Tracking the fate of mustard allergens using Novel Detection and Food Processing Approaches

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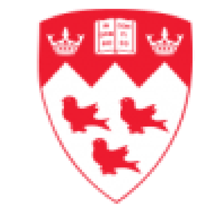

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ABSTRACT

Mustard, a popular condiment used in food industry can induce life-threatening reactions in allergenic individuals. Processing can alter protein structure and decrease the allergenicity. Combination of processing techniques (thermal, roasting, or enzymatic hydrolysis) is proven effective in reducing allergenicity of some proteins. Our aim in this study is to present an optimal processing technique for eliminating the allergenicity of protein allergens in the Canadian mustard.

INTRODUCTION

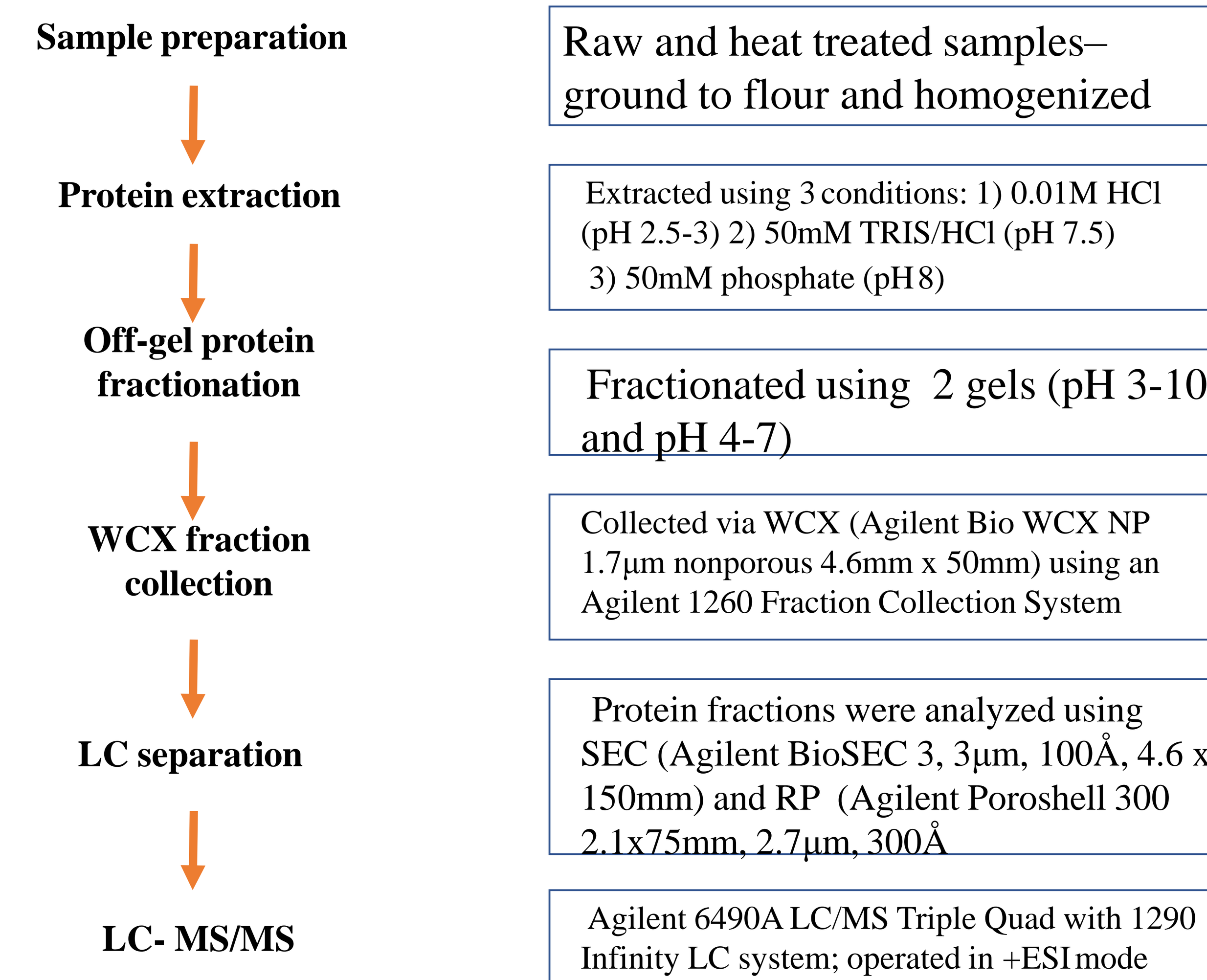
Mustard has a High priority list of foods by Health Canada, Nutritious and valuable source of protein and oil, Low eliciting dose (0.05 mg lead to a reaction), Elicits severe reactions such as anaphylaxis, Responsible for higher number of recalls in Canada as compared to Europe, Canada is the principal exporter of mustard to the world. The structure of the native allergen may experience changes upon exposure to different food-processing methods as Matrix interactions and/or solubility changes, may affect allergenicity of the final product

