APPLICATION NOTE #20144

# Bead Mill Based Plant Protein Extraction Efficiency is not Dependent upon Starting Sample Quantity

Maliha Ishaq, Drexel Neumann, James A. Atwood PhD

Omni International, Kennesaw, Ga.

Recent advances in bead milling technology, including the Bead Ruptor Elite has enabled the rapid and efficient lysis of plant material. The Bead Ruptor Elite supports bead mill based homogenization of samples in volumes ranging from 0.5 mL to 50 mL. Bead milling in the Bead Ruptor Elite for volumes from 0.5 mL to 30 mL is achieved through a vertical intra-tube bead motion which induces high bead impact forces to rapidly dissociate samples. For larger sample volumes up to 50 mL, bead milling in the Bead Ruptor Elite is achieved through a spiral grinding motion as the 50 mL tube is positioned horizontally. The study herein seeks to determine if intra-tube bead movement effects sample disruption and protein recovery from plant samples processing in 50 mL, 30 mL, and 7 mL tubes.

## Materials and Methods

Two grams of fresh peas and spinach were added to 7 mL (Cat # 19-678), 30 mL (Cat #19-6358), and 50 mL (Cat# 19-345-050) bead tubes filled with 1.5 g, 7 g and 15 g ceramic bead media respectively, in triplicate. Samples in 30 mL and 50 mL volumes were diluted to a concentration of 100 mg/mL in 50 mM Tris-HCl, pH 7.6. In the case of samples in 7 mL tubes, the samples were diluted at a ratio of 200 mg/mL due to volume constraints. Pea samples were homogenized in the Bead Ruptor Elite for 30 seconds at 5 m/s while spinach samples were homogenized for 1 min at 5 m/s. In all cases, full homogenization was achieved in less than one minute. One milliliter from each sample was taken to be representative of the sample and placed in a 1.5 mL microtube and centrifuged at 8,000 g for 8 min. The supernatant was then removed and transferred to a 1.5ml microtube, followed by vortexing. Protein concentrations were determined through absorbance at 280 nm using a Nanodrop spectrophotometer. Samples were analyzed in triplicate. 15 µl of each pea protein sample was then mixed with 10 µl of Laemmli sample buffer and heated at 90°C for 10 mins. Proteins were then separated by electrophoresis on a 4-20% Tris Glycine SDS PAGE gel at 200 V for 30 minutes. Proteins were stained with coomassie and visualized on a Gel Doc EZ system (BioRad).

## Results

For mechanical homogenization methods such as bead beating, tube orientation can have a significant impact on processing efficiency. The Bead Ruptor Elite is flexible with tube carriages capable of carrying tubes ranging from 0.5 mL to 50 mL (Figure 1 tube carriages). The Bead Ruptor 24 50 mL tube carriage is unique in that the tubes are oriented horizontally relative to the tube carriage plate. This position impacts the intra-tube bead motion, creating a spiral grinding motion in which the beads move along the edges of the tube walls during processing. The goal of this study was to evaluate the homogenization efficiency of this bead motion for common plants. Total protein extraction yields indicated that there was no effect associated with the modified bead motion when homogenizing peas and spinach in 7 mL, 30 mL or 50 mL tubes (Figure 2). Protein extractions yields were consistent run-to-run and full homogenization was achieved in less than 1 minute in all cases.

In order to evaluate the soluble protein repertoire, pea protein samples were further analyzed by SDS-PAGE (Figure 3). Reproducible protein bands were observed across all samples indicating good homogenization reproducibility across varying tube sizes.

**Figure 1** Tube Carriages

*96 Strip Well Tube Carriage*

*1.5 mL Microtube Carriage*

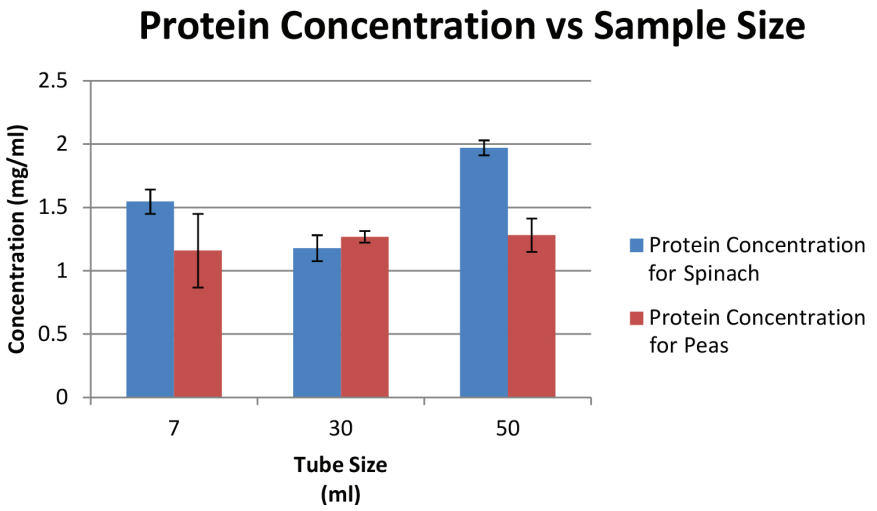
*2 mL Tube Carriage*

*7 mL Tube Carriage*

*30 mL Tube Carriage*

*50 mL Tube Carriage*

OMNI International
The Homogenizer Company®





*Bead Ruptor Elite*

**Figure 2.** Protein yields from pea and spinach as a function of tube size: Normalized pea and spinach samples were homogenized on the Bead Ruptor Elite in varying tube sizes and protein concentrations were determined.

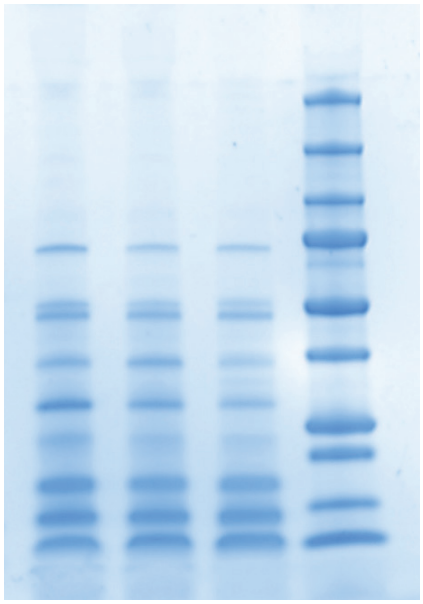


**Figure 3.**
Pea protein extraction analyzed by SDS PAGE. Lane 1: 7 mL tube. Lane 2: 30 mL tube. Lane 3. 50 mL tube. Lane 4: Protein ladder

## Conclusion

The Bead Ruptor Elite supports bead milling in large volumes up to 50 mL. With increased capacity the system can support homogenization of larger plant based samples. Bead mill homogenization in the Bead Ruptor Elite is rapid and efficient across a variety of tube sizes producing highly reproducible protein yields.

OMNI International
The Homogenizer Company®

935-C Cobb Place Blvd. NW
Kennesaw, GA 30144
800.776.4431 • 770.421.0058
**www.omni-inc.com**

CERTIFIED ISO 9001:2008 COMPANY