THE USE OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY FOR THE DETERMINATION OF HAEM CONCENTRATIONS IN BEEF, PORK AND POULTRY

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SUMMARY

A High Performance Liquid Chromatography (HPLC) method was used to determine the haemoglobin (Hb) and myoglobin (Mb) concentrations of beef and pork *longissimus dorsi* (L.D.) and turkey breast meat. The ability to separate and quantify Hb and Mb is demonstrated and the use of ultrafiltration to concentrate extracts from low haem pigment muscles is shown.

Introduction

The colour of meat is accepted as a major determinant in its purchase (Hood, 1980) and, therefore, understanding the chemical and physical parameters influencing colour is of great importance to the meat industry. Three variables, (1) concentration of haem pigments (2) their chemical state and (3) physical light-scattering properties, are responsible for the appearance of fresh meat (MacDougall, 1983). Several methods involving extraction followed by absorption spectrophotometry have been proposed for the determination of meat pigment concentration but quantification of the separate Hb and Mb concentrations is difficult. Recent work using HPLC (Oellingrath *at al.*, 1990) has shown separation of these proteins to be possible in both standard solutions and beef extracts. The aim of this paper is to provide further verification of the method and, in particular, to extend it to extracts of pork and poultry as well as beef.

Materials and Methods

The methodology was based on that of Oellingrath *et al.* (1990). Samples (10g) of beef, pork and turkey were homogenised (Ultra Turrax, Janke & Kunkel GmbH, Staufen, FRG) in 0.1 M sodium phosphate buffer at pH 7.0 (10 ml for pork and turkey , 25 ml for beef) and centrifuged at 50,000g for 30 min at 4°C. The supernatants were first filtered through Whatman No. 541 filter paper followed by several layers of GF/A glass fibre paper. A few mg (<10) of $K_3Fe(CN)_6$ and KCN were added to the standards to keep the proteins in the form of cyanometmyoglobin and cyanohaemoglobin (Warriss, 1976) and 10μl aliquots were injected onto a hydrophobic interaction column (Bio - Gel Phenyl - 5PW, 75 x 7.5 mm) operated by a Hewlett Packard HP 1090 HPLC with UV detector. A linear gradient of 0 - 100 % 0.1 M sodium phosphate buffer, pH 7.0 (eluent B) was run in 100 - 0 % 1.7 M $(NH_4)_2SO_4$ 0.1 M, sodium phosphate buffer, pH 7.0 (eluent A) for 15 min with a flow rate of 1 ml/min and the absorbances read at 420nm.
Standards of horse skeletal myoglobin and bovine haemoglobin (Sigma Chemical Company, Dorset, UK) were prepared in 0.1 M sodium phosphate buffer, pH 7.0 while chicken myoglobin and haemoglobin were extracted from chicken hearts and blood according to the extraction method detailed above. Concentration of the haem proteins was achieved using Centriprep 3 ultrafiltration membrane concentrators (Amicon Ltd. Gloucestershire, England) and the efficiency of protein concentration was estimated using diluted equine myoglobin and bovine haemoglobin standards. In all cases the moisture content of the meat was taken to be 75%.

Results and Discussion

As previously reported by Oellingrath *et al.* (1990), HPLC using a hydrophobic interaction column can be used to separate beef Hb and Mb. In addition, the concentration of haem pigment in the original tissue can be quantified by using reference standards. Figures 1 and 2 show that the retention times for Mb and Hb, respectively, are 6.4 and 12.3 min for beef and 7.0 and 11.9 min for pork. The concentration of haem pigments in turkey breast meat is so small as to make quantification difficult although from Figure 3 there appears to be evidence of a double peak at approximately 14 minutes which may be due to Hb. To investigate Hb and Mb from a poultry source, chicken hearts and blood were extracted and injected onto the column. These samples showed that chicken Mb had a retention time of 5.8 min and that chicken Hb eluted as two peaks with retention times of 13.4 and 14.7 min. Turkey extracts were then concentrated by ultrafiltration and applied to the column. The resultant trace (Figure 5) shows turkey Mb to elute as two peaks at 4.0 and 4.5 min and turkey Hb as two peaks at 13.7 and 14.8 min.

Table 1 shows the mean values for the haem pigment concentrations in beef, pork and turkey breast. The values for beef Mb are greater than those obtained by Oellingrath *et al.* (1990) while those of Hb are lower. Preliminary investigations, however, using a different batch of samples gave values of 4.96 mg/g for Mb and 0.71mg/g for Hb which compares well with the expected Mb value of 0.5% of wet weight for ox L.D. (Lawrie, 1985). The values for pork are lower than expected but a different batch of samples gave values of 0.58 and 0.62 mg/g for Mb and Hb, respectively. The Mb proportion in porcine L.D. is 0.06% by wet weight (Lawrie, 1985) and the results presented here confirm this figure although are lower than those found by Warriss (1979) who suggested a value of 1.86 mg/g for porcine L.D. The results also indicate that most of the expected colour of fresh poultry breast meat may be due to Hb and not Mb. Although the appearance of the meat of these three species is markedly different, the concentration of Hb (0.26 - 0.54 mg/g) does not vary as much as the Mb concentration (0.03 - 8.38 mg/g) and, therefore, reflects the blood supply to the muscle *in vivo* and the blood left in the muscle after exsanguination.

The main advantage of the method as described is that it separates Hb and Mb. Therefore, it gives a more precise value for the haem pigment concentration in muscle than the alkaline haematin methods of Hornsey (1956) and Karlsson and Lundstrom (1991) and the cyanometmyoglobin methods of Drabkin (1950) and Warriss (1979), which provide measurements of total haem concentration only. The advantages of this are that the efficiency of bleeding at slaughter can be determined as a function of Hb concentration and this would be beneficial to poultry processors allowing the processing conditions to be optimised to reduce blood splash (Lyon *et al.*, 1986). Given the different pigment elution pattern in turkey observed in the present study it is essential to validate the relative retention times for Mb and Hb in all species of interest. The main disadvantage of any method for the determination of haem concentrations is error introduced at the extraction stage. It is important, therefore, to homogenise each sample until no solid coloured matter remains in the homogenate. It is also important to use lean samples as fat and connective tissue will not contain Hb and Mb and so will affect the concentration calculations.

Conclusions

The method described has enough sensitivity to quantify very low concentrations of haem pigments in a variety of species. It has the added benefit of separating Hb from Mb and, therefore, can give an indication of the efficiency of bleeding during slaughter. The method is no more complex than that of any of the other methods used to measure the haem concentrations in muscle.

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