OBJECTIVES

Developing specific, sensitive, and robust methods for detection and accurate quantification of the targeted allergenic mustard protein, in a practical and economic manner

METHODOLOGY

1-Protein extraction and fractionation



METHODOLOGY

2- Enzymatic digestion

Extracted protein samples were enzymatically digested with trypsin; protein extracts were centrifuged, and 200μL of supernatant analyzed by LC-MS:

LC-MS Conditions for Peptide Discovery and Identification

RPLC separation: column: Poroshell 120 SB C18 2.1x50mm; 0-40% B in 60 or 90 min then to 70% B in 15min A= 95/5 water/ACN + 0.025% TFA; B= 95/5 water/ACN + 0.025% TFA; flow rate 0.25 ml/min

LC-MS/MS: Agilent 6490A LC/MS Triple Quad with 1290 Infinity LC system; operated in +ESI mode.

Database searches: MS/MS spectra were searched in green plant databases (i.e. SwissProt and NCBI)

3- Analysis of Samples

Sample preparation:

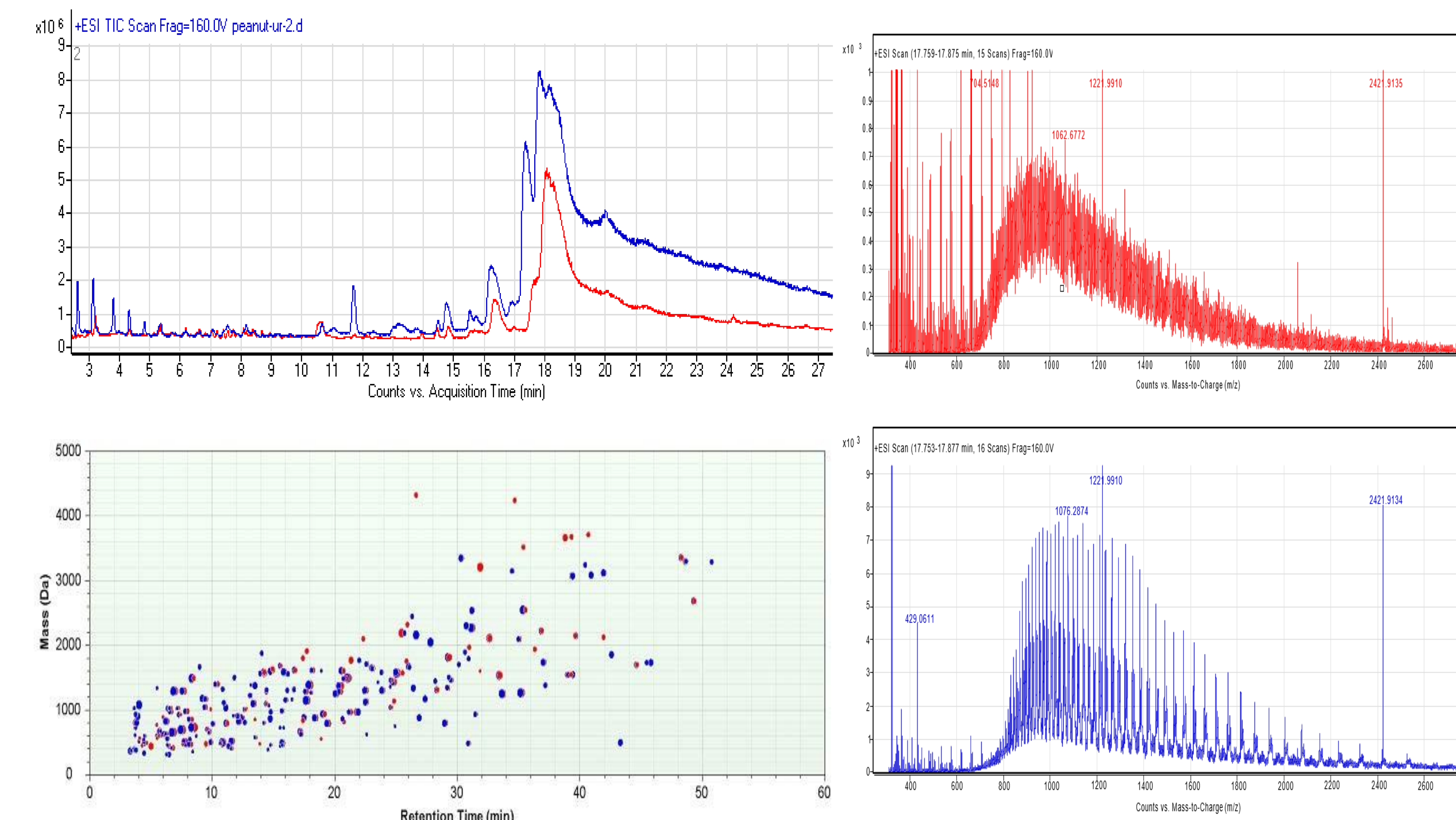
- 30g of various food samples were ground to a powder and homogenized
- proteins were extracted using 50mM TRIS/HCl (pH 7.5)
- extracts were digested with trypsin
- digested extracts were analyzed by LC/MS

