Protein precipitation method for salivary proteins and rehydration buffer for two-dimensional electrophoresis

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Abstract
Precipitants for salivary proteins and rehydration buffers for two-dimensional electrophoresis (2-DE) analysis were, respectively compared and evaluated. Five different protein precipitants: TCA, TCA-acetone-DTT, TCA-acetone-mercaptoethanol, acetone and alcohol were used to precipitate proteins of the saliva samples. The efficiency of the precipitants was evaluated from protein content of the precipitate reflecting protein recovery. The precipitate with the highest protein content was subsequently solubilized using different rehydration buffers (RBI, RB2, RB3 and RB4) before being subjected to the 2-DE. The efficiency of the different rehydration buffers was compared with respect to the resolution and focusing time taken to attain the maximum voltage. Each of the saliva samples was subjected to the above experiments, carried out in triplicates. The precipitant containing TCA-acetone-DTT exhibited the highest protein recovery (82.2%) demonstrating significant difference when compared with the other precipitants (p<0.05). The RB4 containing DTT (reducing agent) and 0.5% IPG buffer 3-10 non-linear (carrier ampholyte) exhibited more protein spots indicating better separation resolution. The results obtained suggested that protein recovery depends on the precipitant used in the precipitation and resolution of proteins separation is influenced by the reducing agent and the ampholyte used in the rehydration buffer. © 2008 Asian Network for Scientific Information.

Language of original document
English

Author keywords
Carrier ampholytes; Precipitants; Reducing agent; Salivary proteins

Index Keywords
Carrier ampholytes; Focusing time; Non-linear; Precipitants; Protein contents; Protein precipitation; Protein recovery; Protein spots; Salivary proteins; Two dimensional electrophoresis; Two-dimensional electrophoresis (2-DE)

Engineering controlled terms: Acetone; Body fluids; Electric potential; Electrolytes; Electrophoresis; Phase transitions; Precipitation (chemical); Reducing agents; Two dimensional

Engineering main heading: Proteins