

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

CASE STUDY

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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 suffer from allergies, and this percentage is growing.

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 treatment require diverse and well-characterized allergenic source materials. This study describes an effective method for allergen characterization.

Overview

Keyword: Allergen, citrus, allergen isolation, citrus homogenization

Aim of the study: Identification of grinding method for citrus allergen isolation

Application: Western blot analysis

Sample name: Green lemon, yellow lemon, orange, grapefruit

Sample type: Citrus

Material: FastPrep-24™ 5G instrument, CoolPrep adapter, 2mL Lysing Matrix A,C & E tubes, IKA grinder

Buffer: PBS

Protocol and Parameters

1. Add citrus peel and pulp with 500µg of PBS in a 2mL Lysing Matrix A,C, or E tube.
2. Load the Lysing Matrix tubes in a CoolPrep Adapter containing dry ice.
3. Process with the FastPrep-24™ 5G instrument at a speed setting of 6.0 m/s for 40s.
4. Centrifuge the Lysing Matrix tubes for 20 min at 18,000 x g, 4  C to pellet debris.
5. Keep the supernatant at -20  C prior to analysis.

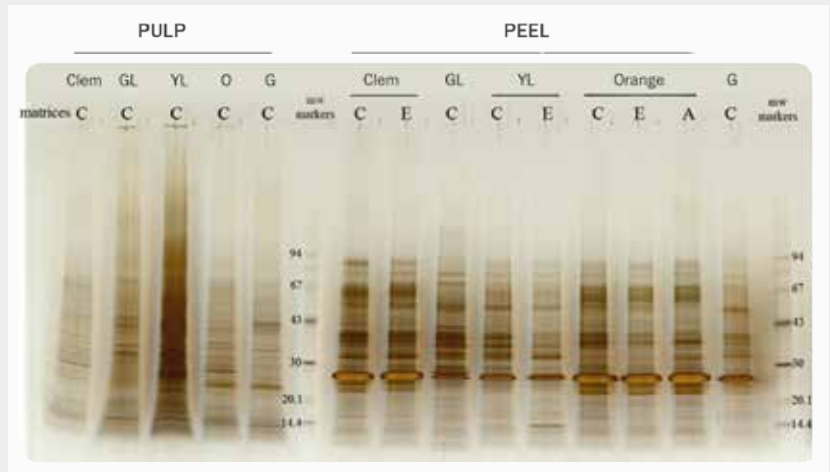


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Results

Comparison of Citrus Protein Extraction with Different Lysing Matrix:

- Citrus proteins extracted 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 instrument 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® system is quantitative, higher protein yield and qualitative, wide variety composition of protein extracts pectins free. The FastPrep-24™ 5G system is a powerful tool to get rapidly and with a very high reproducibility protein extracts ready for electrophoresis (SDS-PAGE). Protein extracted with the FastPrep-24™ 5G instrument have been conserved their immunoreactivity.



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