

An analysis of variance of the discolouration data in Table 5 (representing 6 muscles from 5 animals and at 2 ageing times) show a significant difference in discolouration for the muscles. Table 6 summarises the results of this analysis.

Table 6. Muscle mean discolouration.

M. long. dorsi.	M. psoas major.	M. semiten.	M. semimem.	M. gluteus	M.vastus
.079	0.382	0.168	0.164	0.203	0.166

Standard error of muscle mean 0.015

The difference is largest between M. longissimus dorsi which is generally stable and the unstable M. psoas major. The other four muscles have intermediate values between these two extremes.

In general, figures for 3 week storage are higher than 4-6 days storage (see Table 5) with the exception of M. longissimus dorsi which shows the opposite effect. This is more clearly shown in Table 7 in which muscle mean discolourations at the two ageing times are compared.

Table 7. Effect of storage time on muscle mean discolouration.

	Time 4-6 days (a)	3 weeks (b)	Difference (b - a)	Approx. significance level.
M. l.d.	.090	.068	-0.022	0.05
M. p.m.	.348	.416	+0.068	0.01
M. st.	.167	.168	+0.001	N.S.
M. sm.	.153	.175	+0.022	0.05
M. g.m.	.169	.237	+0.068	0.01
M. v.l.	.154	.178	+0.024	0.05

An approximate significance test using the normal distribution shows that susceptibility to discolouration increases with ageing time in the cases of M. psoas major, M. semimembranosus, M. gluteus medius and M. vastus lateralis.

M. semitendinosus shows no change, and M. longissimus dorsi shows a reduced susceptibility at the longer ageing time.

The muscles from one side of the carcase after 4-6 days ageing are compared with muscles from the other side after 3 weeks ageing and this should be considered in the interpretation of these data. However it is unlikely that any systematic error is introduced as a result of this discrepancy in experimental design.

Discussion.

The accumulation of metmyoglobin does not occur uniformly over the surface of steaks cut from individual muscles. Surface discolouration can therefore be quite variable and a large number of replicate determinations is normally required to measure the degree of discolouration accurately. A sample size of 10 or more is necessary to provide a coefficient of variation better than 4%, particularly where discolouration has already taken place. Reflectance data are more variable and the coefficient of variation greater at the latter stage for the majority of samples. This increase is important where colour changes are being studied and the number of determinations necessary to obtain any desired accuracy should be based on the final rather than initial data.

Samples of 10 replicate determinations provide sufficient accuracy to study the factors which are of interest in the present work. Significant differences are shown between muscles in initial $(K/S)_{572}/(K/S)_{525}$ values even though these are numerically small. More important are the muscular effect and the influence of ageing time on susceptibility to discolouration which are demonstrated.

The increased susceptibility to discolouration with time is greatest for M. psoas major and M. gluteus medius which are also the least stable muscles initially. On the other hand M. longissimus dorsi which is the only muscle to show reduced susceptibility to discolouration on storage, is initially the most stable muscle with respect to colour.

The difference in rates of discolouration at 25°C between these muscles is reproduced also at lower temperatures. Figure 1 shows a comparison of the rates of discolouration for M. longissimus dorsi and M. psoas major from a single carcase at 0°C and 10°C.

Colour deterioration due to the accumulation of metmyoglobin is the resultant of the effects of autoxidation and enzymatic reduction (Stewart et al '65).

Bacteria increase autoxidation rate by reducing the partial pressure of oxygen below the critical level of 30 mm and also by causing denaturation of myoglobin. Bacteriological effects were reduced to a minimum in the present work, but would normally become increasingly important with storage time, and would thus augment the greater intrinsic susceptibility to discolouration of meat aged for three weeks.

Stewart et al '65 described a method for measuring metmyoglobin reducing activity MRA, in which potassium ferricyanide is first added to samples of the ground meat. The rate of metmyoglobin reduction which occurs under anaerobic conditions, is then followed by reflectance spectrophotometry.

MRA has been found to decrease with storage time and to be very low in beef after prolonged storage of 6-8 weeks (Hood, unpublished results). The MRA of M. longissimus dorsi is generally higher than that of unstable muscles like M. psoas major and M. gluteus medius.

Loss in MRA may account, at least in part, for the generally greater susceptibility to discolouration which occurs on storage. M. longissimus dorsi is the exception to this rule; it also has a more stable MRA, and an increase in MRA has occasionally been recorded for this muscle.

The effect of storage time on discolouration rate is small and relatively unimportant compared with intermuscular differences. Colour stability remains quite high particularly at refrigeration temperatures provided there is an adequate supply of oxygen. Where oxygen supply is limited however meat with low MRA readily forms metmyoglobin even at 0°C. (Hood, unpublished results).

