AN ALTERNATIVE SOLUBILISATION BUFFER FOR RECALCITRANT PLANT PROTEINS

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Proteomics analysis has been routinely applied to study protein compositions of plants, animals and microorganisms in order to understand their cellular functions. One of the prerequisites for an effective proteomic analysis is a good protein solubility. However, extracts from plant materials, such as oil palm origin, contain interferences such as phenolics, polyphenol and lipids. One of the typical interferences is the inability of the proteins to dissolve completely after enrichment with trichloroacetic acid/acetone or ammonium acetate/methanol (Fan *et al*., 2009; Gomez *et al*., 2009; Hao *et al*., 2015). A complete dissolution of proteins is highly crucial to enable further downstream mass spectrometric analyses.

The use of different buffers, detergents and surfactants to dissolve proteins is strictly dependent on their compatibility with downstream proteomic analyses. Many studies have employed denaturing buffers containing urea and thiourea (urea/thiourea) to solubilise proteins from recalcitrant plant tissues (Gang *et al*., 2014; Wu *et al*., 2009). However, some of the major drawbacks of urea/thiourea are the additional carbamylation modification of *N*-termini and lysine residues, resulted in elevating the false discovery rate of identified proteins (Gundry *et al*., 2009; Scheerlinck *et al*., 2015). Meanwhile, less common detergents such as sodium deoxycholate has been primarily used to solubilise membrane proteins (Odahara, 2004; Masuda *et al*., 2008).

Our objective is to introduce the patented sodium deoxycholate buffer (DOC) (Malaysian Patent Application No. PI 2018701612) as an alternative to the routinely used urea/thiourea buffer in solubilising recalcitrant proteins after their extraction from plant tissues.

THE TECHNOLOGY

The composition of protein solubilisation buffer to be developed is a mixture of these components:

a) sodium deoxycholate;
b) dithiothreitol; and
c) tris (hydroxymethyl)aminomethane.

Sodium deoxycholate is a water-soluble, ionic detergent. It is widely incorporated as a component of cell lysis buffers (*e.g*. RIPA buffer). In the present technology, the stock solution of sodium deoxycholate is prepared in a concentration range of 10%-40%. Dithiothreitol or DTT, is frequently used to reduce the disulfide bonds of proteins and, more generally, to prevent formation of intramolecular and intermolecular disulfide bonds between cysteine residues of proteins. In the present technology, a stock solution of DTT is prepared at a concentration of 1%-4%. Tris (hydroxymethyl) aminomethane or Tris, is an organic compound which is used in biochemistry and molecular biology as a component of buffer solutions. In the present technology, the stock solution of Tris is prepared at a concentration ranging from 1 to 10 M.

NOVELTY OF THE PRODUCT

1. Sodium deoxycholate is incorporated as the key protein solubilisation agent to dissolve proteins. These proteins are not limited to membrane proteins but comprised of proteins from other plant compartments and tissues as well.

2. The new use of sodium deoxycholate would circumvent the need of removing or decreasing urea and thiourea concentration to a level that permits its compatibility with protein digestion using trypsin enzyme for mass spectrometry analysis (*Figure 1*).

3. Sodium deoxycholate has been experimentally proven to be compatible with tryptic digestion and thus, it would not interfere with the protein digestion. In addition, as reported previously, the addition of sodium deoxycholate enhances the digestion efficiency. Thus, the *dual functionality* of sodium deoxycholate are in the protein solubilisation and also enhancement of protein digestion for mass spectrometry analysis.

