Maize Endosperm Protein Extraction and Analysis

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[Abstract] Alcohol-solubility is the most characteristic feature of the zein proteins, the major storage protein in maize. Using sodium borate buffer system with added reducing agent, total proteins are isolated, and zein proteins are separated from non-zein proteins. The extraction effect is intuitive on a SDS-PAGE isolation system. In addition, a simple and rapid approach to extract zeins is introduced, taking full advantage of alcohol-solubility of zeins directly.

Materials and Reagents

1. Mature corn kernels
2. Petroleum ether
3. Ethanol
4. β-mercaptoethanol
5. Sodium dodecyl sulfonate (SDS)
6. Urea
7. Glycerol
8. HCl
9. Tris(Hydroxymethyl)aminomethane (Tris)
10. Phenylmethanesulfonyl fluoride (PMSF)
11. Methylene diacrylamide
12. Bromophenol blue
13. CHAPS (Sigma-Aldrich, catalog number: V900480-5G)
14. Dithiothreitol (DTT)
15. Liquid nitrogen
16. ddH_2O
17. SDS-PAGE gel (15% separation)
18. Coomassie brilliant blue (R250) staining buffer
19. 30% acrylamide (see Recipes)
20. Sodium borate buffer (see Recipes)
21. 5x protein loading buffer (see Recipes)
22. IPG solution (see Recipes)

Equipment

1. A mortar and pestle
2. Centrifuge (Eppendorf, model: 5415D)
3. Shaker (Zhicheng, model: ZHWY-111C)
4. Concentrator plus (Eppendorf, catalog number: 5305000.193)
5. Gel Doc^{TM} XR + System (Bio-Rad, catalog number: 170-8195)

Procedure

A. Using sodium borate buffer system to extract zeins

1. Soak 3-5 mature corn kernels in ddH_2O for 10 min, then remove the pericarp and embryo and dry the kernels for 10 min at 37 °C.
2. Grind kernels into powder using a mortar and pestle within liquid nitrogen.
3. Transfer the powder into a 2 ml Eppendorf tube. Dry it in Concentrator plus for 1 h till achieve constant weight.
4. Add 1 ml petroleum ether. Vortex and place in the shaker at 250 rpm for 1 h.
5. Centrifuge for 15 min at 12,000 rpm at room temperature (RT) and discard the supernatant.
6. Dry it in Concentrator Plus for 1.5 h until no smell of organic liquid is detectable.
7. Fill a new 2 ml eppendorf tube with 50 mg dried powder from the step 6.
8. Add 1 ml sodium borate buffer and 20 µl β-mercaptoethanol as well as 1% PMSF. Mix and incubate with shaking at 250 rpm for at least 2 h at 37 °C.
9. Centrifuge for 15 min at 12,000 rpm at RT.
10. Transfer 300 µl supernatant into a new 2 ml eppendorf tube as total protein extraction (Fraction A).
11. Transfer another 300 µl supernatant from the step 9 into a new 2 ml eppendorf tube, and add 700 µl ethanol as well as 1% PMSF. Mix with shaking at 250 rpm for 2 h at RT.
12. Centrifuge product from step 11 for 15 min at 12,000 rpm at RT.
13. Transfer 400-500 µl supernatant into a new 2 ml eppendorf tube and dry it in Concentrator plus for 2-3 h. Resuspend it in 200 µl IPG as zein proteins extraction (Fraction B).
14. Wash the precipitate in step 12 with 70% ethanol twice.
15. Centrifuge for 15 min at 12,000 rpm at RT.
16. Discard the supernatant and air-dry the precipitate until the edges become transparent. Resuspend it in 200 µl IPG as non-zein proteins extraction (Fraction C).

17. Add 10 µl protein extraction from fraction A, or B, or C (see the attached corresponding pictures), 1.5 µl DTT and 3 µl 5x Protein loading buffer in a new 0.2 ml eppendorf tube. Heat 10 min at 99 °C for denaturation.
18. Load 2-5 µl denatured protein sample and perform the SDS-PAGE on a 15% separation gel.
19. Afterwards, the gel is stained with Coomassie brilliant blue R250 (Figure 1, Figure 2, and Figure 3).

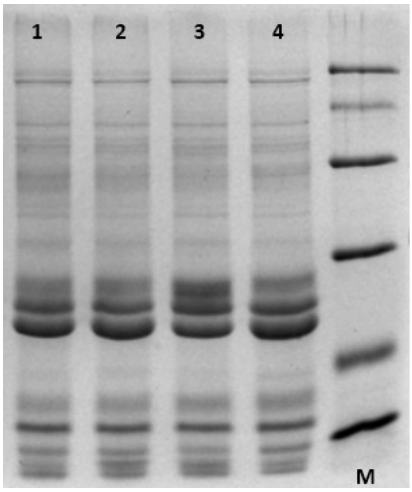

Figure 1. SDS-PAGE (Fraction A). M represents protein standards with molecular weight ranging from 14,400 to 97,400 Da (similarly hereinafter). This figure shows total proteins extraction. 1, 2, 3, 4 represent four different maize cultivars.

