Gel and gel-free approaches for the quantitative characterisation of complex protein mixtures

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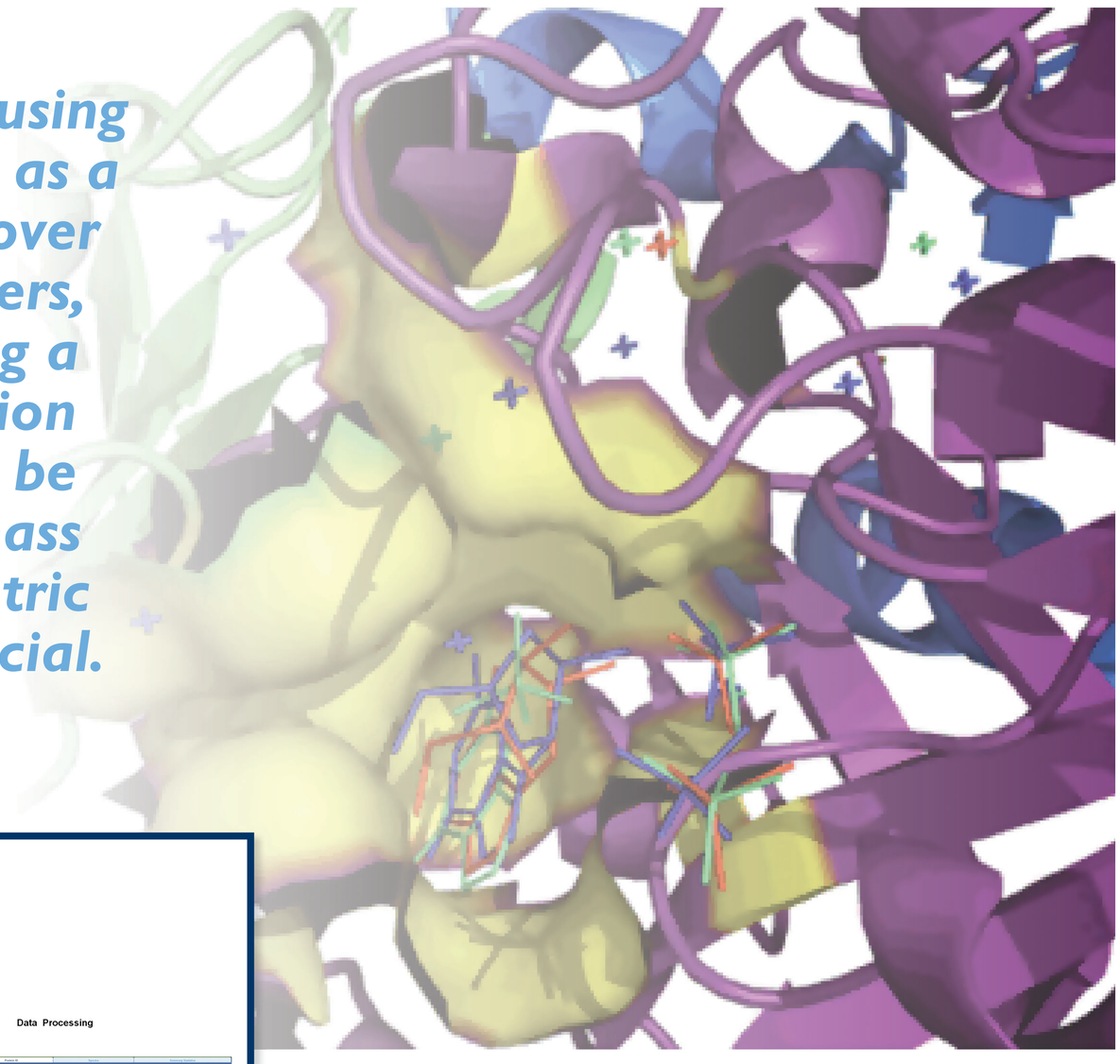
INTRODUCTION

Mechanisms involved in pathogenic processes are identified by evaluating cellular processes and their changes at a protein level. The problem with protein studies is that the proteome changes from cell to cell in response to various factors (e.g. environmental change, nutritional status and drug treatment)[1]. At the same time, the complexity of the proteome is increased by post-translational modifications and spliced isoforms that are especially common in multicellular organisms. This dynamic complexity has made it difficult to rapidly identify all proteins expressed in a cell or tissue[3]. Even though mass spectrometric analysis is capable of reliably identifying thousands of peptides, its capability relies heavily on the ability to resolve each species distinctly prior to mass spectrometric detection. A number of fractionation techniques are available, however, selecting the best one is usually a challenge for proteomics researchers[2].

