

From Ocean To Table: An Integrated Mass Spectrometry Approach To Identify The Fish On Your Plate

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ABSTRACT

The fishery market has grown in sales for the last 15 years. As a result, fish demand is producing a worldwide overexploitation of resources and fraudulent practices in the industry that account for 30% of the sales. In most cases, high priced fish species are substituted for lower value species. Here we described an integrated proteomic approach to authenticate fish species from muscle tissue.

INTRODUCTION

The identification of commercial fish species is a relevant issue to ensure correct labeling, maintain consumer confidence and enhance the knowledge of the captured species, benefiting both, fisheries and manufacturers. Here we propose a proteomic approach, based on top down proteomic analyses using ESI-MS/MS in a high resolution Orbitrap™ mass spectrometer for the identification of fish species with commercial interest. ESI-Orbitrap protein mass fingerprint from thermo-stable proteins purified from fish tissue were used for the identification of a commercial hake filet with no label regarding the fish other than "Product from South Africa." Further identification and characterization of this sample was performed using standard shotgun proteomics and PRM targeted analysis. We believe that fisheries and manufacturers may take advantage of this methodology as a tool for a rapid and effective seafood product identification and authentication, providing and guaranteeing the quality and safety of the foodstuffs to consumers.

MATERIALS AND METHODS

1 gram of fish muscle tissue was homogenized in water. The sample was then centrifuged to remove the insoluble material. Water soluble proteins were then heated at 70°C for 5 min. After the heat treatment the sample was again centrifuged and the supernatant was aliquoted. One of the aliquots was submitted for bottom-up and PRM proteomics analysis. A second aliquot was submitted for top-down analysis. For bottom-up proteomics, the pH of the sample was adjusted to 8, trypsin was added and digestion was performed for 3 minutes using high intensity ultrasound. After digestion the sample was desalted using Thermo Scientific™ Pierce™ Micro-Spin Columns following the instructions of the manufacturer. After desalting, the samples were resuspended in 0.1% formic acid and subjected to LC-MS analysis using a Thermo Scientific™ Easy-nLC 1200 system hyphenated to a Thermo Scientific™ Q Exactive™ hybrid quadrupole-Orbitrap™ mass spectrometer. Peptides were separated using a 15 cm Thermo Scientific™ EASY-Spray™ column. After LC-MS analysis, raw files were submitted for database search using Thermo Scientific™ Proteome Discoverer™ 2.1 software and a composite protein database of all fish species from Uniprot.

WORKFLOW

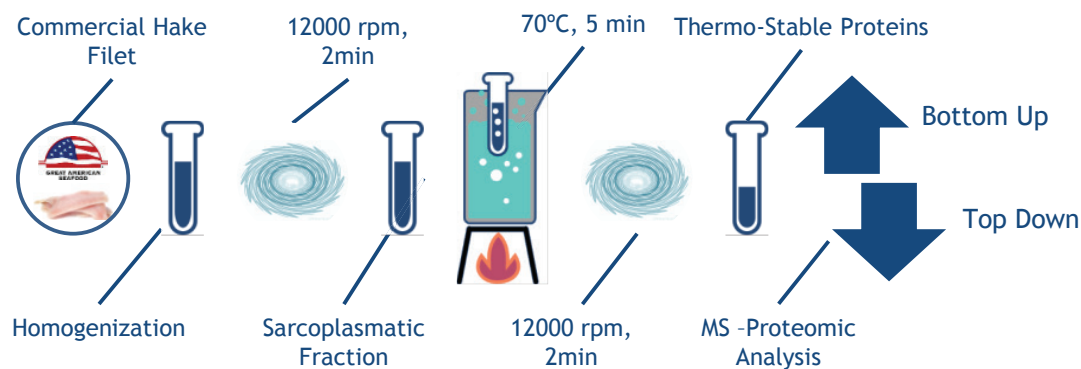


FIGURE 1. General overview of the analytical workflow. Commercial hake filet samples were processed as described in the workflow. First, 1 gram of tissue was physically disrupted with a mortar and later with ultrasound in water. Muscle debris was removed by centrifugation and the supernatant was submitted to a heat treatment for five minutes. After the heat treatment, the sample was centrifuged to remove denaturalized proteins and submitted to either bottom-up or top-down proteomic analysis.

