POSTER NOTE

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Ultra-Fast Analysis of Allergens Using Capillary Electrophoresis Coupled to Mass Spectrometry and Ultra Violet Photodissociation

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ABSTRACT

The identification of proteins in food matrices that cause sensitization in individuals or allergic reactions in those individuals already sensitized, represents a major concern for the food industry. Here we explore the application of CE/MS/MS as an accurate and sensitive way to rapidly test for protein allergens in food. The workflow is based on a simple protein extraction step and the use of capillary electrophoresis (CE) hyphenated to a Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer with an UVPD source for the characterization of the extracted proteins. The high mass accuracy and resolution of this instrument, combined with efficient isoform separation and the availability of different fragmentation modes, allow for rapid allergen identification.

INTRODUCTION

The main goal of this work was to develop a simple and fast strategy allowing simultaneously the identification of allergens and the authentication of fish species. The method consists of a two-step workflow, taking advantage of the pH and temperature stability of food allergens. In a first step, we isolated the thermo-stable proteins from the sarcoplasmic protein fraction of the reference Hake species. This was followed by a step of top-down proteomic analysis of the purified proteins. Samples were cleaned up and loaded onto CE system for 10~30mins separation. The proteins were then analyzed via top-down fragmentation in the HCD cell of a benchtop quadrupole Orbitrap™ mass spectrometer. EThcD and UVPD fragmentation modes were also explored. For all samples, unique mass to migration time maps were built representing a unique signature for each fish allergen. The most abundant protein in the electropherograms was a 11 kDa protein, identified as parvalbumin (PRVB). This thermo-stable protein is considered to be the major fish allergen. We were able to efficiently separate several PRVB protein isoforms. Overall, this strategy offers a very reliable top-down proteomics method, and provides the basis for the development of CE chips that could be used in the food industry to detect allergens and to authenticate food.

MATERIALS AND METHODS

Reference samples from commercial fish species were included in this work. Protein extraction was carried out by mechanically homogenizing 1 g of muscle tissue. Water soluble proteins were centrifuged, the supernatant heated to 70 ºC for 5 min and centrifuged again. Soluble proteins were cleaned using stage tips, and then transferred onto an Agilent 7100 capillary electrophoresis system. An Orbitrap Fusion Lumos Tribrid mass spectrometer was coupled to the CE system using a custom made ion source incorporating an electro-osmotic flow driven methanol/water/formic acid sheath liquid to improve the MS signal. Proteins were loaded and separated using a cation-coated capillary at -30kV or neutral-coated capillary (100 cm X 50um I.D) at +30kV. MS/MS acquisition was achieved using ETD, EThcD, HCD or UVPD fragmentation at 120K@m/z 200. Data analysis was performed using Thermo Scientific™ Xcalibur 4.0, QualBrowser and ProSight Lite software.

