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A HPLC-METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF HEMOPROTEINS IN PORCINE MUSCLES

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INTRODUCTION

Methods for measuring the concentration of hemoproteins are important for the evaluation of meat quality. The blood content of meat has also to be judged at the post mortem examination of slaughter animals (Meat Inspection Legislation). If hemoglobin is used as an index of residual blood in muscles, it is possible to study the effects of different slaughter procedures on bleeding efficiency. It is important to know the exact blood content of muscles for fixing the time from stunning to bleeding, for the judgement of different stunning methods and for the judgement of stunning together with killing.

There exist various methods to measure total pigment concentration in meat, using extraction and photometric measurement of one of several derivatives (Trout, 1991). There are also some techniques described for determining the hemo- and myoglobin content of meat (Hofmann and Blüchel, 1992). One of these techniques is the separation of a water extract of muscle tissue by Sephadex gel chromatography (Bünnig and Hamm, 1969). Sephadex gel chromatography has some disadvantages. Pigment extraction and chromatography take a long time. Therefore the method is too slow for routine analysis. Because of the shortcomings of the Sephadex gel chromatography, a new HPLC-method was developed.

MATERIALS AND METHODS

Muscle meat used for the experiments was taken from the hind legs of pigs 30 to 35 minutes after electrical or CO_2 stunning. Samples were obtained from the *m.adductores* 5g of the pre-rigor lean pork muscle was homogenized in 25ml cold (1∘C) 0.1M phosphate buffer (Adachi *et al.*, 1988), containing 0.1M sodium chloride, pH7.0 (Warriss, 1979). After 60 minutes at 4∘C the homogenate was centrifugated at 8000rpm (6.500g), 4∘C for 10 minutes. The extract was filtered through a paper filter. The soluble proteins were fractionated by means of a gel permeation HPLC. 20µl were applied on a TSK-G3000-SW column and eluted at a flow rate of 0.5ml/min at room temperature, using again phosphate buffer (0.1M $NaH2_2PO_4$+0.1M NaCl). Myoglobin was detected after about 18.5 minutes, hemoglobin after about 20 minutes by measuring the absorbency of 280nm (Rittinghaus and Franzen, 1980).
A total of 70 male and female pigs were stunned with two different techniques (CO2: n=35, electrical: n=35). They were bleeded with another two different techniques (hanging: n=36, lying: n=34).

RESULTS AND DISCUSSION

Table 1 present the mean values for the hemo- and myoglobin concentration. Myoglobin content was 1.22-5.11mg/g and hemoglobin content was 0.20-1.46mg/g. The gel-permeation-HPLC was more reproducible than the Sephadex gel chromatographie. The hemoglobin fraction showed no contamination with myoglobin. The method works with direct measuring of the absorbency of the hem pigments and did not need any convertation to derivatives.

CONCLUSION

The method described in this paper is a more rapid procedure for the quantification of hemoproteins in porcine muscles than the Sephadex gel chromatographie. The repeatability of the analysis of hemo- and myoglobin is much more better, which shows that this method is much more accurate.

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Table 1. Mean hemo and myoglobin concentration.

	CO_2 stunning		Electrical stunning	
	bleeded hanging	laying	hanging	lying
hemoglobin, mg/g	0.36	0.46	0.35	0.40
myoglobin, mg/g	3.06	3.26	3.13	3.03