RESULTS

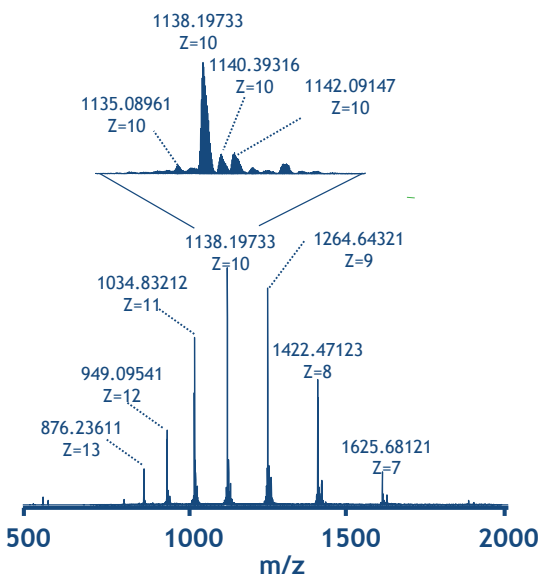
Accession	Description	Coverage %	#AAs	MW [kDa]	# Unique Peptides
P86765	Parvalbumin beta 2 OS=Merluccius merluccius	65.74	108	11.27	8
P86764	Parvalbumin beta 1 OS=Merluccius hubbsi	65.74	108	11.29	8
P86768	Parvalbumin beta 1 OS=Merluccius paradoxus	65.74	108	11.39	8

TABLE 1. List of the top three proteins out of over ~200 proteins identified from the bottom-up proteomic analysis using Protein Discoverer 2.1. As can be noticed, the very high protein sequence homology among three very different species of hake does not allow for accurate species identification.

	1	11	22	31	41	51	61	
P86765	AFAGILADAD	ITAA	LAACK	AEGSFKHGE	FFTKIGLKGK	S_AADIKVVF	GIIDQDKSDF	VEEDELKFL
P86764	AFAGILADAD	ITAA	LAACK	AEGTFKHGE	FFTKIGLKGK	S_AADIKVVF	GIIDQDKSDF	VEEDELKFL
P86768	AFAGILAEAD	ITAA	LAACK	AEGTFKHGE	FFTKIGLKGK	SPA_DIKKVF	GIIDQDKSDF	VEEDELKFL
			★		★★			
	71	81	91	101	111			
P86765	QNFSAGARAL	TDAETATFLK	AGDSGDGDKI	GVDEFAAMVK	G			
P86765	QNFSAGARAL	TDAETATFLK	AGDSGDGDKI	GVDEFAAMVK	G			
P86768	QNFSAGARAL	TDAETATFLK	AGDSGDGDKI	GVDEFAAMVK	G			

FIGURE 2. Blast alignment of the three calcium binding proteins among the three species emphasize their highly conserved sequence information. Blue stars indicate where the amino acid sequence varies among the three proteins. Only three different peptides could allow for the specific identification of fish under study: AEGTFK, SPADIK and SPAADIK. However the short sequence of these peptides does not allow a straight identification of the species because their mass to charge ratios are below the typical scanning range in DDA experiments in case of +2 charge state peptide, or if they are in their +1 charge state usually +1 charge ions are not targeted for fragmentation.

RESULTS



Observed Mass: 11365.68

Theoretical Mass for P86769: 11365.77

Mass Diff. (ppm): 7.84

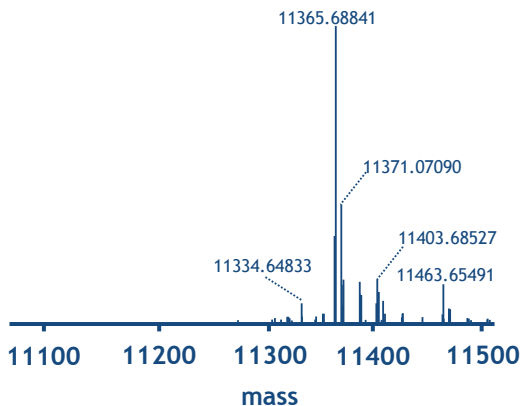
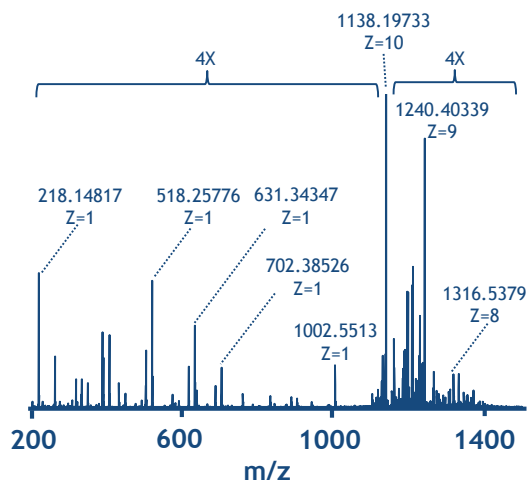