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WORKFLOW

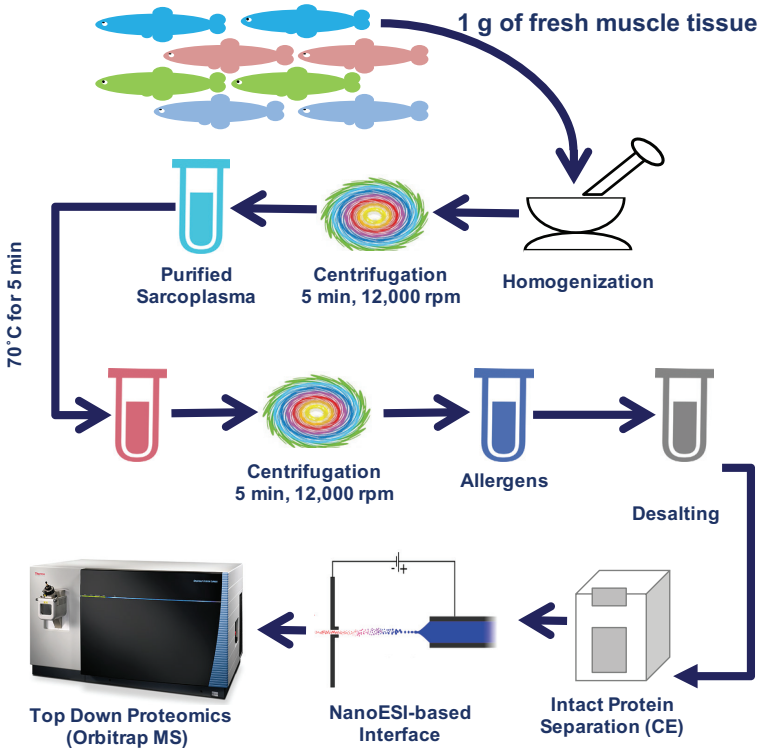


FIGURE 1. General overview of the analytical workflow with reference muscle tissue samples. The thermo-stable proteins, β-parvalbumins are separated by CE top-down MS.

RESULTS

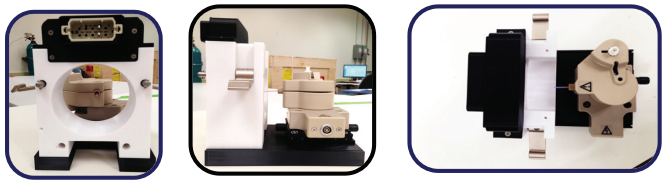


FIGURE 2. Photographs of the ion source including the 3-D printed stage and EMASS-II source. The stage was specially designed for an Orbitrap Fusion Tribrid MS.

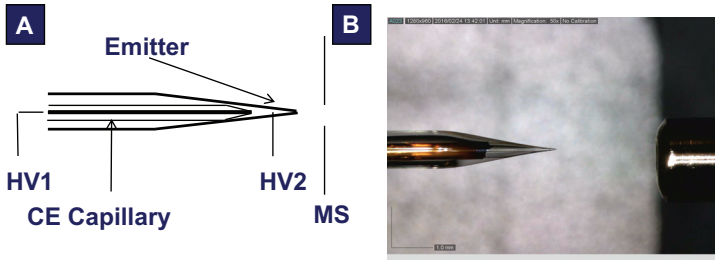


FIGURE 3. A) Design of sheath flow CE interface. The fused silica capillary is introduced into a borosilicate emitter. HV1 is used for CE separation. HV2 is applied to the sheath liquid for electrospray. B) Photograph of the etched capillary in the emitter aligned with MS inlet.

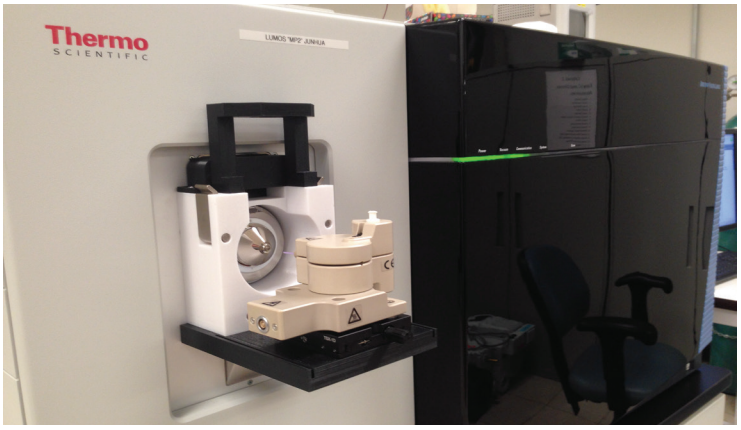


FIGURE 4. Photograph of sheath flow CE interface aligned with Orbitrap Fusion Lumos Tribrid MS.