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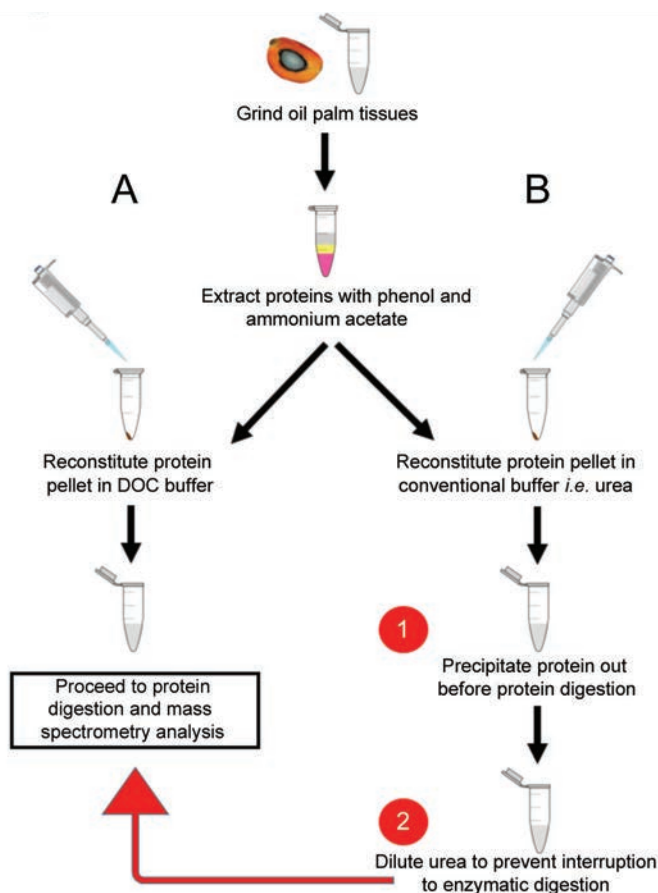

Figure 1. Comparison of workflows using A. Sodium deoxycholate buffer and B. urea/thiourea buffer (Lau et al.*, 2015). Additional steps (1 and 2) are required when urea/ thiourea buffer is used to prepare proteins for proteomics analysis.*

ECONOMIC ANALYSIS

Cost-benefit analysis was calculated to determine the viability of the technology.

Economic analysis	Value
Net presence value (NPV) @ 10%, RM	3 741 039
Internal rate of return (IRR), %	37.80
Discounted payback period (DPP)	4 years and 10 months
Benefit-cost ratio	1.38

IMPACT

The two buffers evaluated in this study were able to solubilise the proteins extracted from oil palm fruit mesocarps to a variety of extends. When chromatograms (*Figure* 2) and gene ontologies (*Figure* 3) of the proteins solubilised in urea/ thiourea and sodium deoxycholate buffers were compared, sodium deoxycholate was equally applicable to the oil palm mesocarp proteins. All the identified proteins from both buffers were categorised into the same biological processes, cellular components and molecular functions (*Figure 3*). The detailed statistical analysis on the oil palm proteomic datasets was in agreement with the mass spectrometric analyses that there were only minor differences between the urea/thiourea and sodium deoxycholate buffers (*Figure 4*).

CONCLUSION

This technology demonstrated the assessment of the utilisation of sodium deoxycholate in solubilising the whole mesocarp proteins from oil palm for proteomics studies. There were no significant variations in the chromatogram patterns, regardless of solubilisation buffers used. The mass spectrometric and statistical analyses had also concluded that sodium deoxycholate is applicable in oil palm mesocarp protein solubilisation and the efficiency is comparable to that of urea/thiourea buffer. Hence, the inexpensive sodium deoxycholate is a valuable solubilisation buffer in studying the oil palm mesocarp proteins using proteomic approach, and not limited to the application in membrane proteins and enhancement of protein digestion, nor plant tissues.

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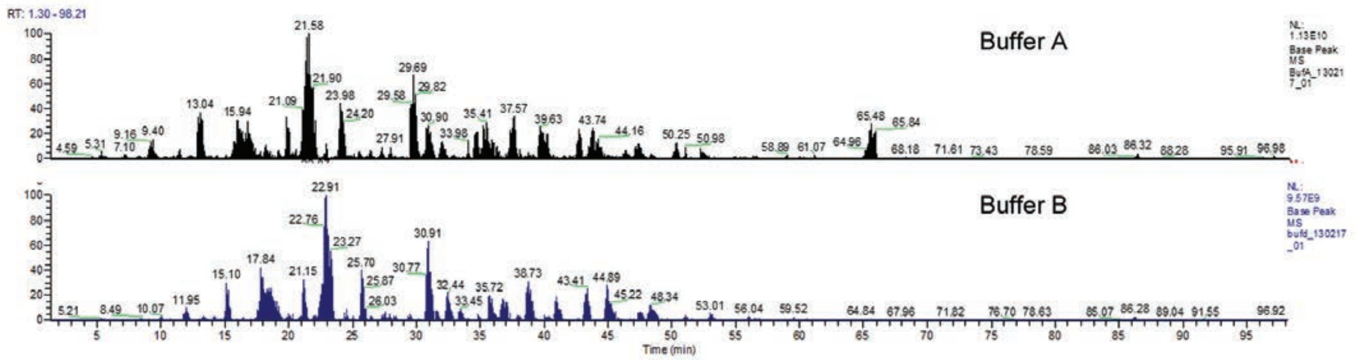

