



PROTEIN EXTRACTION EFFICIENCY OF SOFT AND HARD SEEDS USING THE PRECELLYS LYSING KITS

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/ CONTEXT

Sample preparation for protein extraction is a critical first step to achieve reliable analytical results. In this study we compared different bead kits using two different suspension liquids and two kinds of plant organ, soft and hard seeds, namely peanut and rice, respectively.

/ MATERIALS

- Precellys®24 protocol: 6500rpm, 3x60s, 20s pause in between cycles
- Precellys lysing kits: CK14, CK28-R, MK28-R, CKMix50-R 2mL tubes
- Soft seed: One cotyledon of peanut (410 mg)
- Hard seed: ten seeds of rice (220 mg)
- Suspension media: saline buffer (1M NaCl, 50 mM Tris, pH 8.5) vs. ddH2O (control), 1ml/tube
- Centrifugation at 18000rpm, 25min, 4°C (Microfuge®18 Beckman) separated the top oil phase from the fiber/beads pellet (bottom phase), leaving a medium aqueous phase, which was used for protein quantification (analyzed by the BCA assay). Mean values were derived from triplicate samples.

/ RESULTS

Lysing Kits	Peanuts (%)	Rice (%)	Number of proteins identified from rice using ESI-MS-MS**
MK28-R	36.98	6.41	212 IDs
CK28-R	38.55	6.11	202 IDs
CKMix50-R	41.96	8.03	178 IDs
CK14	42.08	8.25	151 IDs

Table 1. Protein extraction efficiency as a percentage of total protein content, using saline extraction buffer. Extraction efficiency is based on the theoretical amount of protein, 25.8g, 6.12g/100g of peanut, rice respectively. (USDA National Nutrient Database for Standard Reference Release 26, Feb 7, 2014. <http://ndb.nal.usda.gov/ndb/foods>).

**A MudPIT method for LC-MS/MS was used to identify proteins from rice (Delahunty CM, Yates JR. MudPIT: multidimensional protein identification technology.

Biotechniques 2007; 43: 563-569).

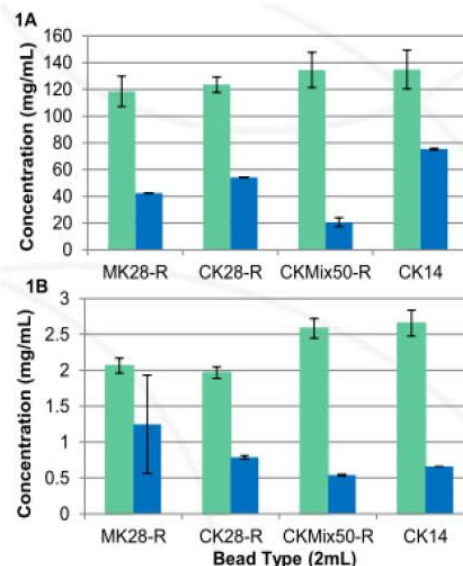


Figure 1. Protein concentration was quantified in the medium aqueous phase after peanut (A) and rice (B) homogenization in saline buffer (green bars) versus ddH2O (blue bars) using 4 different lysing matrices.

Protein extraction efficiency in peanuts and rice was compared after bead-beating with 4 different lysing kits. The use of saline buffer increased protein extraction efficiency as expected (Robert LS, Nozzolillo C, Altosaar I. Homology between legumin-like polypeptides from cereals and pea. Biochem J 1985; 226: 847-852). The CK14 beads gave the best protein yields compared to the other lysing beads for both hard and soft seeds.

/ CONCLUSION

Protein extraction efficiency from rice and peanuts was validated using ceramic (CK14, CK28-R, CKMix50-R 2mL) and stainless steel (MK28-R 2mL) lysing beads. The smallest bead diameter (CK14, 1.4 mm) correlated with the highest protein yields. The Precellys24 is a high-throughput homogenizer that can generate high quality extracts for proteomics when coupled with the right lysing kit.

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