

Case study - Citrus

Isolation of citrus allergen using FastPrep-24™ 5G homogenizer.

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Introduction

Allergy is a hypersensitivity disorder of the immune system. According to epidemiological studies, at present, 20-30% of the population in many countries around the world suffers from allergies, and this percentage is growing trend.

Allergens are proteins with a broad range of molecular weights (5-50 kDa) exhibiting different features of solubility and stability, able to cause IgE-mediated hypersensitivity after contact with the immune system.

The development of new types of allergy treatments needs diverse and well-characterized allergenic source materials. This study describes an effective method for allergen characterization.

Overview

- **Keywords:** Allergy, citrus, allergen isolation, citrus homogenization
- **Aim of the study:** identification of grinding method for citrus allergen isolation
- **Application:** West blot analysis
- **Sample type:** Citrus
- **Sample name:** Green lemon, yellow lemon, orange, grapefruit
- **Material:** FastPrep-24™ 5G, CoolPrep adapter, 2ml Lysing Matrix A, C & E tubes, IKA grinder
- **Buffer:** PBS

Protocol and Parameters

1. Add citrus peel and pulp with 500µl of PBS in 2 ml Lysing Matrix A, C or E tubes.
2. Load Lysing Matrix tubes in a CoolPrep Adapter containing dry ice.
3. Process with the FastPrep-24™ 5G: 40 sec at a speed setting of 6.0 m/s.
4. Centrifuge the Lysing Matrix tubes 20 min at 18.000 x g, 4 °C to pellet debris.
5. Keep the supernatant at -20 °C prior to analyses.

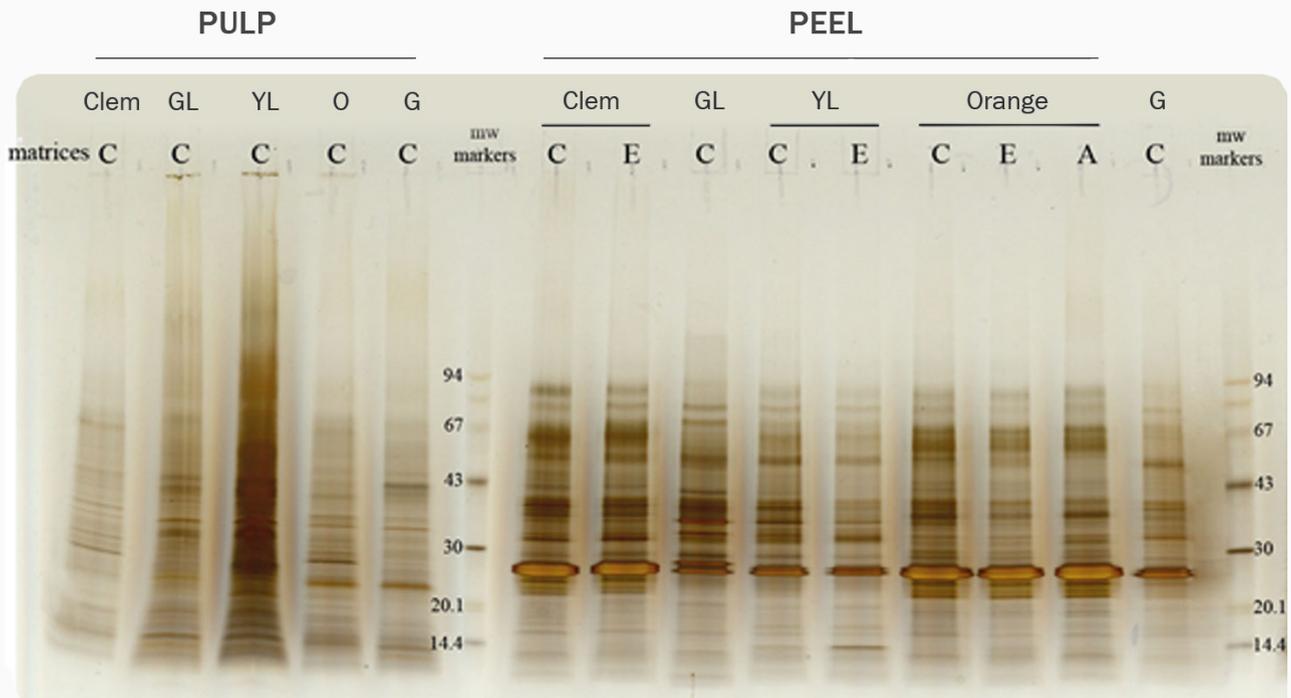


Results

Comparison of citrus protein extraction with different lysing matrix:

Citrus protein extracts with FastPrep-24™ 5G and lysing matrix A, C and E: Pulp, Peel.

(Clem: clementine, GL: green lemon, YL: yellow lemon, O: orange, G: grapefruit)
Protein migration on SDS-PAGE 8-18% acrylamide gradient. Staining with silver nitrate.



Conclusion

- Protein extraction from peel and pulp of citrus samples with the FastPrep-24™ 5G showed to be highly effective with the 3 types of lysing matrix tested.
- The protein yield with IKA grinder method was very low with high concentration of pectins, preventing protein migration on SDS- PAGE gel.
- The effectiveness of the FastPrep® method is **quantitative, higher protein yield, and qualitative**, wide variety composition of protein extracts **pectins free**.
- The FastPrep® system is a powerful tool to get rapidly and with a very **high reproducibility protein extracts** ready for electrophoresis (SDS-PAGE) analysis.
- Protein extracted with the FastPrep-24™ 5G instrument have conserved their immunoreactivity.

Successful sample preparation using the MP Biomedicals FastPrep® product line has been highlighted in thousands of scientific articles. To access articles and other materials, visit www.mpbio.com/FastPrepLibrary.



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