Quantification of Hemoproteins in Seal Meat and Other Muscle Foods

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SUMMARY: Myoglobin and hemoglobin of muscle foods are generally separated from each other by precipitation of hemoglobin in a phosphate buffer solution. They are then converted to their corresponding cyano derivatives and are quantified using spectrophotometric methods. Although the myoglobin obtained was always pure, the hemoglobin fraction showed contamination with different proportions of myoglobin as evidenced by a gel electrophoretic separation technique. The degree of contamination of hemoglobin fraction with myoglobin depended on the initial content of each component as well as the concentration of phosphate buffer used. Separation of hemoproteins on Sephadex G-75 revealed that the hemoglobin fraction may contain up to 78% myoglobin. Thus, the quantification method of individual hemoproteins of muscle foods by phosphate buffer precipitation is inadequate and the procedure may only be used for myoglobin purification.

INTRODUCTION: Hemoproteins are the principle oxygen reservoir in the muscle tissues and blood of live animals. In meats, they serve as a source of iron, but also act as a catalyst for autoxidation of their lipid components. Thus quantification of hemoproteins is of interest. Methods of myoglobin determination involve the extraction of hemoprotein pigments from the tissue followed by their separation from hemoglobin, among other proteins. Various procedures for the extraction of heme pigments from muscles have been developed. Hornsey (1956) used acidified 80% acetone to convert both hemoglobin and myoglobin pigments to acid hematin. Other researchers have employed various aqueous solutions for the extraction of hemoproteins (Poel, 1949; Ginger and Schweigert, 1954; Rickansrud and Henrickson, 1967). Examination of different buffer solutions for the extraction of hemoproteins was reviewed by Warris (1979). Methods of Ginger and Schweigert (1954) and Rickansrud and Henrickson (1967) are usually used for myoglobin quantification. In these procedures, after separation of heme compounds, the hemoglobin fraction is preferentially precipitated from its mixture with myoglobin by using a 3 M phosphate buffer at pH 6.6.

Separation of hemoglobin and myoglobin in their mixtures extracted from muscles could also be achieved by column chromatography. Warris (1976) used a Sephadex stationary phase for separation of hemoproteins from each other. The relative quantities of these pigments, as their cyano derivatives, were then determined.

The objectives of this investigation were to determine the concentration of myoglobin in seal muscles and to re-examine the validity of procedures available in the literature for quantification of myoglobin and hemoglobin in seal meat and other sources of muscle foods.

MATERIALS AND METHODS: Seal carcasses, after trimming most of their subcutaneous fat, were deboned using a POSS deboner (model PDE 500, POSS Limited, Toronto, Ontario). Small portions of mechanically separated seal meat (MSSM) were vacuum packed and kept frozen at -20°C until use. MSSM was washed once, twice or three times with water at 5°C for 10 min while stirring manually at a water to meat ratio of 3:1 (v/w). The washed meat was then filtered through cheese cloth filters with 1 mm diameter holes.

Commercial beef and pork meat samples were bought from a local supermarket and were ground in a Waring blender (Model 33BL, Dynamics Corp., New Hartford, USA), vacuum packed in plastic bags, and stored at -20°C until use.

ANALYSES: Total hemoprotein pigments were determined after three extractions of meat samples with acidified 80% acetone solutions in water according to Hornsey (1956). Absorbance of the extract was read at 640 nm and total hemoprotein pigments were then calculated.

Myoglobin content in the pooled mixture obtained after three homogenizations with water of 5 g of meat sample after removal of other protein by lead acetate and then by phosphate buffer precipitation of hemoglobin at pH 6.6 was determined as its cyanoprotein derivative (Rickansrud and Henrickson, 1967). The absorbance of the cyanoprotein derivative of myoglobin was read at 540 nm and the myoglobin content of the sample was calculated. The myoglobin content was also determined according to Warris (1976, 1979). The extracted pigments in 0.04 M phosphate buffer at pH 6.8 were converted to their cyanomet derivatives and then freeze dried, redissolved in 1 ml of water and dialized against 0.5 M NaCl solution to precipitate other sarcoplasmic proteins. Hemoglobin and myoglobin were separated from each other on a Sephadex G-75 column. The eluent was 0.1 M phosphate buffer containing 0.1 M NaCl. Absorbance of hemoglobin fractions was read at 420 and 540 nm, respectively. The amount of myoglobin in the samples was then calculated from the relative absorbance values at these wavelengths and the total hemoprotein pigments obtained by Hornsey's method (1956).

SDS-polyacrylamide gel electrophoresis was carried out using a Laemmli buffer system with a 5% (w/v) stacking gel and a 15% (w/v) separating gel.

RESULTS AND DISCUSSION: Myoglobin content in MSSM, beef and pork using Ginger and Schweigert (1954) and Rickansrud and Henrickson (1967) method was 13.20, 2.50 and 0.46 mg/g sample, respectively. The data for beef and pork was close to those reported be Rickansrud and Henrickson (1967) and Ginger and Schweigert (1954). However, the method of Rickansrud and Henrickson (1967) lends itself for preparation of highly purified myoglobin from the muscle tissues. However, its value for quantification of myoglobin in muscle foods of either low- or high-myoglobin content is questionable.

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Table 1: Hemoproteins of Muscle Foods.[a]

Sample	Total, mg/g[b]	Myoglobin, % of Total	
		Column Separation Method	Precipitation Method
MSSM	59.36 ± 1.41	82.61	22.24
Washed MSSM[c]	19.30 ± 0.31	77.88	3.42
Beef	4.46 ± 2.3	92.60	56.05
Pork	1.93 ± 0.19	86.01	23.83

[a] Results are mean values of 6 to 8 replications ± standard deviation.
[b] According to Hornsey (1956).
[c] Washed 3x with H_2O at a meat to water ratio of 1:3 (w/v).

Table 2: **Influence of Buffer Concentration on the Myoglobin Content of Muscle Tissues Determined by a Precipitation Method.[a]**

Phosphate Buffer Concentration, M	Relative Myoglobin Content
3.0	100.0
2.5	113.0
2.0	118.6
1.0	141.9

[a] Results are mean values of 8 replicates.

Table 3: **Effect of Dilution of the Pigment Extract Solution on the Content of Assayable Myoglobin by a Precipitation Method.[a]**

Hemoproteins Concentration in Solution, mg ml^{-1}	% of Total Determined
5.67	26.6
2.83	32.0
1.20	32.6
0.34	39.7

[a] Results are mean values of 8 replicates.