Sample Preparation Procedure for

Diary Products

Organic Solvent Extraction (OSExtr) – (incl. Variant with Protein Precipitation)

General Information

This protocol for diary products was provided by CVUA Stuttgart and used to create and validate a reference database for feta and mozzarella cheese [1, 2].

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

Dairy products, e.g. milk, yogurt or cheese.
For pure milk it is necessary to perform protein precipitation (steps 1 – 4).
For yoghurt or (fresh-)cheese (already "precipitated" casein) please start with step 5.

Chemicals and Material

- 2.5 ml reaction tubes and tips.
- Spatula
- Centrifuge with centrifuge tubes and temperature control
- Small beaker
- Heating block
- Benchtop centrifuge
- Dilute acetic acid (50 %)
- Aqua dest/bidest
- HCCA Matrix solution (please see »Tipps and Recommendation«, page 2)
- OS Solvent (acetonitrile 50 %, water 47.5 % and trifluoroacetic acid 2.5 %)

References

[1] Männig, A., Hiller, E., Rau, J. (2017): MALDI-TOF MS zur Tierartenbestimmung bei Milch und Käse. 46; Poster at the "Deutscher Lebensmittelchemikertag" 25. – 27.09.2017, Würzburg. https://maldi-up.ua-bw.de/docs/CVUAS_Maennig_et_al_Tierarten_MALDITOF_2017.pdf

[2] Rau, J., Korte, N., Dyk, M., Wenninger, O., Schreiter, P., Hiller, E. (2020a): Rapid Animal Species Identification of feta and mozzarella cheese using MALDI-TOF mass-spectrometry. Food Control, 107349. https://doi.org/10.1016/j.foodcont.2020.107349

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Protein / Peptides Precipitation

1. Separation of Fat/Skimming

For milk with especially low fat content, e.g. mare milk, this step can be skipped.

- Add approx. 30 ml milk to a centrifuge tube
- centrifuge at 3,345 rpm and 8 °C for 12 minutes
- To remove fat, please carefully decant the liquid into a small beaker; the fat should cling to the wall of the centrifuge tube and is to be discarded.

2. Precipitation of Protein / Peptides (mostly casein)

- Add a few drops of dilute acetic acid to the skimmed milk
- Heat the liquid to 40 – 45 °C (e.g. in a heating block) for at least 15 minutes until sufficient casein is precipitated.

3. Separation of Casein and Whey

- Transfer the liquid with the precipitate to 1.5 ml reaction tubes
  For mare milk you will need to pool the content of about 6 Eppendorf tubes, since mare milk does not seem to contain as much casein as other milk types. For other milk types less tubes are required
- Centrifuge at 12,000 – 14,000 rcf in a benchtop centrifuge for 2 minutes
- Discard the supernatant (= whey) and merge the pellets (= casein)

4. Washing

- Add 1 ml aqua dest. and stir with a spatula
- Centrifuge at 12,000 – 14,000 rcf in a benchtop centrifuge for 2 minutes
- Discard the supernatant

Extraction Procedure

5. Organic Solvent Extraction Procedure

- Transfer a small amount (about 3 mm^3) of the sample or protein precipitate into a 1.5 ml tube
- Add 200 µl OS
- Homogenize by stirring with a spatula
- Mix thoroughly by vortexing for 10 – 20 seconds
- Centrifuge at 12,000 – 14,000 rcf in a benchtop centrifuge for 2 minutes
- Pipet 1 µL supernatant onto a target sample spot (We recommend spotting the supernatant in duplicate or triplicate)
- As soon as the sample spot has dried, overlay the sample with 1 µL HCCA matrix solution (to prevent oxidation reactions leading to peak shifting)
- Allow the sample spot to air dry before analysis ➔ MALDI measurement

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