# MEAT PIGMENT DETERMINATION BY A SIMPLE AND NON-TOXIC ALKALINE HAEMATIN METHOD - AN ALTERNATIVE TO THE HORNSEY AND THE CYANOMETMYOGLOBIN METHODS

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## SUMMARY

A method is described for the determination of total pigment content in porcine meat by conversion to haematin (ferriprotoporphyrin hydroxide), and using the non-ionic detergent Triton® X-100 to increase the absorptivity. The method is presented as an alternative to the well-known Hornsey and cyanometmyoglobin methods. The alkaline haematin method requires a single reagent, sodium hydroxide and the detergent Triton X-100. This combination is probably less poisonous and more stable than the reagents used in the Hornsey and the cyanometmyoglobin methods. Reference solutions of alkaline haematin can be prepared easily.

## INTRODUCTION

Meat colour is an important factor of meat quality. When discussing meat quality in pigs, the term PSE (Pale, Soft, Exudative) or watery meat is applied. The pale colour of the porcine Longissimus dorsi (LD) muscle for example, can be due to either or a combination of two causes. Firstly, there is PSE meat where the muscle proteins have become denatured due to rapid glycolysis post mortem while the body temperature is still high. The denatured proteins reflect more light than in normal meat and the surface looks pale. Secondly, the meat can look pale because of a low pigment content. When measuring meat colour with optical instruments, where a pigment influence can be suspected from the wavelength used, the pigment effect needs to be quantified.

The Hornsey haematin chloride method (Hornsey 1956) is the most widely used spectrophotometric procedure for the determination of total pigment in meat (Bünnig and Hamm 1970; Warriss 1979; Pikul et al. 1982; Dransfield et al. 1985; Monin and Sellier 1985). Another common method for pigment determination is the cyanometmyoglobin method (Drabkin et al. 1950; Ginger et al. 1954; Warriss 1976). The principle of these two methods, and their advantages and disadvantages are discussed later in this paper.

The alkaline haematin method described here is based on a method using alkaline haematin detergent for the determination of haemoglobin in blood, as developed by Zander et al. in 1984 (see also Wolf et al. 1984 and O'Halloran 1987). The reagent is a solution of the non-ionic detergent Triton X-100 dissolved in sodium hydroxide.

No references to the use of alkaline haematin for the purpose of analysing haematin in meat have been found in the literature. An alkaline haematin method to determine total haematin concentration was therefore developed.

## MATERIALS AND METHODS

**Extraction buffer.** A phosphate buffer of low ionic strength was used for extraction (0.05 M $K_2HPO_4/KH_2PO_4$, pH 7.4, ionic strength 130 mM; 4 parts 0.1 M $K_2HPO_4$, 1 part 0.1 M $KH_2PO_4$ and 5 parts distilled water). This solution must be stored at about 4°C. The chemicals used were of pro analysi grade and obtained from Merck, Darmstadt, FRG.

**Detergent solution.** The detergent used was an aqueous solution of 10 % Triton® X-100 in distilled water. This solution can be stored at room temperature and stays stable for over 2 years (Wolf et al. 1984). Triton X-100 for scintillation techniques analytical grade was used and obtained from Merck, Darmstadt, FRG.

**Standard haematin solution and standard curve.** To evaluate the haematin concentration in the meat samples, a haematin standard curve was prepared by using haematin chloride dissolved in a solution of extraction buffer, detergent solution and sodium hydroxide in the same proportions as in the final meat extract. As a control, haematin chloride was also solved in filtered meat extract, also with detergent solution and sodium hydroxide in the same proportions. Haematin chloride was used instead of haematin, because of its better solubility in alkaline solutions and greater purity (Zander, personal communication 1988). The standard series should cover the range of concentrations that can be of interest. The haemin standard was obtained from Serva, Heidelberg, FRG.

**Sample preparation.** The muscle used was *M. longissimus dorsi* (LD) from slaughter pigs. The samples were taken from the last rib and backwards 24 hours post mortem and kept at -20°C. Samples were thawed overnight in a refrigerator. Muscle samples were freed from fat and connective tissue and minced twice using a mincer with plate hole size 3 mm (Electrolux Assistent, Electrolux Svenska Försäljnings AB, Stockholm, Sweden).

