bstract: Improving Protein Extraction From <I>Brachiaria Ruziziensis</I> Leaf Samples by Usin ...

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224-3 Improving Protein Extraction From *Brachiaria Ruziziensis* Leaf Samples by Using a Plant Protease Inhibitor Cocktail and a Chaotropic Reagent.

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Presentations
PosterVirtual_ASA_Bioanalyzer.pdf (409.3 kB)

Protein extraction from biological samples generally requires denaturation or cold manipulation to attenuate proteasecaused degradation. As a result, procedures not always lead to desired precision degrees in the protein level determination. The purpose of this study was to compare a traditional, buffer-based protein extraction protocol with the utilization of a protease-blocking protein extraction kit, aiming at allowing protein manipulation under room temperature prior to proteomic approaches. The protease inhibitor mixture contained pepstatin A, AEBSF, E-64, leupeptin, 1-10-phenanthroline, and bestatin, showing broad specificity for the inhibition of aspartic, aminopeptidases, cysteine, serine, and metalloproteases. Protein samples in this treatment were resuspended in the chaotropic reagent. *Brachiaria ruziziensis* ground frozen leaf tissues were utilized for the comparison, and extracts were analyzed in a Bioanalyzer (Agilent 2100), covering polypeptides ranging from five to 80 KDa. Results indicated that extraction with the traditional method did not yield detectable protein fragmentation, so that only one pattern was distinguished in the electropherogram. Samples extracted with the kit, in turn, provided quality electropherograms, with well-defined and reproducible peaks. In conclusion, the utilization of the protein extraction kit is preferable over the traditional protocol for producing quality protein profiles of *Brachiaria ruziziensis*.

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