OBJECTIVE

The research aims to establish a reliable set of methods for profiling proteins in a complex mixture in order to allow for the mining of low abundant species. To achieve this, several fractionation techniques were applied to samples of bovine hepatic tissue. These included two-dimensional gel electrophoresis (2DE gels), solution phase isoelectric focusing (IEF), offline strong cation exchange (SCX) chromatography and offline high pH reverse phase (RP) chromatography. All fractions collected from the solution-based methods were further separated via low pH reverse phase (RP) chromatography before being introduced for mass spectrometric analysis.

When using proteomics as a tool to discover biomarkers, selecting a fractionation method to be used prior to mass spectrometric analysis is crucial.



MATERIALS AND METHODS

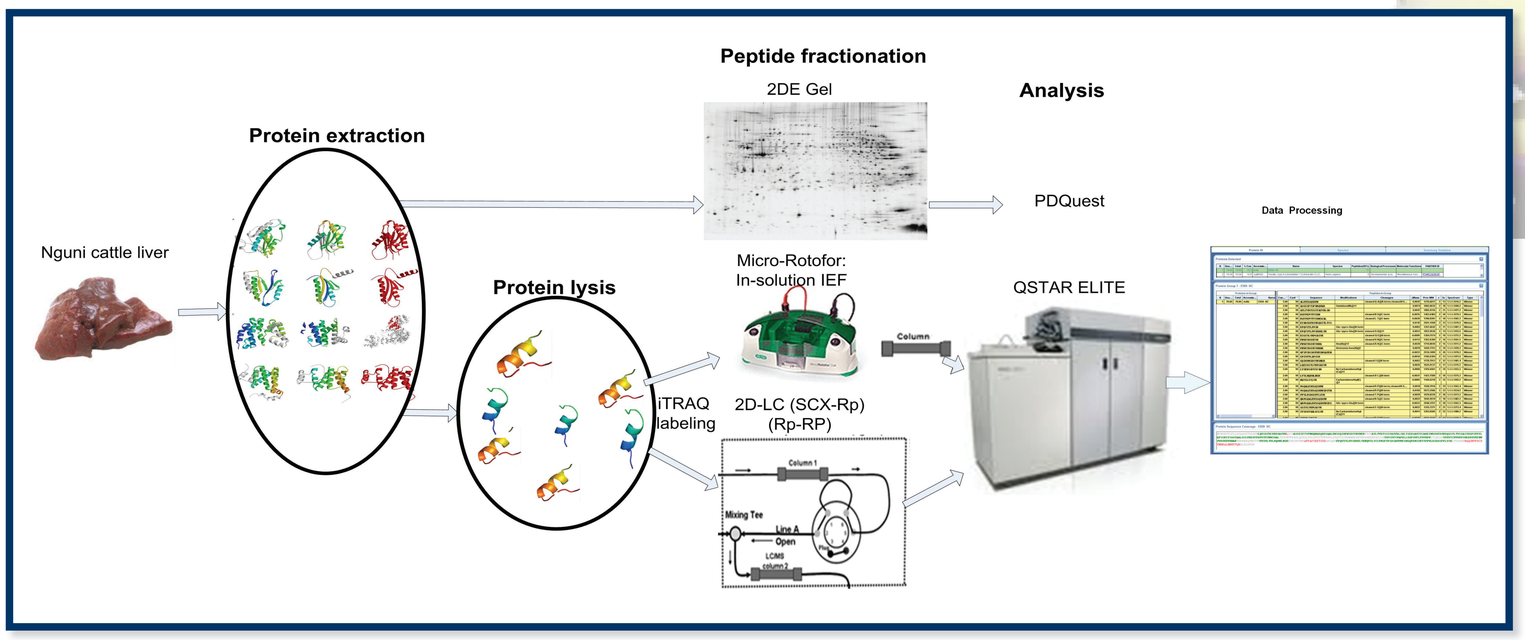


Figure 1: Study design to analyse a complex mixture of proteins extracted from hepatic tissue. To determine which fractionation method between 2DE gels, solution phase IEF, SCX-RP and RP-RP results in the highest number of protein identities

RESULTS

When the number of protein identities in the different fractionation techniques was compared (**Figure 2**), it was found that the RP-RP chromatography method resulted in the highest number. The 2DE gels that were stained with the Oriole fluorescent stain had the lowest number of protein followed by the SCX-RP 30 min gradient and 15 min gradient. The 2DE gel stained with Silver stain, all the Micro-Rotofor runs (50µg, first 100 µg and second 100 µg) and the 15 min gradient RP-RP chromatography runs, were all considered to have resulted in the same number of protein identities. Running 18 cm gels is more expensive than the 7 cm gels no matter which stain is used (**Figure 3**). The RP-RP method was found to be the most cost-effective method to run when the consumable cost and running time of the solution-based methods were compared (**Figure 4**).

