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Quantification of Hemoglobin and Myoglobin in Pork Muscle: Effect of Rinse&Chill Technology on Blood Removal

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Objectives

The objective of this study was to use ion exchange chromatography to measure hemoglobin (Hb) and myoglobin (Mb) content in ground and salted pork lean obtained from early post-mortem sow carcasses treated with Rinse&Chill (RC) technology (MPSC Inc., Hudson, WI) and evaluate its efficacy to decrease blood content in the muscle, when compared to a conventional treatment.

Materials and Methods

RC technology involved vascular rinsing the carcass early postmortem using a chilled (3°C) isotonic solution (98.5% water; balance: glucose, polyphosphates, glycerine, and maltose). Sows were electrically stunned (550 V) prior to exsanguination. Six sows were used as the conventional treatment and 6 sows received the RC process. At 30 to 60 min post-mortem, the Boston butt and picnic shoulder were deboned, ground (9.5 mm diameter plate) and mixed with 1% NaCl (w/w). Samples (~100 g) were vacuum-packaged and stored at –80°C until analysis. After thawing, samples were frozen with liquid nitrogen and ground into a fine powder for extraction and determination of total heme, Mb, and Hb. Total heme was extracted with acid acetone and determined spectrophotometrically (ε_{640}nm = 4.80 $mM^{-1}cm^{-1}$). Mb and Hb were extracted with 0.01 M PBS buffer (pH 7.4) and after exchange into a 0.01 M Tris buffer (pH 8.6), the heme pigments were separated by ion exchange chromatography in a diethylaminoethyl cellulose (DE52 resin, 2 g) column. Mb was eluted with 0.05 M Tris buffer (pH 8.0) and Hb with 0.5 M NaCl solution. The amount of Mb and Hb in the corresponding eluates was determined spectrophotometrically (ε_{418}nm = 125 $mM^{-1}cm^{-1}$ for Mb and ε_{414}nm = 128 $mM^{-1}cm^{-1}$ for Hb). ESI-MS was used to measure the mass of polypeptides in the Mb and Hb fractions. Total heme, Mb, and Hb content in muscle extracts from the 2 treatments were compared using an unpaired student *t* test.

Results

ESI-MS analysis of the Mb fraction indicated a mass of 16,954 Da which was consistent with the mass of pig Mb without its heme moiety. The Hb fraction had masses of 15,040 and 16,036 Da which was consistent with the mass of the α and β chain of pig Hb, respectively, without their heme moiety. These results indicated that the chromatographic separation was satisfactory. Heme and Mb content in the muscle extracts were statistically similar, when comparing conventional to RC process. The corresponding means for heme content were 187.5 and 209.2 µmol/kg muscle and for Mb, 151.9 and 168.1 µmol/kg muscle. Hb content in the RC treatment was significantly lower, 39.6% on average, than that for the conventional treatment. The percentages of Mb and Hb by weight (relative to each other) in the conventional treatment were 80.9 and 19.1%, respectively. For the RC treatment, Mb was 86.4% and Hb was 13.6%.

Conclusion

The ion exchange chromatography method presented here allowed the measurement of Mb and Hb in pork lean with a satisfactory recovery compared to the total heme determination. Using Hb content in muscle extracts as a measure of blood content suggested that the RC method removed 40% more blood from the muscle compared to the conventional method, leaving still about 60% of the blood in the muscle of RC-treated pigs. The possibility to quantify Hb and Mb separately offers a method to quantitatively assess blood content in muscle.