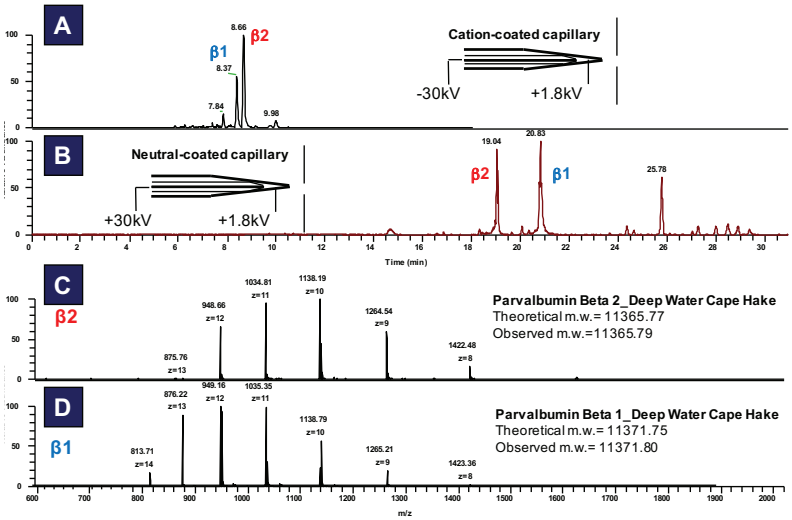


FIGURE 5. A) Allergens from Hake fish were separated with cation coated capillary (PEI). The isoforms were separated and driven by electroosmotic flow (EOF). B) The allergens were separated with neutral coated capillary (Gaurant). The isoforms were separated only by electrophoresis. C) Parvalbumin β2 was detected and deconvoluted to full mass of 11365.79Da D) Parvalbumin β1 was detected and deconvoluted to full mass of 11371.80Da.

