Sample Preparation Procedure for

Fish and Seafood

Trifluoroacetic Acid Extraction (TFAextr)

General Information

This protocol for fish and seafood, provided by CVUA Karlsruhe, is based on the description of Mazzeo et al. [1] with modification, e.g. sample weight. It contains no purification step with magnetic beads. This method was used to create and validate a reference database for muscle meat of fish and seafood, which will be continuously expanded.

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

Field of Application

Muscle meat of fish, crustaceans and bivalve molluscs in natural form, raw, cooked or frozen.

Chemicals and Material

- 1.5 ml reaction tubes
- Pipettes and tips for volumes from 1–500 µl
- Silica beads (0.1 mm resp. 0.5 mm diameter)
- Micropestle, compatible with the tubes used
- Bead mill (optional)
- Benchtop centrifuge
- HCCA matrix solution (please see »Tipps and Recommendation«, page 2)
- Trifluoroacetic acid (TFA) 0.1 %

References

[1] Mazzeo, M. F., Giulio, B. D., Guerriero, G., Ciarcia, G., Malorni, A., Russo, G. L. & Siciliano R. A. (2008): Fish Authentication by MALDI-TOF Mass Spectrometry. J Agric Food Chem 56(23): 11071–11076.

[2] Stephan, R., Johler, S., Oesterle, N., Näumann, G., Vogel, G. & Pflüger, V. (2014): Rapid and reliable species identification of scallops by MALDI-TOF mass spectrometry. Food Control 46: 6–9.

Fish and Seafood:

Trifluoroacetic Acid Extraction (TFAextr)

Extraction Procedure

- Add approx. 5 mg muscle without skin from the depth of the tissue into a 1.5 ml reaction tube
- Extract proteins either manually:
  - » Add 100 µl TFA (0.1 %) and approx. 100 mg silica beads into the tube
  - » Homogenize/grind the sample using a micropestle
  - » Add 400 µl TFA (0.1 %) and mixing thoroughly by vortexing for 10 – 20 sec.

  or mechanically:
  - » Add 500 µl TFA (0.1 %) into the tube
  - » Disrupt the cells using a bead mill with a frequency of 40 Hz for 3 minutes
- Leave for about 10 minutes (to solve proteins)
- Centrifuge at 15,900 – 18,400 rcf in a benchtop centrifuge for 10 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 which might cause peak shifts)
- Allow the sample spot to air dry before analysis ➔ MALDI measurement

https://doi.org/10.48414/aspects2020/13

Sample Preparation Procedure for

Fish and Seafood

Magnetic Beads Extraction (TFAextr C18)

General Information

This protocol for fish, provided by CVUA Karlsruhe, is based on the description of Mazzeo et al. [1] with modification for e.g. sample weight and the purification step with magnetic beads. This method was used to create and validate a reference database for muscle meat of fish and seafood, which will be continuously expanded.

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

Field of Application

Muscle meat of fish, crustaceans, bivalve molluscs and fish eggs in natural form, raw, cooked or frozen.

Chemicals and Material

- 1.5 ml reaction tubes
- Pipettes and tips for volumes from 1-500 µl
- Silica beads (0.1 mm resp. 0.5 mm diameter)
- Magnetic stand
- Micropestle, compatible with the tubes used
- Bead mill (optional)
- Benchtop centrifuge
- HCCA matrix solution (please see »Tipps and Recommendation«, page 2)
- Trifluoroacetic acid (TFA) 0.1 %
- Desorption solution (50 % acetonitrile in 0.1 % TFA)
- Magnetic beads (surface modified with C18 alkyl groups)

References

[1] Mazzeo, M. F., Giulio, B. D., Guerriero, G., Ciarcia, G., Malorni, A., Russo, G. L. & Siciliano R. A. (2008): Fish Authentication by MALDI-TOF Mass Spectrometry. J Agric Food Chem 56(23): 11071 – 11076.

[2] Stephan, R., Johler, S., Oesterle, N., Näumann, G., Vogel, G. & Pflüger, V. (2014): Rapid and reliable species identification of scallops by MALDI-TOF mass spectrometry. Food Control 46: 6 – 9.

[3] MoBiTec 2012, M-Beads-Proteomics S-C4, S-C8, S-C18

Fish and Seafood:

Magnetic Beads Extraction (TFAextr C18)

Protein Extraction

1. Protein Extration

- Add approx. 5 mg muscle without skin from the depth of the tissue into a 1.5 ml reaction tube
- Extract proteins either manually:
  - » Add 100 µl TFA (0.1 %) and approx. 100 mg silica beads into the tube
  - » Homogenize / grind the sample using a micropestle
  - » Add 400 µl TFA (0.1 %) and mixing thoroughly by vortexing for 10 – 20 sec.

  or mechanically:
  - » Add 500 µl TFA (0.1 %) into the tube
  - » Disrupt the cells using a bead mill with a frequency of 40 Hz for 3 minutes
- Leave for about 10 minutes (to solve proteins)
- Centrifuge at 15,900 – 18,400 rcf in a benchtop centrifuge for 10 minutes

Prurification of Protein Extract with Magnetic Beads

2. Conditioning / Resuspension Magnetic Beads

- Pipette 20 µl of the magnetic beads solution into a 1.5 ml reaction tube, place the tube on the magnet, remove the supernatant with a pipette and discard it
- Remove the reaction tube from the magnet, add 100 µl TFA (0.1 %) and resuspend the magnetic beads
- Place the tube on the magnet, remove the supernatant with a pipette and discard it, repeat this washing step a second and a third time
- Add 10 µl TFA 0.1 % and resuspend the magnetic beads

3. Protein/Peptide adsorption

- Add 25 µl sample solution from step 1 into the tube containing magnetic beads and leave for 10 minutes at ambient temperature
- Place the tube on the magnet, remove the supernatant with a pipette and discard it
- Remove the reaction tube from the magnet, add 50 µl TFA (0.1 %) and re-suspend the magnetic beads
- Place the tube on the magnet, remove the supernatant with a pipette and discard it, repeat this washing step a second and a third time

4. Protein/Peptide desorption

- Add 10 µl desorption solution to the sample-coated magnetic beads from step 3, resuspend and incubate for 8 minutes at ambient temperature
- 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 which might cause peak shifts)
- Allow the sample spot to air dry before analysis ➔ MALDI measurement

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