**Pigment determination.** Samples (5 g) of minced meat were weighed into centrifuge tubes, and 50 ml chilled (4°C) extraction buffer added to each. The samples were then homogenized (Ultra Turrax, Janke and Kunkel GmbH, Staufen, FRG) for 10 sec, the centrifuge tubes being immersed in ice-water before and after the procedure. The detergent solution was added (2.5 ml) and the tubes were covered and stored overnight at 4°C. The following day the samples were stirred using a wooden spatula and filtered at room temperature, using Whatman no. 42 filter paper. About 30 min after starting the filtration, 4 ml of the filtrate was mixed with 200 µl detergent solution. Finally 250 µl 5.0 M sodium hydroxide was added. The absorbance was read after 5 min (directly or within 12 hours according to O'Halloran 1987) at 575 and 700 nm using a Gilford 300 N spectrophotometer (Gilford Instruments Lab. Inc., Oberlin, Ohio). The haematin concentrations were calculated by using the regression equation from the standard curve (1) and expressed as ppm haematin per gram fresh meat.

$$ppm = \text{dilution factor} \times (96.261 \times (A_{575} - A_{700}) - 0.075) \quad (1)$$

## RESULTS

**The absorption spectra** of the alkaline haematin method, with and without the detergent solution and sodium

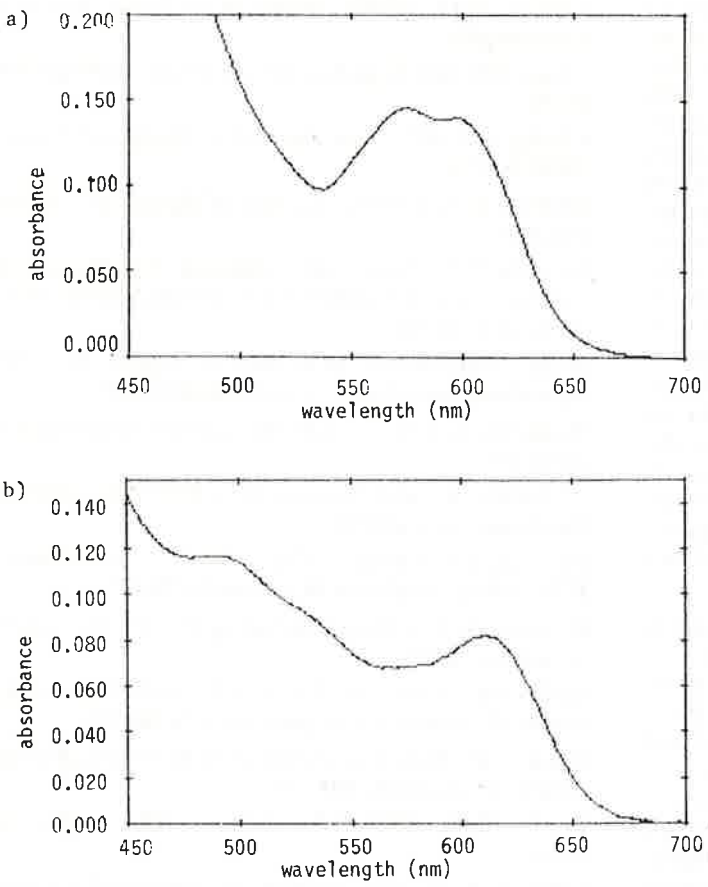


Fig. 1. Comparison of the spectra of alkaline heamatin, a) with and b) without the detergent solution for haemin

hydroxide in the range 450 to 700 nm for haematin chloride, are shown in Fig. 1. The same spectrum was also obtained irrespective of if the pigment was from whale or horse myoglobin or porcine haemoglobin. This was not the case when Triton X-100 was omitted. Due to turbidity of the solutions, the absorbance increased when not using the detergent solution or sodium hydroxide, or when using neither of these reagents, which gave invalid results (not shown).

**Linearity and standard curve.** The linearity of the interval of normal haematin concentration in porcine LD (range 15.0 to 50.0 ppm) was checked by constructing a standard curve. This showed that the Lambert-Beer law was followed within the range of normal haematin concentrations. When only using the absorbance at 575 nm, the standard curve made with the filtered meat extract showed lower regression coefficient compared with the standard curve without meat extract. It thus seems to be a non-specific absorption from the meat. To correct for that the absorbance at 700 nm was subtracted, yielding approximately the same regression coefficient both with and without meat extract.

## DISCUSSION

Alkaline haematin generated by means of the haemoglobin method of Zander et al. (1984) shows a distinct absorption peak at 575 nm, and a shoulder around 600 nm. We have obtained the same results when using pure haematin chloride, whale and horse myoglobin, and porcine haemoglobin. According to Zander et al., all sorts of non-ionic detergents interact with haemoglobin in this way. In the original alkaline haematin method developed for blood, no pre-extraction step was used. With the high pH (13) of the reagent solution, problems arose with denatured muscle proteins when used directly on minced meat. As the pigment protein, myoglobin, is a sarcoplasmic protein, it can be readily extracted from meat by using a chilled buffer of low ionic strength (.2 M) (Helander 1957; Reynafarje 1963), e.g. a phosphate buffer. It should be pointed out, that the pH value will influence the absorbance level. Due to the great buffering capacity of porcine muscle (Warriss 1979), it is of great importance that pH is the same in the meat samples and in the solution used for the standard curve. The non-specific absorption obtained from the meat extract might be due to unclear solutions not detected by the human eye. We corrected for that contribution by subtracting the absorbance at 700 nm, i.e. where no absorption is obtained from the haematin (see Fig. 1).

