SLAS2014

Evaluating the Efficacy and Reproducibility of Automated Homogenization Technologies

Drexel Neumann, Larry Maletta, Pete Tortorelli, James A. Atwood III
Omni International Inc., 935-C Cobb Place Blvd., Kennesaw, GA 30144, USA

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Overview

- Three homogenization platforms were evaluated for protein extraction from two tissue types
- Protein quantification measurements were compared to determine extraction reproducibility as a function of homogenization platform and tissue type.
- Proteins were separated by 1D-PAGE to evaluate protein repertoire as a function of homogenization platform

Introduction

Studies targeted at the analysis of proteins, nucleic acids, and small molecules typically start with a homogenization step to liberate the analytes of interest. Increasing amounts of data suggest that experimental variability and analytical sensitivity is in large part determined by differences in the extraction efficiencies of analytes through the homogenization process1. This is particularly true when studies involve large sample numbers, which represent not only an analysis bottleneck but an increased opportunity for error propagation. Here we evaluate the potential for an automated homogenization system to increase sample throughput and improve reproducibility by comparing two automated homogenization technologies to the widespread approach of hand held rotor stator based homogenization.

Methods

Samples: *Rattus norvegicus* liver and brain. Tissues were manually sectioned into twenty four 50 – 150 mg samples and diluted to 50 mg/mL in 100mM Tris-HCl, pH 7.6.

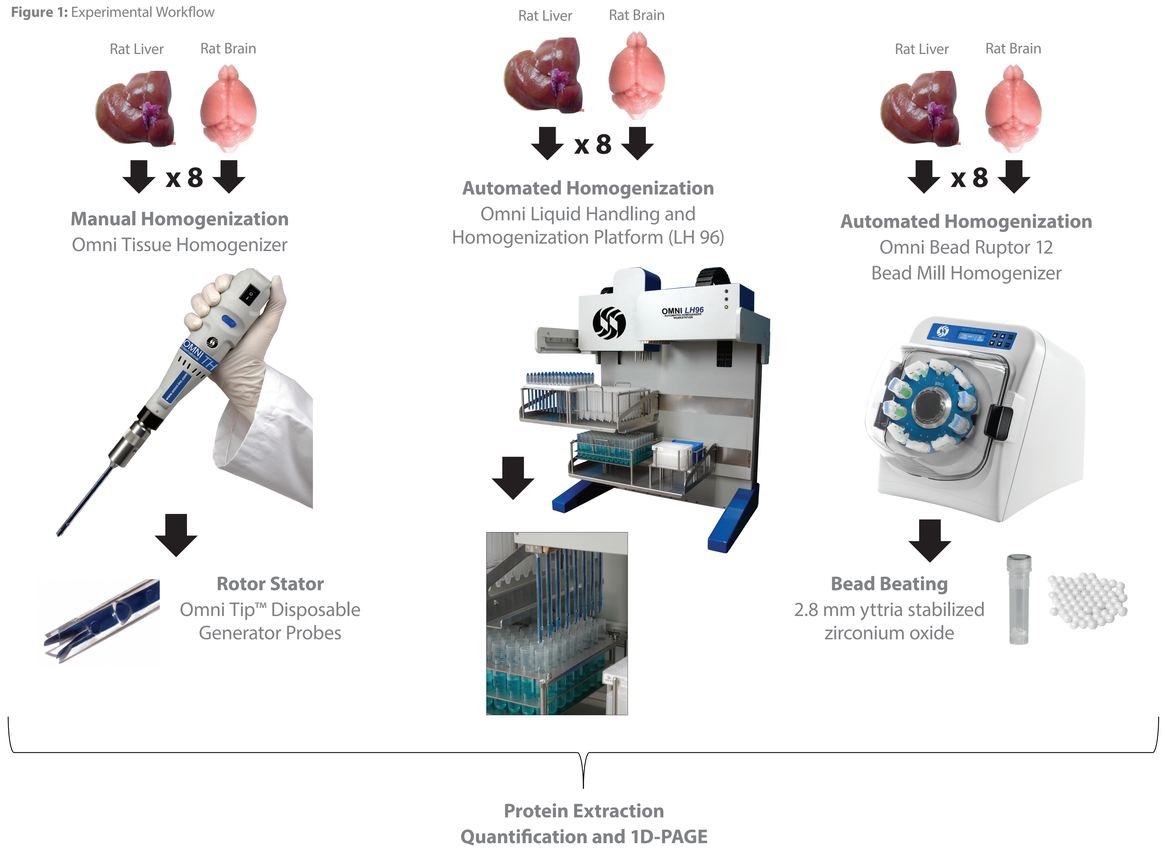
Homogenization: Eight samples of each tissue type were homogenized on one of three homogenization platforms. Manual rotor stator homogenization was performed using the Omni International **Tissue Homogenize**r (Cat# TH115) fitted with a 7 mm disposable Omni Tip™ (Cat# 3075H) generator probe operated at 30,000 rpm. Rotor stator homogenization was performed in a completely automated fashion using the Omni International **Liquid Handling and Homogenization Platform (LH96)** using 7 mm disposable Omni Tip™ generator probes operated at 30,000 rpm for 15 seconds. Lastly, samples were homogenized via bead beating on a medium setting on the Omni International **Bead Ruptor 12** in 2 mL polypropylene tube containing 5 x 2.8 mm yttria stabilized zirconium oxide beads (Cat# 19-628) for 45 secs. Post homogenization, homogenates were divided into two parts with half aliqouted for protein extraction and half aliquoted for DNA extraction.