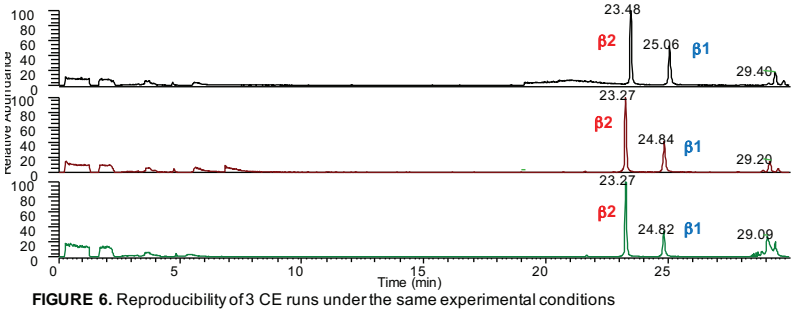


FIGURE 6. Reproducibility of 3 CE runs under the same experimental conditions

Parvalbumin β2

HCD

PROTEIN COVERA 44%

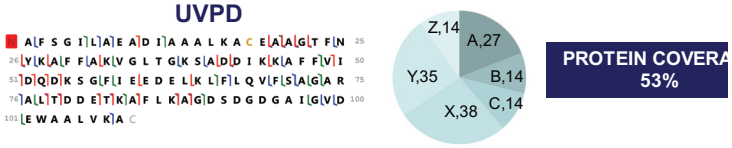
ETD

PROTEIN COVERA 64%

ETDhcD

PROTEIN COVERA 81%

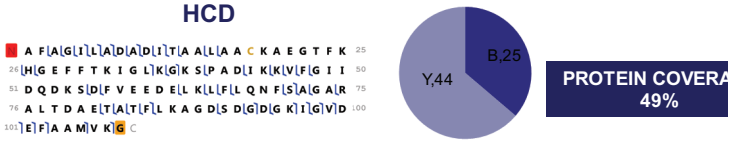
UVPD



PROTEIN COVERA 53%

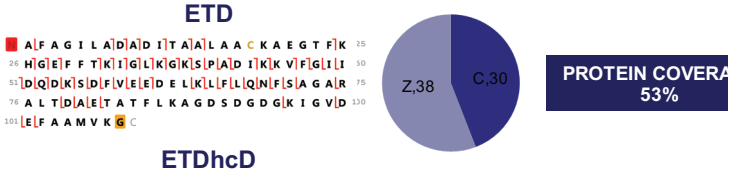
Parvalbumin β1

HCD



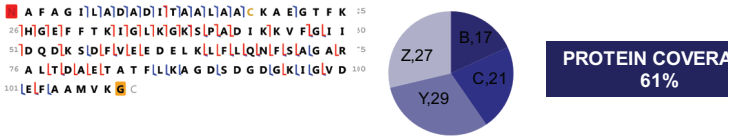
PROTEIN COVERA 49%

ETD



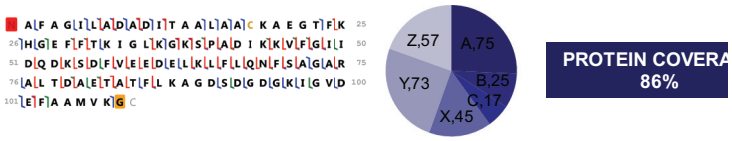
PROTEIN COVERA 53%

ETDhcD



PROTEIN COVERA 61%

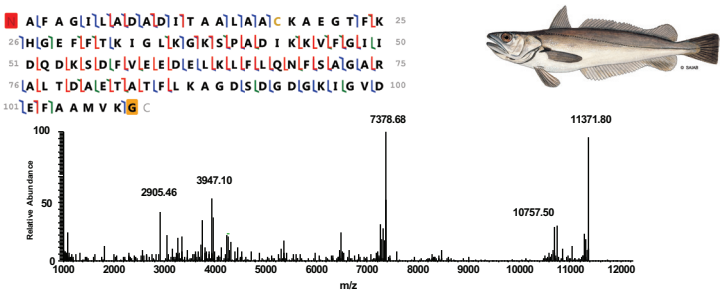
UVPD



PROTEIN COVERA 86%

FIGURE 7. Evaluation of different fragmentation strategies implemented on the Orbitrap Fusion Lum Tribrid MS for research purposes. Parvalbumin β2 (top figure) or Parvalbumin β1 (bottom figure) wer fragmented using HCD, ETD, ETDhcD and UVPD fragmentation modes. Data were analyzed with ProSight Lite at 10ppm mass tolerance. The candidate sequence was Merluccius Paradoxus (P867

Merluccius Paradoxus (Deep Water Cape Hake)



Merluccius Merluccius (European Hake)

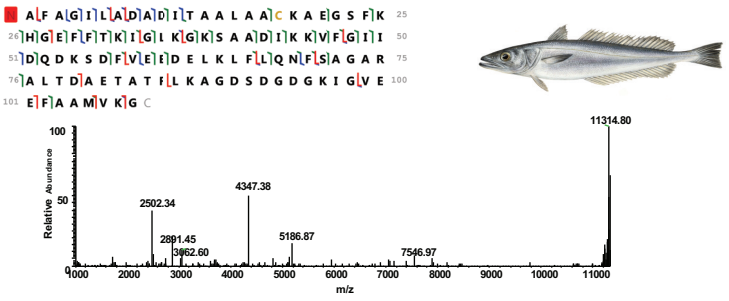


FIGURE 8. Characterization and identification of two Hake species with CE-MS, Top Merluccius Paradoxus and bottom Merluccius Merluccius.

CONCLUSIONS

- Successfully coupled the commercial CE to the Orbitrap Fusion Lumos mass spectrometer with the CMP ion source on a 3-D printed stage.
- Parvalbumin protein isoforms were separated with cation coated capillary within 10 mins and neutral coated capillary within 30 mins in MS compatible conditions.
- Parvalbumin β1 and β2 were identified from the Hake fish Merluccius Paradoxus within 4.1 and 1.8 ppm mass difference.
- 81% protein coverage of Parvalbumin β2 was achieved by using ETDhcD fragmentation,
- 86% protein coverage using UVPD fragmentation.
- Two Hake species were successfully characterized and identified.

REFERENCES

1. Commercially available from CMP Scientific, Brooklyn, NY
2. Commercially available from Alcor Bioseparations LLC, Palo Alto, CA
3. Carrera M., Canas B., Gallardo J.M., Journal of Proteomics, 2012, 75, 3211-3220

ACKNOWLEDGEMENTS

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