Myofibril Fragmentation Index

EXTRACTION

1. Sample extraction should be done in duplicate.
2. In a cold room (2˚C), scissor mince 4 grams of muscle. Minced sample should be free of fat and connective tissue.
3. Put sample in a Eberbach blender container (Eberbach Semi-micro 350 ml stainless steel container with pressure fit lid, A. Daigger #LC22337A) and add 40 ml cold (2˚C) MFI buffer. Using a blender (Waring commercial, 2 speed blender, A. Daigger #LC22302A), homogenize on high (22,000 rpm) for 30 seconds.
4. Pour the homogenate (with the aid of a funnel) into a 50 ml conical bottom centrifuge tube.
5. Centrifuge at 1,000 x g for 15 minutes (2˚C).
6. Discard the supernatant. If there is a fat cap (layer of fat, connective tissue, and myofibrils) above the supernatant, save the fat cap with the pellet.
7. Using a glass stir rod, resuspend the pellet (and fat cap) in 40 ml cold (2˚C) MFI buffer. (DO NOT USE A VORTEX MIXER).
8. Centrifuge at 1,000 x g for 15 minutes (2˚C).
9. Discard the supernatant and fat cap.
10. Resuspend the pellet in 10 ml cold (2˚C) MFI buffer and mix well by using a Vortex Mixer.
11. To remove connective tissue, pour the sample through a polyethylene strainer. Rinse the centrifuge tube with an additional 10 ml cold (2˚C) MFI buffer and pour through the polyethylene strainer. (A Tupperware© strainer, 2" diameter, 1"height, 1 mm pore size, works well. Place the strainer on a funnel that has been placed a conical centrifuge tube).

PROTEIN ASSAY

1. Protein assay should be conducted in duplicate for each sample suspension.
2. Place 0.25 ml of each sample into 13x100 mm glass tubes.
3. Add 0.75 ml MFI buffer.
4. Add 4 ml Biuret reagent and mix on a vortex mixer.
5. Incubate for 30 minutes at room temperature and in the dark.
6. Simultaneously, Bovine Serum Albumin (BSA) standards should be run to establish a standard curve used in determining protein concentration. The following concentrations are preferred: 0 (blank), 2.5, 5.0, 7.5, and 10.0 mg/ml. To these 1 ml standards, add 4 ml Biuret reagent and incubate for 30 minutes. Standards should be run in duplicate.
7. Read the absorbance at 540 nm using a Bausch and Lomb Spectronic 20 Spectrophotometer© with a large slit with (20 nm). If the spectrophotometer is properly calibrated, the absorbance of the standards should be approximately 0, 0.15, 0.30, 0.45 and 0.60 for the 0, 2.5, 5.0, 7.5, and 10.0 mg/ml BSA standards, respectively.

8. Using the standard curve, calculate the protein concentration (mg/ml) of the samples.

MFI MEASUREMENT

1. MFI should be measured in duplicate for each sample suspension.
2. In a 13x100 mm glass tube, dilute an aliquot of the sample suspension to equal 0.5 mg/ml protein in 8 ml MFI buffer.
3. Cap tube and mix sample immediately before reading the absorbance (540 nm) on the Spectronic 20 spectrophotometer. Use MFI buffer for the blank.
4. Multiply the absorbance reading by 200 to obtain the Myofibril Fragmentation Index..

MFI BUFFER (2 LITERS), pH 7.0

You will need 250 ml MFI buffer per sample.

100 mM KCl, 20 mM potassium phosphate, 1 mM EGTA, 1 mM $MgCl_2$, 1 mM NaN_3

KCl	14.91 g
KH_2PO_4	2.72 g
K_2HPO_4	3.50 g
EGTA	0.76 g
$MgCl_2$	0.41 g
NaN_3	0.13 g

Dissolve in distilled deionized water. Adjust pH to 7.0. Bring to a final volume of 2 liters. Store at 4˚C. Do not use anhydrous magnesium chloride, as this chemical causes a yellow tint.

BIURET REAGENT

You will need 16 ml Biuret reagent per sample.

Dissolve 1.5 g Cupric Sulfate ($CuSO_4 \circ 5H_2O$) and 6 g sodium potassium tartrate (Rochelle Salt, $NaKC_4H_4O_6 \circ 4H_2O$) in about 500 ml distilled deionized water in a 1000 ml volumetric flask. With constant stirring, add 300 ml of freshly prepared, carbonate free 10% NaOH. Bring up to 1 liter with distilled deionized water and store in a brown polyethylene bottle. Store at room temperature. Discard if a black or red precipitate appears. This pre-mixed Biuret reagent may also be purchased from Sigma (#6901).

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Reference: Culler, R.D., F.C. Parrish, Jr., G.C. Smith, and H.R. Cross. 1978. Relationship of myofibril fragmentation index to certain chemical, physical, and sensory characteristics of bovine longissimus muscle. J. Food Sci. 43:1177.