Myofibril Fragmentation Index

EXTRACTION

1. Extraction should be conducted in duplicate.
2. In a cold room (2°C), scissor mince 4 gram of muscle. Minced muscle sample should be free of fat and connective tissue.
3. Put sample in an Eberbach blender and add 40 mL cold (2°C) MFI buffer. Homogenize on high for 30 seconds.
4. Pour homogenate into 50 ml centrifuge tubes.
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. Resuspend the pellet (and fat cap) in 40 mL cold (2°C) MFI buffer using a glass rod (DO NOT VORTEX).
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 vortex until well mixed.
11. Pour the sample through a polyethylene strainer to remove connective tissue. Rinse the centrifuge tube with an additional 10 mL cold (2°C) MFI buffer and pour through the polyethylene strainer.

PROTEIN ASSAY

12. Protein assay should be conducted in duplicate for each suspension.
13. Place .25 mL of each suspension into 13X100 mm glass tubes.
14. Add .75 mL MFI buffer.
15. Add 4 mL biuret reagent and vortex.
16. Place in the dark at room temperature for 30 minutes.
17. Simultaneously, Bovine Serum Albumin (BSA) standards should be run to establish a standard curve. The following concentrations are preferred 0 (blank), 2.5, 5.0, 7.5, and 10.0 mg/ml. Standards should be run in duplicate.
18. Read the absorbance at 540 nM with a Bausch and Lomb Spectronic 20 with a large slit width. If the Spectronic 20 is properly adjusted and blanked, the absorbance of the standards should be approximately 0, .15, .30, .45, and .60 for 0, 2.5, 5.0, 7.5, and 10.0 mg/ml, respectively.
19. Determine the protein concentration of each suspension.

MFI MEASUREMENT

20. MFI should be measured in duplicate for each suspension.
21. In 13X100 mm glass tubes, add appropriate quantities of the suspension and MFI buffer to make 8 mL of a .5 mg protein/mL solution.
22. Cap, vortex, and shake well immediately prior to reading the absorbance at 540 nM with a Bausch and Lomb Spectronic 20 with a large slit width. The Spectronic 20 should be blanked against MFI buffer.
23. MFI = 200 X Absorbance.

MFI BUFFER (2L)

100 mM KCl, 20 mM potassium phosphate (pH 7), 1 mM EGTA, 1 mM MgCl2, and 1 mM NaN3

KCl.....14.91 g
KH2PO4.....2.72 g
K2HPO4.....3.50 g
EGTA.....0.76 g
MgCl2.....0.41 g
NaN3.....0.13 g

Dissolve in distilled deionized H2O
pH to 7.0
Transfer to a 2 L volumetric and bring up to volume.

You will need 250 mL of MFI buffer per sample

BIURET REAGENT (2L)

Dissolve 1.5 g Cupric Sulfate (CuSO4*5H20) and 6 g sodium potassium tartrate (Rochelle Salt, NaKC4H4O6*4H20) in about 500 mL ddH20 in a 1000 mL volumetric flask. With constant stirring, add 300 mL of freshly prepared, carbonate free 10% NaOH. Bring up to 1 litter with ddH20 and store in a brown polyethylene bottle. Discard if a black or red precipitate appears.

You will need 16 mL of biuret reagent per sample

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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.