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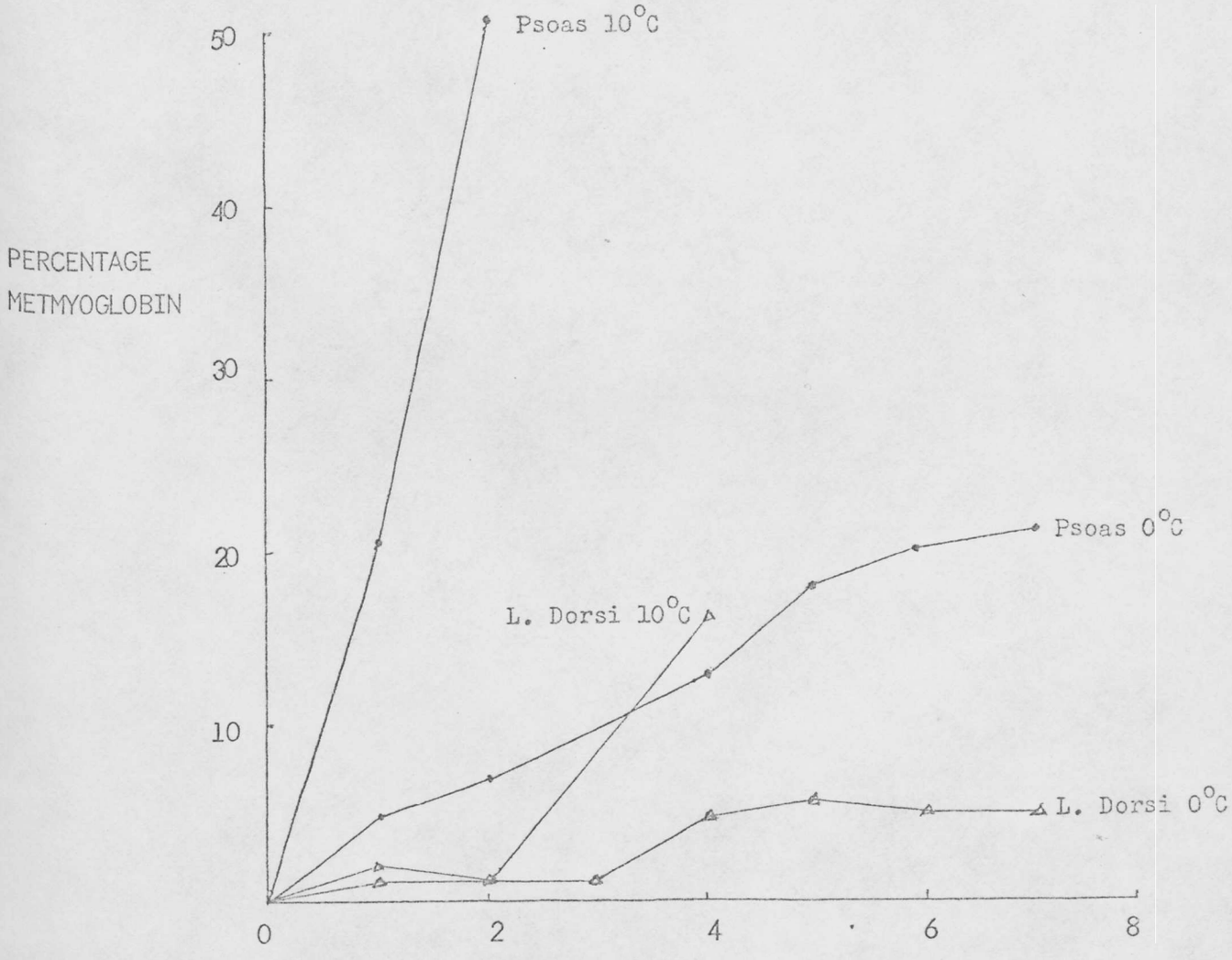
This can be simply demonstrated by placing two steaks from muscles with low MRA in contact in a refrigerator. A brown area of metmyoglobin forms on the surface of each at the point of contact.

Speculating on the absence of metmyoglobin formation below the surface of oxygenated meat, where the partial pressure of oxygen is optimal for metmyoglobin accumulation, Zimmerman & Snyder '67 suggested that such a layer does not occur in meat. However a layer of metmyoglogin is readily observed a few mm below the surface in meat with low MRA.

FIGURE 1. RATE OF DISCOLOURATION OF M. PSOAS MAJOR AND M. LONGISSIMUS DORSI FROM A SINGLE CARCASE AT 0°C AND 10°C.



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