Analysis of Myoglobin Derivatives in Meat or Fish Samples Using Absorption Spectrophotometry

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A general method is described for determining relative and absolute pigment concentrations of myoglobin derivatives in fresh whole and comminuted meats and fish, both untreated and treated with atmospheres containing carbon monoxide. Carboxymyoglobin, metmyoglobin, and deoxymyoglobin plus oxymyoglobin can be determined using simple extraction and absorption spectrophotometry. The method does not permit independent measurement of deoxymyoglobin.

In the process of examining the effects of carbon monoxide and carbon dioxide enriched atmospheres on the preservation and shelf-life extension of meats and fish, it became of interest to determine the amount of CO bound to the myoglobin of the muscle tissue. Many current methods used for the examination of meat pigments employ reflectance spectroscopy (Dean and Ball, 1960; Snyder, 1965; Stewart et al., 1965; Snyder and Armstrong, 1967; Franke and Solberg, 1971; Satterlee and Hansmeyer, 1974; Stange et al., 1974). However, since we were interested in the extent of penetration and the total absorption of the CO, it was more appropriate to reexamine existing techniques based on pigment extraction (Lemberg and Legge, 1949; Ginger et al., 1954; Broumand et al., 1958).

This paper details a simple spectrophotometric method useful for the determination of absolute and relative concentrations of oxymyoglobin (MbO_2), carboxymyoglobin (MbCO), and metmyoglobin (MetMb) in meat or fish samples which is an extension of earlier techniques involving extraction.

EXPERIMENTAL SECTION

Pigment Analysis. The pigments were extracted by blending weighed 40–60-g samples with 80 mL of pH 6.0, I = 0.2, phosphate buffer. The resulting slurry was centrifuged for 30 min at 3 °C and 15 000g. The aqueous layer was filtered through glass wool and the volume was recorded; it was then centrifuged for an additional 30 min at 15 000g. Usually a clear, nonturbid supernatant was obtained. The spectrum of this solution was recorded between 700 and 500 nm (Cary Model 15 spectrophotometer). This yielded a spectral envelope including contributions from MbO_2, MetMb, and MbCO, if any (Figure 1). Any deoxymyoglobin (Mb) initially present is observed as oxymyoglobin since it becomes oxygenated during the extraction process. The solution in the cuvette was then saturated with CO by slow bubbling with the gas, after which the spectrum was redetermined. Care should be taken not to bubble excessively as it may cause denaturation, indicated by an increase in the absorbance around 690 nm due to light scattering. The addition of a small drop of octanol to the cuvette is useful in suppressing foaming, hence denaturation, and does not appear to interfere.

Subtraction of the MetMb contribution from the spectral envelope after saturation with CO allows calculation of the total reduced myoglobin as MbCO. The absorbance of bovine MetMb in the 560–580 region at pH 6.0 is two-thirds that at 630 nm. The relative proportions of MbO_2 and MbCO present in the original extract were also determined from these two scans. Separate experiments with standardized bovine myoglobin solutions established that the minimum which lies between the α and β bands for MbO_2 was 0.80 (±0.01) times that of the minimum between the α and β bands for MbCO solutions of identical concentration. This difference (δ in Figure 1) is the change that a sample containing no MbCO will undergo upon saturation with CO. Typical samples containing some MbCO initially will undergo proportionately less change upon saturation with CO. Thus, by obtaining the value of the minimum for pure MbCO, corrected for MetMb, and comparing the absorbance of the minimum of the extract with the computed minimum for pure MbO_2, the percentages of MbO_2 and MbCO may be calculated. The fractional change in absorption of this minimum upon saturation with CO was found to obey Beer's Law and was linearly related to the relative concentrations of the species present, at pH 6 (Figure 2). For a pigment concentration of about 10^{-4} M, the magnitude of δ is approximately 0.2 absorbance units/cm, allowing determination of the concentrations to within ±5% absolute deviation.

Relative concentrations can be converted to amounts per sample weight by determining the extraction efficiency by consecutive extraction of representative samples. Thus, 84% of the soluble myoglobin was recovered in the first supernatant for lean ground beef samples. This efficiency was quite consistent for a sample type, but varied somewhat for samples having more fat or much higher bacterial loading, for example. Similar values have been obtained for tuna fish samples.

Appreciable absorbance (>0.05) at 690 nm is due to turbidity rather than that of any myoglobin derivative. If turbid samples cannot be avoided, as with some fish samples, this wavelength-dependent error may be corrected using the method of Goldbloom and Brown (1966).

Calculations. The equations given in this section assume the use of a 1 cm cuvette for spectral measurements. The calculations can be further refined using iteration, but the precision obtainable by the method does not warrant it. Similarly, some minor corrections have been ignored.

All observed absorbances were first corrected for baseline error by subtracting the absorbance at 690 nm.

