



Efficient method of protein extraction from *Theobroma cacao* L. roots for two-dimensional gel electrophoresis and mass spectrometry analyses

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ABSTRACT. *Theobroma cacao* is a woody and recalcitrant plant with a very high level of interfering compounds. Standard protocols for protein extraction were proposed for various types of samples, but the presence of interfering compounds in many samples prevented the isolation of proteins suitable for two-dimensional gel electrophoresis (2-DE). An efficient method to extract root proteins for 2-DE was established to overcome these problems. The main features of this protocol are: i) precipitation with trichloroacetic acid/acetone overnight to prepare the acetone dry powder (ADP), ii) several additional steps of sonication in the ADP preparation and extractions with dense sodium dodecyl sulfate and phenol, and iii) adding two stages of phenol extractions.

Proteins were extracted from roots using this new protocol (Method B) and a protocol described in the literature for *T. cacao* leaves and meristems (Method A). Using these methods, we obtained a protein yield of about 0.7 and 2.5 mg per 1.0 g lyophilized root, and a total of 60 and 400 spots could be separated, respectively. Through Method B, it was possible to isolate high-quality protein and a high yield of roots from *T. cacao* for high-quality 2-DE gels. To demonstrate the quality of the extracted proteins from roots of *T. cacao* using Method B, several protein spots were cut from the 2-DE gels, analyzed by tandem mass spectrometry, and identified. Method B was further tested on *Citrus* roots, with a protein yield of about 2.7 mg per 1.0 g lyophilized root and 800 detected spots.

Key words: Cocoa; Plant protein extraction; Root; Two-dimensional gel electrophoresis