Protein extraction: Insoluble material was pelleted by centrifugation at 8,500 x g for 5 minutes. The supernatants was then extracted for quantification and 1D-PAGE.

Protein Quantification: 1µl of each supernatant was analyzed in triplicate to determine protein yields at 280 nm on a NanoDrop spectrophotometer (Thermo Fisher Scientific).

Protein Separation: 10 µL of each supernatant was mixed with 5 µL of Laemmli Sample Buffer (BioRad), heated to 95°C for 5 minutes then separated on a 4-20% TGX Tris-Glycine SDS gel for 30 minutes at 200 V in a Mini-PROTEAN Tetra Cell (BioRad). Proteins were then stained with Coomassie G-250 for 1 hr, destained overnight and visualized analyzed under white light in a Gel Doc EZ system (BioRad).

Figure 1: Experimental Workflow



Results

Two tissue types were homogenized in multiple replicates using a traditional rotor stator manual homogenizer and two automated homogenization platforms, the LH 96 and Bead Ruptor 12 to evaluate protein yield and reproducibility. Protein abundance measurements (Figure 2-3) indicated that both bead mill and rotor stator technologies enabled the extraction of proteins at acceptable yields for downstream applications such as mass spectrometry. Bead Milling resulted in an protein yield average percent standard deviation of 22.5% while rotor stator based homogenization decreased the percent standard deviation to 12% across both tissue types.