For the MetMb concentration, the net absorbance at 630 nm must be corrected for tail absorption of the MbCO and MbO_2 species. This was empirically found to be about 4% of the 580 nm absorption.

$$[\text{MetMb}] = \frac{A_{630} - 0.04(A_{580})}{\epsilon_{\text{MetMb},\,\lambda\,630}} \quad (1)$$

The total reduced myoglobin, Mb_T, can be determined as MbCO after CO saturation of the sample. This ab-

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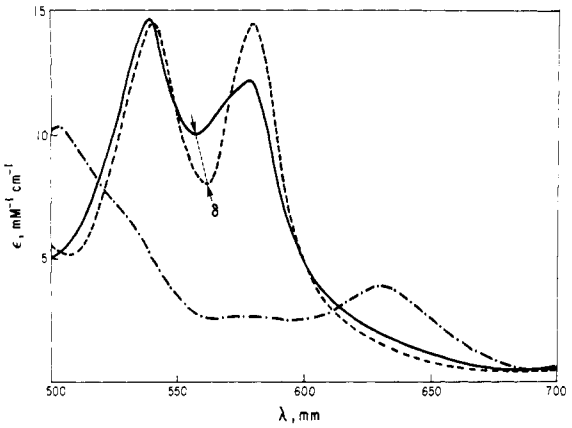

Figure 1. Spectra of metmyoglobin (·—·), oxymyoglobin (-------), and carboxymyoglobin (—). The delta symbol (δ) depicted at the 560-nm region represents the magnitude of the absorbance change in converting from oxymyoglobin to carboxymyoglobin. A typical extract of samples treated with CO will have its absorbance minimum at some point between these extremes.

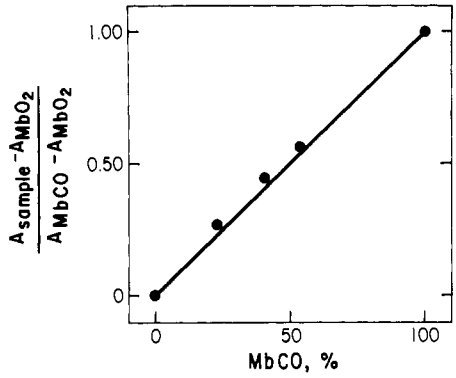

Figure 2. The relation between sample absorbances at the 560-nm region minima and carboxymyoglobin concentration expressed as percent of total reduced myoglobin. The subscript (MbCO) refers to the sample absorbance after saturation with CO.

sorbance at 578 nm must first be corrected empirically for the MetMb contribution.

$$[Mb_T] = \frac{A_{578(CO\ sat.)} - 0.66(A_{630})}{\epsilon_{MbCO,\ \lambda\ 578}} \quad (2)$$

The amount of the reduced myoglobin which is present as MbCO in the sample is calculated from the measured net absorbances of the sample before and after CO saturation and the calculated net absorbance of an MbO_2 solution of equal concentration. All readings are taken at the minimum between the α and β bands in each case (signified by primed wavelengths) ignoring the small shift in the wavelength at which this minimum occurs depending upon the ligand bound.

$$[MbCO] = [Mb_T]\left\{\frac{A_{560'(sample)} - A_{560'(MbO_2)}}{A_{560'(CO\ sat.)} - A_{560'(MbO_2)}}\right\} \quad (3)$$

These absorbancies must be corrected for the MetMb contribution near 560 nm which is the same as that at 578 nm (i.e., $0.66A_{630}$). Empirically, $A_{560'(MbO_2)} = 0.80A_{560'(MbCO)}$, which yields a simplified form of eq 3 upon substitution:

$$[MbCO] = [Mb_T]\left[5.0\left\{\frac{A_{560'(sample)}}{A_{560'(CO\ sat.)}}\right\} - 4.0\right] \quad (4)$$

Finally

$$[MbO_2] = [Mb_T] - [MbCO] \quad (5)$$

Table I. Millimolar Absorptivities of Bovine Myoglobin Derivatives in Phosphate Buffer (pH = 6.0, I = 0.2) at 20 °C

Myoglobin derivative	λ, nm	ϵ, mM^{-1} cm^{-1}
Mb (deoxy)	560	13.8
MbO_2	580	14.4
	542	14.4
MbCO	578	12.2
	540	14.6
MetMb	630	3.9

The total pigment present can be calculated as a summation of the individual components and serves as a useful internal check on the calculations for a given series of samples. Maximum accuracy is obtained if relative molar absorptivities are determined for the individual myoglobin derivatives of the particular species being investigated. Those observed for bovine myoglobin in this study are presented in Table I. Only the absorbances at 560, 580, and 630 nm are required for the calculations.

