MOLECULAR CHARACTERIZATION OF FOOD-GRADE MEAT PROTEIN HYDROLIZATES

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INTRODUCTION

- The animal by-products industry has always been a vital part of the world food production chain, providing valuable new products while reducing pollution loads.
- Traditional exploitation of the protein rich solids includes use in FOODS, PET FOODS, LIVESTOCK FEEDS, FERTILIZERS. Fats are transformed into SOAPS, OLEOCHEMICALS, in addition to their use in FOOD, PET FOODS and FEED APPLICATIONS.
- The EU project PROSPARE aims to exploit unmarketable animal residues based on efficient bioconversion technological methods and biocatalysts with subsidiary production of renewable energy and biologically valuable substances.
- The project aims at showing how to obtain and characterize value added peptide mixtures, starting from different raw materials, and ultimately how to make them available to industry for further larger scale processing. Such peptide mixtures can be exploited in the food, feed and green chemicals chains. In addition to the above also raw fat materials can be obtained and suitably transformed in biofuels.
- In the frame of the project, Functional Animal Proteins (FAP) produced by new technologies is planned to be used as a food protein substance in instant foods and processed meat products.

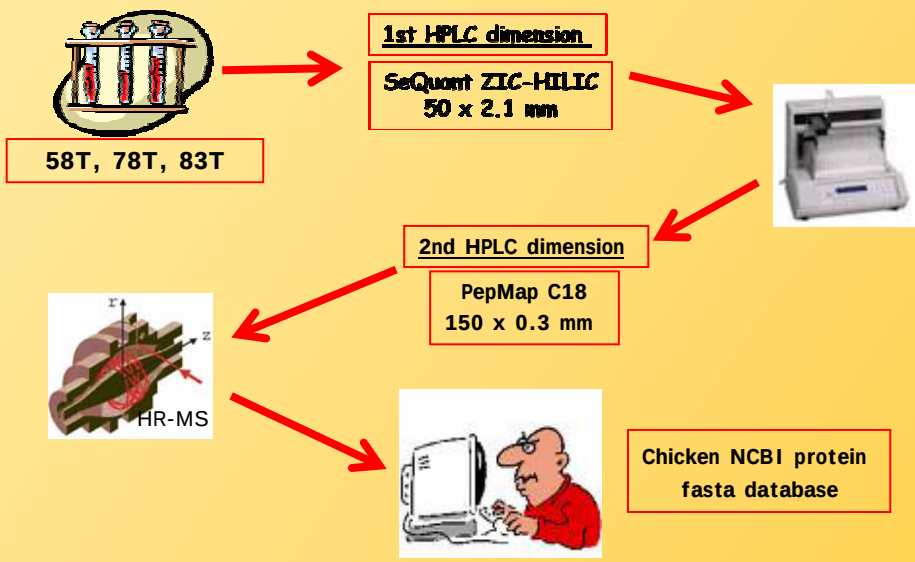
MAIN RESIDUES FROM POULTRY SLAUGHTERING

MATERIALS AND METHODS

- Three samples, 78T, 58T and 83T were dissolved in H_2O/CH_3CN 50/50.
- An off-line 2D-HPLC was performed; the first dimension was a HILIC column and the second a RP column.
- Fractions were analysed by MS in a HRMS LTQ Orbitrap equipped with a nano ESI source.
- LTQ Orbitrap operated in Data Dependent Scan mode; a survey scan spectrum was acquired at 15000 resolution in the 400-2500 *m/z* range; then MS/MS spectra for three most abundant ions were recorded at 7500 resolution. Mono and doubly charged ions were excluded from MS/MS experiments.
- Data were processed for protein identification by BioWorks 3.3 software (Thermo Fisher). A specific chicken proteins database was created using entries from NCBI.

AIM OF THE WORK

The first year work has been primarily devoted to set up suitable methods for performing analyses on the molecular composition and on the functional properties of protein hydrolizates. Characterization and identification of sequences of the most abundant peptides in the mixtures was performed with the main purpose to provide a detailed molecular information which can eventually be linked to desirable functional and technological properties.



Scheme 1

RESULTS

27 fractions from HILIC column were collected and analyzed on a RP column (Scheme 1). In figure 1, the gradient elution used for HILIC column is shown together with the UV profiles of 78T, 58T and 83T. In Table 1 and figure 2 the elution program for RP column and the elution MS profile from the 58T sample are shown, respectively.
High resolution data obtained from Data Dependent Scan in Orbitrap were processed for protein identification with a specific chicken protein database. HR-MS/MS spectrum of the 1855.86 MW triply charged peptide is shown in figure 3. This peptide derived from actin.