DISCUSSION AND CONCLUSION

The 7 cm 2DE gel was the cheapest to run no matter which stain was used (without MS analysis), however, if ~20 gel spots are analysed on the MALDI-TOF, then the RP-RP method becomes cheaper to run. The results also indicated that the RP-RP method yielded the highest number of confident protein identifications.

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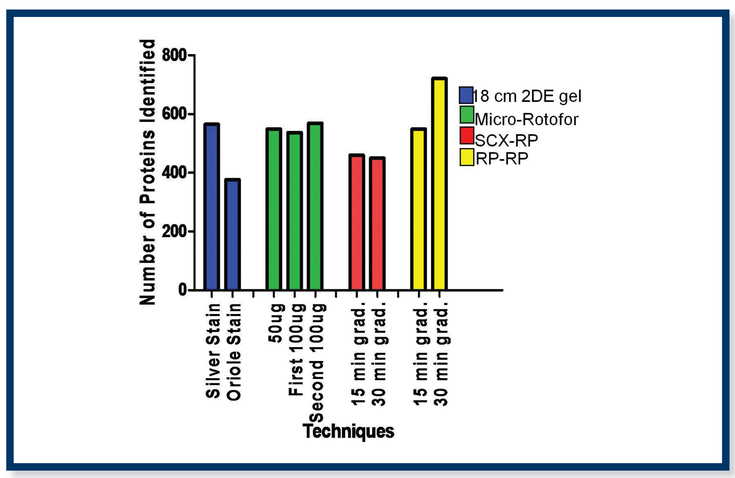

Figure 2: Number of proteins identified in gel and solution-based fractionation methods

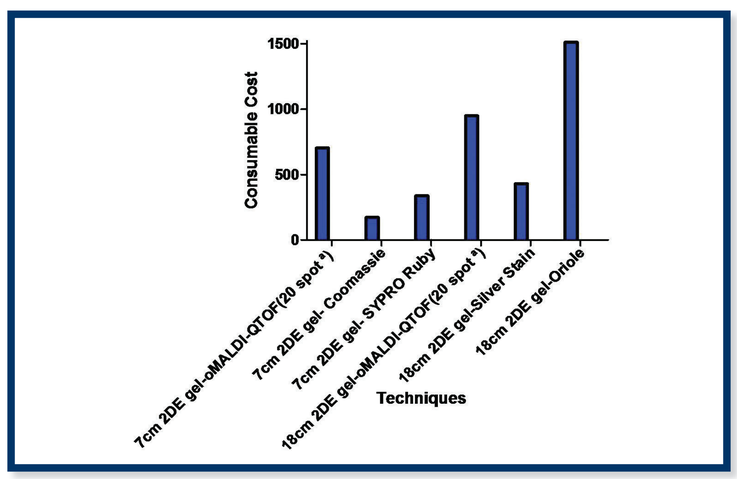

Figure 3: Consumable costs for 7 cm and 18 cm 2DE gels

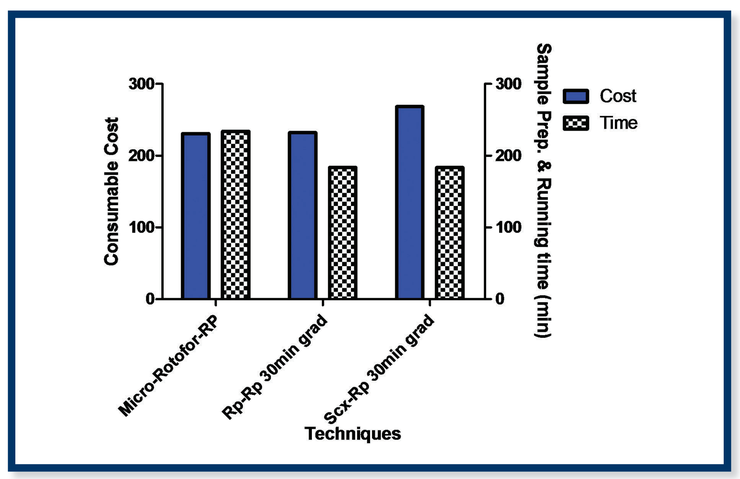

Figure 4: Comparison of consumable costs, sample preparation and running time between solution-based methods

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