RESULTS AND DISCUSSION

The method is simple and, though not as rapid as direct reflectance spectrophotometry, has the advantages of not requiring special spectrophotometer attachments and of allowing determination of interior pigment composition. The relative proportions of pigments observed were stable over a span of hours and, since the extraction requires less than 2 h, could be considered as an accurate reflection of the distribution of the myoglobin derivatives at the time of extraction. A major disadvantage is the lack of capability for direct measurement of deoxymyoglobin. All uncomplexed myoglobin is converted to the oxygenated form as a result of aeration during the extraction process. No interaction of MetMb and carbon monoxide was observed; the indicated amount of MetMb was independent of the duration and rate of CO bubbling. Amounts of hemoglobin ordinarily present in ground meats do not interfere with this method.

The procedure is of less value for the examination of lightly pigmented meats, such as pork, poultry, and some fish. However, we have been able to apply it to both light and dark muscle of skipjack and yellowfin tuna. Useful complementary procedures are available, including one which allows the determination of metmyoglobin and total myoglobin in tuna muscle (Sano and Hashimoto, 1958) and one which determines combined hemoglobin and myoglobin in poultry (Saffle, 1973).

The determination of the proportions of MbCO and MbO_2 is accurate to about 5% absolute, due to the relatively small change of the valley minimum in the 560-nm absorbance region. The total quantity of the reduced pigment (as MbO_2 and MbCO) and the MetMb can be found to greater precision, since the MetMb absorption band at 630 nm lies in a region of little overlap and the conversion of all reduced forms to the carboxy derivative allows an accurate determination of their concentrations from the 578-nm absorption band. The precision of the total pigment determination for each trial is better than ±5% average relative deviation based on over 100 triplicate extractions.

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Characteristics of Proteins from Normal, High Lysine, and High Tannin Sorghums

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The purpose of this paper was to study the characteristics of proteins from normal, high lysine, and high tannin *Sorghum bicolor* (L.) Moench. Endosperm preparations were obtained from four inbred lines of sorghum representing a normal, low tannin variety (P-721-N), its mutagenically derived high lysine counterpart (P-721-O), an inbred Ethiopian variety high in lysine (IS-11167), and a high tannin line (IS-4225). Endosperm proteins were separated into five soluble fractions by the Landry–Moureaux method. Whole endosperms and their respective protein fractions were subjected to amino acid analysis. Polyacrylamide gel electrophoresis patterns were determined for the fractionated proteins. The high lysine endosperms had lower levels of kafirins (fractions II and III) than the lysine-deficient, alcohol-soluble protein fraction, when compared with the normal sorghum endosperm preparations. Both high lysine varieties contained elevated levels of albumins and globulins (fraction I) and glutelins (fraction V), which were the highest in lysine content. Differences from normal were observed in the distribution pattern of proteins from a high tannin sorghum. There were no significant differences among the constituent proteins of the identical fractions of these four varieties of sorghum as determined by gel electrophoresis. These results support the general hypothesis that genes affecting protein quality do so by changing the relative quantities of Landry–Moureaux fractions and not by changing the quality of proteins within these fractions.

In recent years a large and concerted effort has been mounted to enhance the nutritional quality of almost all agriculturally significant cereal grains. A considerable portion of this effort is directed toward improving cereal protein quality and is particularly aimed at attaining the most favorable levels of the essential amino acids in cereal grain proteins (Nelson, 1969). *Sorghum bicolor* (L.) Moench ranks fourth in the world production of cereal grains grown for human consumption and is a primary food source for the populations in the semiarid regions of Africa and Asia. The proteins in sorghum, like other cereal grains, may be characterized as albumins, globulins, prolamins, and glutelins (Jambunathan and Mertz, 1973). These four classes of proteins are distinguishable in all cereal grains on the basis of their solubility in water (albumins), salt solutions (globulins), alcohol (prolamins), and alkaline detergents (glutelins). The alcohol-soluble prolamins are extremely low in lysine, which is generally the first limiting amino acid in cereal grains (Mertz et al., 1964). Sorghums are similar to other cereal grains in many of their nutritional characteristics and yet differ in several important respects, the foremost among which is the presence of tannins. These poorly characterized polyphenolic compounds are present in certain genotypes and adversely affect protein availability and digestion (McGinty, 1969; Jambunathan and Mertz, 1973; Axtell et al., 1975). Jambunathan and Mertz (1973) found that three high tannin varieties of sorghum gave significantly lower growth responses in weanling rats when compared with three low tannin varieties. In this paper we will attempt to examine the proteins of sorghum from a nutritional and biochemical viewpoint and determine the potential for improving sorghum grain protein quality by genetic screening and selection. The objective of this paper was to study the characteristics of proteins from normal, high lysine, and high tannin sorghums.

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MATERIALS AND METHODS

Four inbred lines of sorghum were selected: (1) P-721-N (normal), a low tannin line; (2) P-721-O (opaque), a high lysine variety derived from P-721-N by chemical mutagen treatment (Mohan, 1975); (3) IS-11167, an Ethiopian variety high in lysine (Singh and Axtell, 1973); and (4) IS-4225, a high tannin line. All four varieties were grown at the Agricultural Experiment Station in Puerto Rico during the 1974–1975 winter–spring season.

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