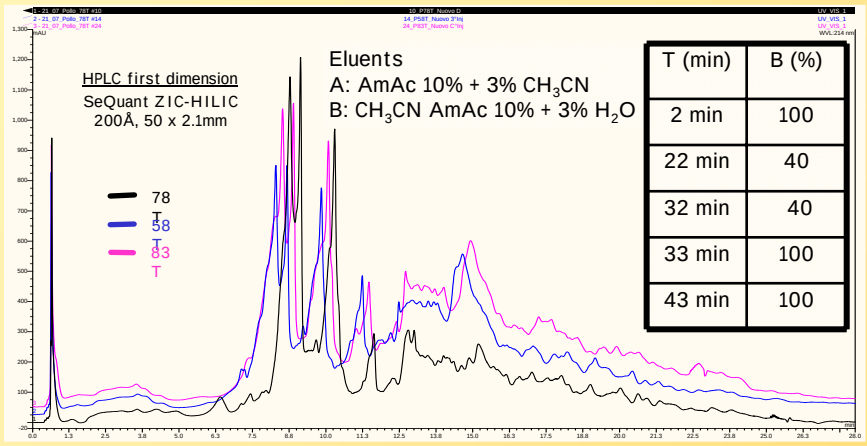


Fig. 1: UV elution profiles (214 nm) of 78T, 58T and 83T samples and elution program from HILIC column.

Eluents
A : H_2O FoAc 0.05%
B : CH_3CN FoAc 0.05%

T (min)	%B
0 min	5
3 min	5
30 min	40
33 min	40
35 min	90
38 min	90
39 min	5
50 min	5

Tab. 1: Eluition program from RP column.

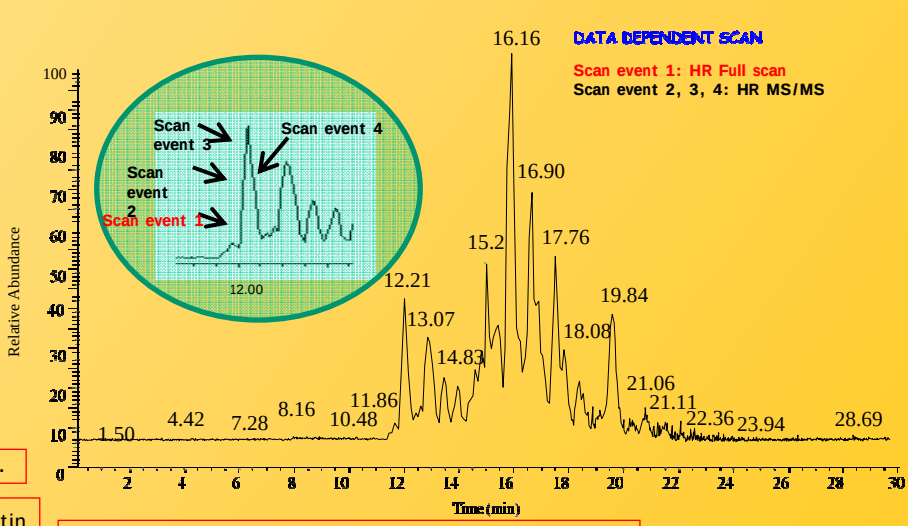


Fig. 2: Elution chromatographic profile of a 58T sample from RP column.

Results from chicken protein database showed 58T and 83T samples showed the presence of similar proteins of which actin and myosin are most abundant. The most important difference can be marked observing 78T sample; infact we observed a lower number of proteins (10 proteins in sample 78T, 28 proteins in the sample 83T and 17 in 58T) and we can not detect the presence of actin and myosin.

Most abundant proteins detected:

- **83T:** actin, myosin, alpha 1 globin, tyrosine kinase, exonuclease
- **58T:** actin, myosin
- **78T:** alpha 1 globin, elongation factor, RNA polimerasi II, Beclin 1

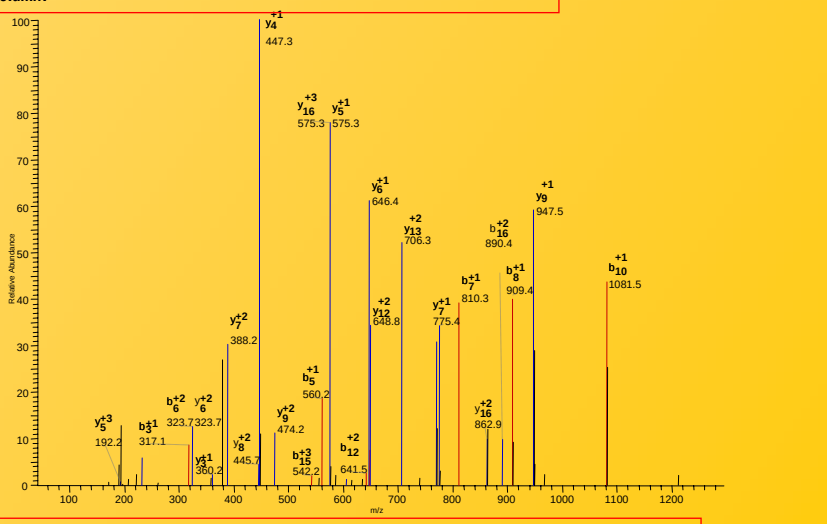


Fig. 3: HR MS/MS spectrum of triply charged peptide (MW 1855.86) used to identify actin.

CONCLUSIONS

- Preliminary results showed interesting features of samples studied in PROSPARE project.
- The analyses of hydrolizates oligopeptide fractions showed qualitative and quantitative differences between samples derived from different composition of row starting material and also due to different digestion processes.
- Identification of the sequences of the most abundant peptides in the mixture was successful, allowing a more detailed molecular characterization of FAP samples.