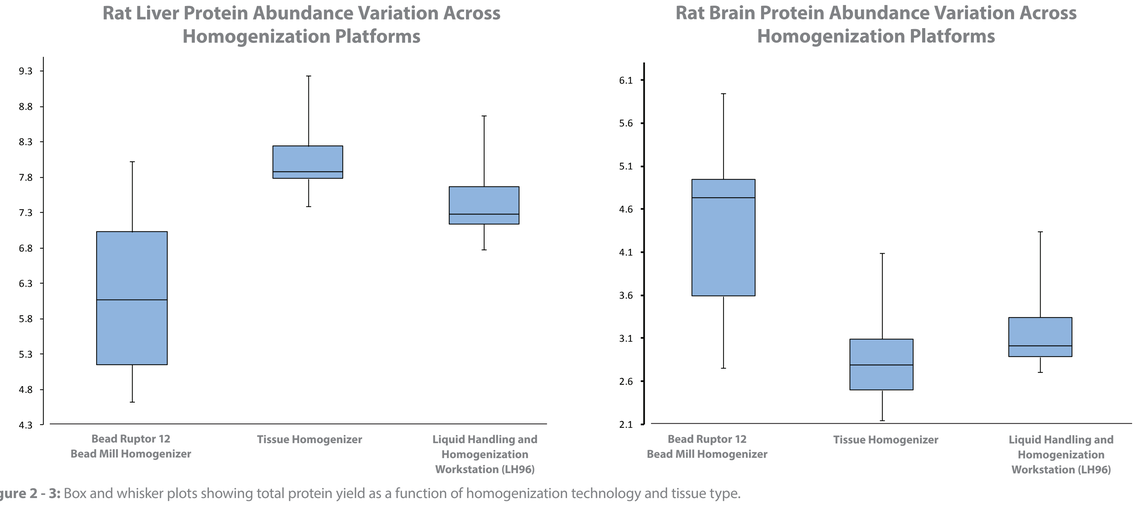


Figure 2 - 3: Box and whisker plots showing total protein yield as a function of homogenization technology and tissue type.

Results

In addition to protein yield measurements, sample processing times were recorded for each instrument platform.

Table 1: Sample throughput by instrument

Instrument	Samples/Cycles	Time/ Cycle	Total Time
Bead Ruptor 12	12	45 sec.	45 sec.
LH96	8	15 sec.	15 sec.
Tissue Homogenizer	1	30 sec.	4 min.

Extracted proteins were lastly separated by electrophoresis to evaluate the total protein repertoire and to determine the level for reproducibility associated with each homogenization technology (Figures 4- 9). Electrophoresis revealed that samples prepared by a single homogenization technology had a high degree of lane-to-lane reproducibility. While little sample-to-sample variation was observed between the rotor stator based platforms (TH and LH 96), sample homogenization through bead milling on the Bead Ruptor 12, while producing a comparative protein yield did result in the appearance of unique protein banding.

Figure 4: Sample: Liver
Instrument: Tissue Homogenizer

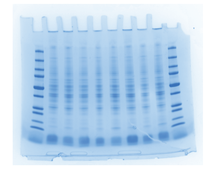


Figure 5: Sample: Brain
Instrument: Tissue Homogenizer

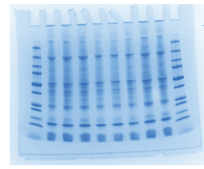


Figure 6: Sample: Liver
Instrument: Liquid Handling and Homogenization Workstation

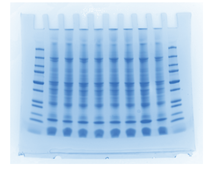


Figure 7: Sample: Brain
Instrument: Liquid Handling and Homogenization Workstation

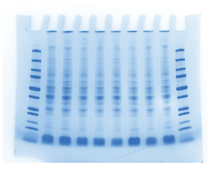


Figure 8: Sample: Brain
Instrument: Bead Ruptor 12

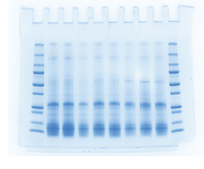
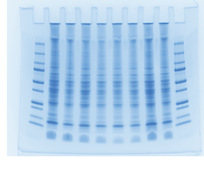


Figure 9: Sample: Brain
Instrument: Bead Ruptor 12



Conclusions

- Homogenization can be automated while ensuring high protein yields and reproducibility
- Homogenization via rotor stator technologies resulted in the lowest protein yield variation in this study.
- For soft tissues both automated rotor stator and bead milling technologies represent attractive solutions for increasing sample throughput

References

1. Piehowski P, *et al.* J Proteome Res. 2013. 12, 2128-2137.