Sample Preparation Procedure for

Plant Products

General Information

The methods in this protocol are modified from the CVUA Stuttgart standard sample preparation procedure for meat, Organic Solvent Extraction (OSExtr), using MALDI-TOF MS. The modified methods improve the quality of mass spectra from plants and plant-based food products. The protocol is based on student projects completed during internships at the CVUA Stuttgart.

A comprehensive collection of reference spectra (msp) and single spectra generated using this protocol is available for exchange on the MALDI-UP homepage (https://maldi-up@ua.bw.de).

Field of application

- All plant parts, including leaf, fruit, seed, flower, stipe,…
- Unmixed plant products and separable plant materials in processed plant products, e.g. fresh and frozen fruits, dried and preserved plant products,…
- Plant-based milk products, e.g. yogurt from berries and nuts.

Materials

- Organic solvent (OS)
  (60% acetonitrile, 37.5% double-distilled water, 2.5% trifluoroacetic acid)
- α-Cyano-4-hydroxycinnamic acid (HCCA) solution (saturated in OS)
- 70% ethanol (70% ethanol, 30% double-distilled water)
- Cold (4°C) acidified acetone (97.5% acetone, 2.5% trifluoroacetic acid)

Methods

1. Optional Washing Step prior to Extraction

- Transfer sample materials in a 1.5 mL reaction tube containing approximately 200 µL 70% ethanol
- Mix for 20s using a vortex mixer and incubate for 5min at RT
- Decant and properly dispose the supernatant
- Collect and clean sample materials with Kimtech wipes
- Air-dry briefly
- Transfer sample materials in another reaction tube for further analysis

Applicable for sample material concentration and for cleaning sample materials from undesirable substances in complex mixtures, e.g. seeds from fruit, fruit pulp, fruit purée, fruit juice or fruit yogurt. Skip this step if irrelevant.

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2.1. Plant Extraction Procedure (Plant-Extr)

- Transfer an approximately 0.25 mm^2 piece of sample material into a 1.5 mL reaction tube containing 200 µL OS
- Grind finely with a micro pestle
- Mix for approximately 20s with a vortex mixer
- Centrifuge for 2min at 14.500 rpm
- Deposit 1 µL of the supernatant and mix immediately with another 1 µL of HCCA onto a target spot
- Air-dry thoroughly
- Deposit 1 µL of HCCA onto the dried sample spot and allow to dry thoroughly

General protein extraction procedure applying for all kinds of plant sample materials.

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2.2. Modified Plant Extraction Procedure using Ribulyser (Plant-Extr-R)

- Transfer an approximately 0.25 mm^2 piece of sample materials into a 2.0 mL PCR screw capped micro tube containing 200 µL OS
- Homogenize with Ribulyser (TeSeE™ PRECESS 24™ Homogenizer) for 30s at 6200 rpm
- Centrifuge for 2min at 14.500 rpm
- Deposit 1 µL of the supernatant and mix immediately with another 1 µL of HCCA onto a target spot
- Air-dry the sample spot

- Deposit 1 µL of HCCA onto the dried sample spot and allow to dry thoroughly

Alternative protein extraction procedure applying for all kinds of plant sample materials, especially for materials hardly ground with the micropestle, and reducing the laboratory workload. Comparable measuring results to Plant-Extr.

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2.3. Acetone Precipitation of Proteins modified from Plant Extraction Procedure using Ribulyser (Plant-Extr-AceR)

- Transfer an approximately 0.25 mm² piece of sample material into a 2.0 mL PCR screw capped micro tube containing 200 µL of cold acidified acetone
- Homogenize with Ribulyser for 30s at 6200 rpm
- Centrifuge for 2min at 14.500 rpm
- Decant and properly dispose the supernatant
- Optional: repeat washing steps above (mix the pellet with a vortex mixer for 20s instead of the Ribulyser step) if additional cycles of precipitation are necessary to completely remove the interfering substance
- Remove residual acetone by air-drying
- Resolubilize the pellet by adding 200 µL (or less depending on the purpose) of OS and vortex thoroughly
- Centrifuge for 2min at 14.500 rpm
- Deposit 1 µL of the supernatant and mix immediately with another 1 µL of HCCA onto a target spot
- Air-dry the sample spot
- Deposit 1 µL of HCCA onto the dried sample spot and allow to dry thoroughly

Alternative protein extraction procedure applying for all kinds of plant sample materials, especially for samples with a complex matrix and many interfering substances in MALDI analysis, e.g. pigments in fruit and fruit products. Applicable to purify and concentrate proteins in samples containing low protein amounts. Comparable measuring results to Plant-Extr.