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 & METHODS**

- **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**

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

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).**

**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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