# PROTEOMIC INSIGHTS IN ACROCOMIA ACULEATA: OPTIMIZING SAMPLE PREPARATION TECHNIQUES FOR ANALYSIS OF A BIOENERGY ALTERNATIVE CROP

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Proteomic analysis of non-model plants requires selecting appropriate sample preparation techniques to achieve reliable and comprehensive results. *Acrocomia aculeata*, a palm species distributed throughout Tropical America, serves as an alternative source of oil due to its high oil yield and environmental adaptability. Plant proteomics poses unique challenges due to the complexity of plant tissues and the presence of interfering substances. This study explores innovative sample preparation techniques for proteomic analysis of endosperm and mesocarp tissues in *A. aculeata*, employing phenol-based (P), sodium deoxycholate (SDC), and sodium dodecyl sulfate (SDS) extraction methods combined with in-solution digestion (ISD) and S-Trap device to evaluate the efficiency and effectiveness. The aim this research was optimize protein extraction, solubilization, digestion, and identification, thereby establishing a robust methodology for plant proteomics. Proteins from mesocarp and endosperm underwent five preparation protocols: P-ISD, SDC-ISD, P-SDC-ISD, P-S-Trap, and SDS-S-Trap. In the endosperm, the SDS-S-Trap method demonstrated superiority with the recovery of proteins and peptides, identifying a total of 667 and 239 unique proteins. The P-S-Trap also performed well, identifying a total of 600 and 171 unique proteins, though slightly less efficiently than SDS-S-Trap. The traditional phenol-saturated method was less effective in the endosperm, 319 proteins identified, resulting in significantly lower protein recovery. For the mesocarp, the P-ISD method excelled, identifying the highest number of proteins and effectively extracting proteins from plastids and vacuoles. However, the SDS-S-Trap method also showed strong performance, identifying a total of 2210 and 511 unique proteins and exhibiting high recovery of proteins from specific organelles such as the Golgi and nucleus. The SDC-ISD method had the poorest performance in the mesocarp, identifying 1985 proteins, the lowest number among the methods tested. Quantitative analysis revealed that P-ISD and SDS-S-Trap stands out for its protein identification and diversity across different classes and locations. Despite its efficiency, the P-ISD method had limitations in the endosperm, while SDS-S-Trap showed consistent performance in both tissues. This suggests that the choice of preparation method should be tailored to the tissue type to maximize proteomic coverage and data quality. The SDS-S-Trap method demonstrated superior digestion efficiency, peptide recovery, and overall reproducibility, particularly in the endosperm. Its ability to handle high SDS concentrations facilitated efficient detergent removal and enhanced digestion efficiency, making it advantageous for challenging plant tissues. Conversely, the P-ISD method offered specific advantages in mesocarp protein identification, underscoring the need for a tissue-specific approach. This study highlights the importance of selecting appropriate sample preparation protocols in plant proteomics for non-model species to achieve comprehensive and high-quality data. Integrating different techniques like SDS-S-Trap and P-ISD provides complementary insights into the proteome of *A. aculeata*, paving the way for discovering new biomarkers and deeper understanding of molecular mechanisms in biofuel-producing plants. Optimizing sample preparation methods is crucial for advancing plant proteomics, particularly for non-model species like *A. aculeata*.

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