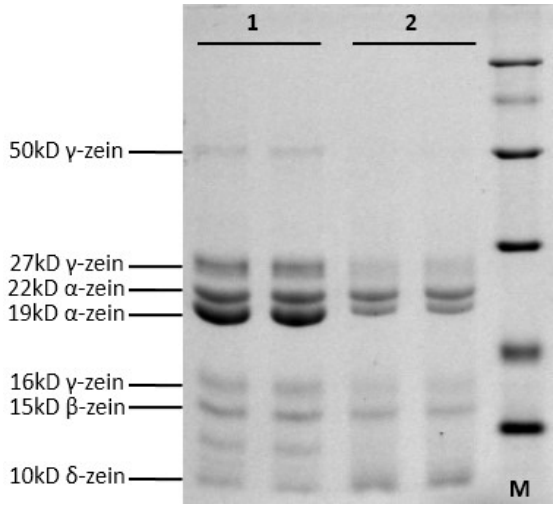

Figure 2. SDS-PAGE (Fraction B). This figure shows zein protein extraction. 1, 2 represent two different maize cultivars (two replicates for each maize cultivar).

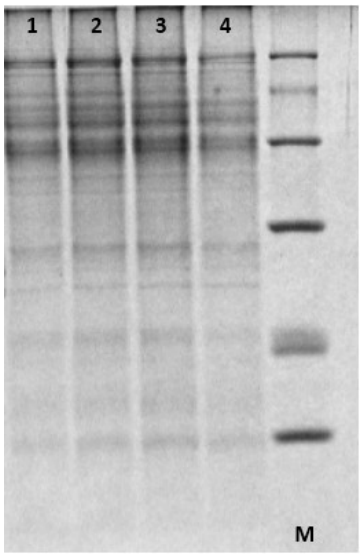

Figure 3. SDS-PAGE (Fraction C). This figure shows non-zein protein extraction. 1, 2, 3, 4 represent four different maize cultivars.

B. Simple and rapid extraction approach

1. Soak 3-5 mature corn kernels in ddH_2O for 10 min, then remove the pericarp and embryo and dry the kernels for 10 min at 37 °C.
2. Grind kernels into powder using a mortar and pestle within liquid nitrogen.
3. Transfer the powder into a 2 ml eppendorf tube. Dry it in Concentrator plus for 1 h till achieve constant weight.
4. Fill a new 2 ml eppendorf tube with 50 mg dry powder from step 3.
5. Add 400 µl 70% ethanol and 8 µl β-mercaptoethanol as well as 1% PMSF.
6. Mix and incubate for at least 2 h at RT. Invert the cube 2-3 times during incubation.
7. Centrifuge for 10 min at 1,300 rpm at RT.
8. Transfer 100 µl supernatant into a new 2 ml eppendorf tube.
9. Add 10 µl 10% SDS, and mix by pipetting.
10. Dry it in Concentrator plus for 1 h.
11. Add 200 µl ddH_2O for elution.
12. SDS-PAGE is performed in 15% polyacrylamide gels, and the gels are stained with Coomassie brilliant blue R250 (Figure 4).

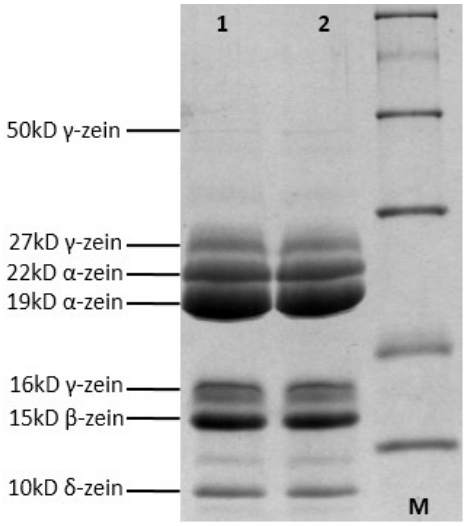

Figure 4. SDS-PAGE (zein proteins). This figure shows zein proteins through simple and rapid extraction approach. 1, 2 represent two replicates of same maize cultivar.

Recipes

1. 30% acrylamide (500 ml aqueous solution)
   145 g acrylamide
   5 g methylene diacrylamide
2. Sodium borate buffer
   12.5 mmol/L sodium borate
   1% SDS
   pH 10.0
3. 5x Protein Loading Buffer
   60 mmol/L Tris-HCl (pH 6.8)
   25% glycerol
   2% SDS
   0.1% bromophenol blue
4. IPG solution
   8 mol/L urea
   2% CHAPS

References

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