Figure 2. Chromatograms of peptides digested from the two solubilisation buffers (A and B). Buffer A: Urea/thiourea and Buffer B: Sodium deoxycholate.

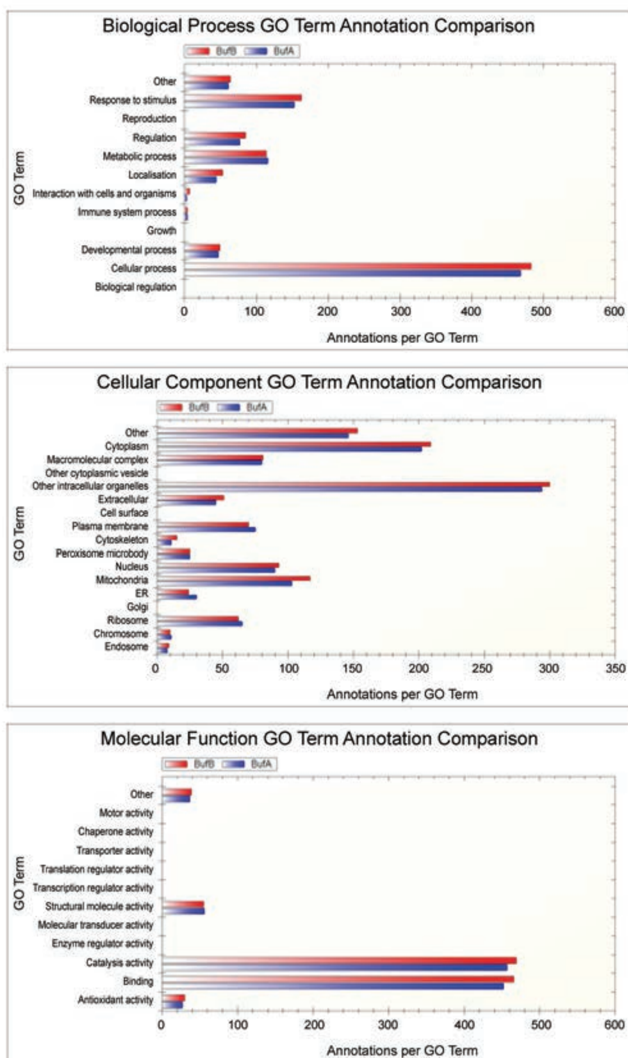

Figure 3. Comparison of biological process, cellular localisation and molecular activity of proteins solubilised in Buffer A (urea/thiourea) and Buffer B (sodium deoxycholate).

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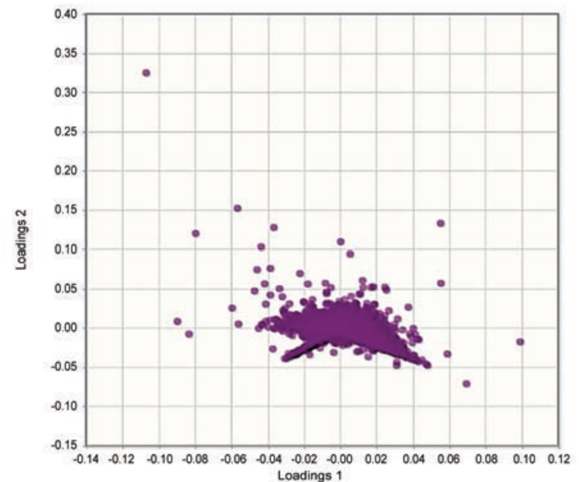

Figure 4. Loadings plot of the first two principal components from PLS-DA representing proteomics data from the evaluation of two solubilisation buffers: Urea/thiourea and sodium deoxycholate. Majority peptides showed no significant difference for each buffer group.

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