The polyether-derived non-ionic detergent Triton® X-100 was used here as it interacts with haematin, causing the development of a specific absorption peak. As described by Helenius and Simons (1975), this detergent does not usually denature proteins, and it appears to be very inefficient in stopping protein-protein interaction. Most proteins preserve their quaternary structure in the presence of high concentrations of non-ionic detergents. However, examples of dissociation are also known. For example, haemoglobin is partially dissociated into subunits by Triton X-100. At the same time, the detergent also increases the solubility of proteins and lipids, in the same way as deoxycholate (bile salt) does (Kirkpatrick et al. 1974; Clarke 1975; Hearing et al. 1976; Schubert et al. 1983).

Sodium hydroxide was used to oxidize the iron of the myoglobin prostetic haem ($Fe^{II}$) group to haematin ($Fe^{III}$). It also reduced the turbidity and a clear extract was obtained. Plasma proteins, such as serum albumins and serum globulins, as well as lipids, are much more soluble in alkaline than in acid solutions (Wu 1922; Klein 1934; Clegg and King 1942; Ponder 1942; Bell et al. 1965).

In the Hornsey method for pigment determination, the haematin (Merck Index 1983) of myoglobin is extracted with a solution of 2-propanon (acetone) and water. The haematin is converted to haemin (Merck Index 1983) by adding concentrated hydrochloric acid. This derivate has absorption maxima at 512 and 640 (Lewis1954; Hornsey 1956; MacDougall and Disney 1967). The advantages of the Hornsey method are its rapidity and simplicity. However, its disadvantages are chiefly that 2-propanon easily evaporates, which will make the spectrophotometric values uncertain and will cause environmental problems for the analyst. Moreover, it is flammable, it may defat the skin and it can also act as an anaesthetic.

The factor 680, used to convert the absorbance reading to ppm haematin in meat (Hornsey 1956), is based on a too low water content of 65-70 % in porcine muscle. Other authors (Callow 1938; Poel 1949; Reynafarje 1963; Warriss 1976; Asghar and Pearson 1980; Lawrie 1985) have reported that the water content in meat is

approximately 75 %. This great difference may be due to the fact that the porcine meat used by Hornsey (1956) had a higher content of adipose tissue. The factor 680 is also based on a millimolar absorptivity coefficient of 4.80, which is calculated from a 50 μM haemin solution. In porcine muscle, the reported haematin concentrations (based on myoglobin content) vary between 8.4 and 115.6 ppm (for ref. see Pikul et al 1982). The coefficient seems, however, to be based only on a 50 μM haemin solution, with no dilution, i.e. no standard curve within the concentration of interest for pig muscle. The linearity of the method is thus not proven. An additional source of error not pointed out by Hornsey is the temperature in the acidic acetone solution. As already shown by Lewis (1954), the solution has to be chilled in order to precipitate the proteins before filtering the extract. As shown by Bünning and Hamm (1970), also the fat content of especially pig muscle can cause an increase in absorbance and thus an overestimation of the pigment concentration. The authors suggested defatting of the muscle and triple extractions to get valid results.

In the cyanometmyoglobin method, all haem compounds are converted to cyanmetmyoglobin by different cyanides. These derivates have absorption maxima at 540 and 545 nm (Drabkin et al. 1950; Ginger et al. 1954; Warriss 1976). The great disadvantage with this method is that the reagents used contain cyanides, which are very toxic substances. Zander et al. (1984) has summarised the disadvantages of this method as follows: (1) when preparing the reagent, the analyst is handling a highly dangerous poison, and this may be a drawback for routine use, when large amounts of reagent are required; (2) the reaction solution is light labile; (3) standardization of the method is based on purified cyanhaemoglobin solutions, whose quality is controlled only indirectly by spectrophotometry; (4) the reaction times of the different haemoglobin species and derivatives differ markedly.

In conclusion, the alkaline haematin method described here has certain advantages, even though it is more time-consuming than the Hornsey and cyanometmyoglobin methods. It is probably possible to further simplify the method, but due to lack of time this could not be attempted in this investigation.

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