FIGURE 3. Intact mass analysis: Left panel shows the mass spectrum obtained after direct infusion of the undigested fish sample. Showing ~11 kDa group of proteins. After protein deconvolution (right panel) using the Extract algorithm the most abundant mass corresponds to Parvalbumin beta 2 from *Merluccius paradoxus*.



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15 A[F]S[G]I[L]A[E]A[D]I[A]A[A]L[L]
16 K[A]C[E]A[A]G[T]F[N]YK[A]F[F]
31 A K V G L T G K S A D D I K K
46 A F F V I D Q D K S G F I E E
61 D E L[K]L F L Q V F S A G A R
76 A L T D[D]E T K[A]F L[K]A[G D]
91 S[D]G[D]G[A]I[G]V[D]E[W]A[A]L[L]
106 V[K]A C
    
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FIGURE 4. The 11365.68 mass was further selected for top-down analysis to verify that the protein sequence belongs to Parvalbumin beta 2 from *Merluccius paradoxus*. Left panel shows the MSMS spectra for the 1138.19733 mass. The right panel shows the sequence coverage obtained that allows for the explanation of 45% of the residues cleavages.

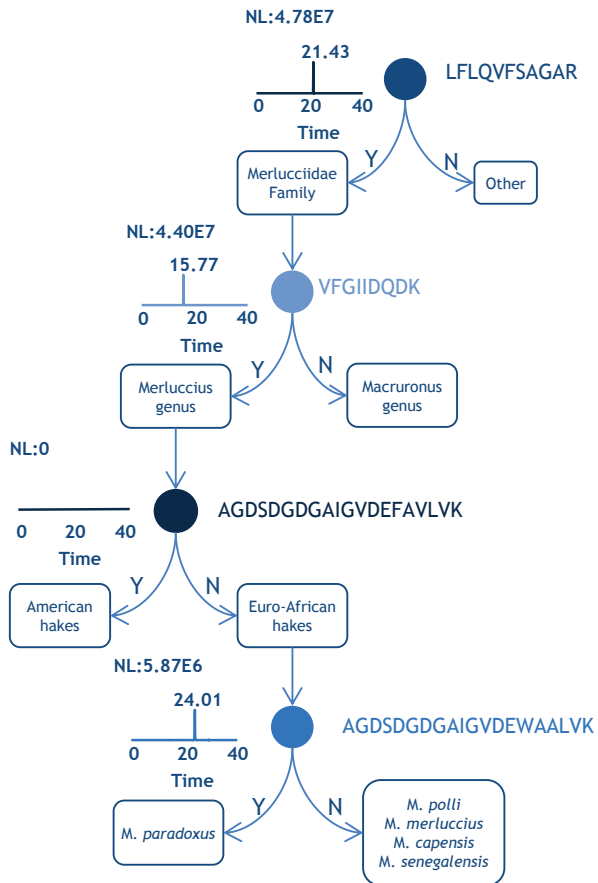


FIGURE 5. A PRM decision tree for a systematic discrimination of Merlucciidae species using specific tryptic peptides from parvalbumins based on previously published peptide biomarkers¹.

CONCLUSIONS

We successfully identified the fish species from an unlabeled commercial hake filet.

Intact MS analysis of thermostable proteins represents a promising technique for fish identification.

The workflow developed here allows for fish authentication in less than 30 minutes.

REFERENCES

1. Monica Carrera, Benito Canas, Daniel Lopez-Ferrer *et al.* Anal. Chem. 2011, 83, 5688–5695

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