RPLC separation: column: Poroshell 120 SB C18 2.1x50mm; 0-40% B in 60 or 90 min then to 70% B in 15min A= 95/5 water/ACN + 0.025% TFA; B= 95/5 water/ACN + 0.025% TFA; flow rate 0.25 mL/m

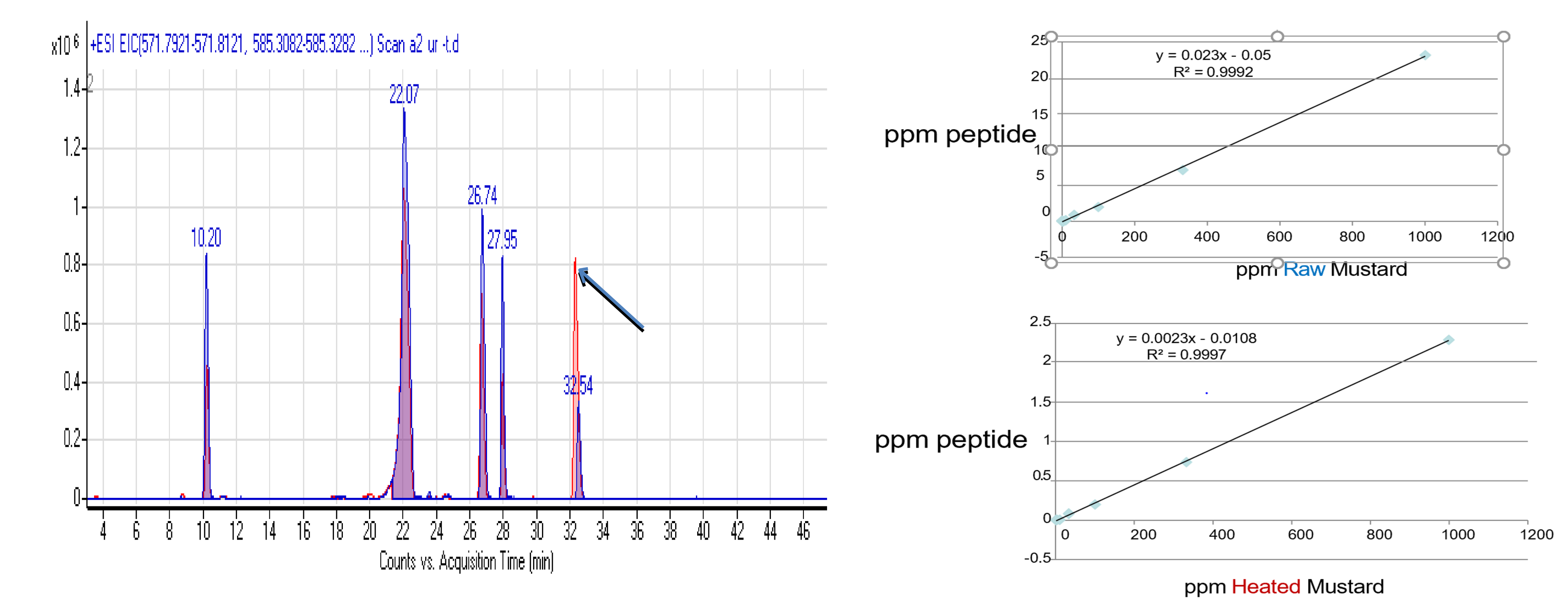
LC-MS/MS: Agilent 6490A LC/MS Triple Quad with 1290 Infinity LC system; operated in +ESI mode

RESULTS

Comparison of protein molecular weights between raw and Heat-treated Mustard



RESULTS



DISCUSSION

Initial work involved characterization of the protein profiles of raw versus heat treated mustard samples; various extraction and fractionation conditions were investigated. Then, Proteins from raw versus heat treated were extracted, then proteolytically digested *in-vitro*, using conditions and enzymes that model the human digestion process (pepsin, trypsin and chymotrypsin) – *data shown here is trypsin only*. Finally: The peptide digests were analyzed using accurate LC-MS/MS; potential representative peptides were identified and sequenced through MS/MS database searches. Observed protein profiles of mustard were different (sometimes significantly) between raw and heated. Relative intensities of some digested peptides were sometimes very different between raw and heated state. Optimization of extraction and purification steps has notably played a key role in improvement of analytical methods. Food processing and food matrix can affect the structure change and allergenic activity (IgE-binding capacity and mediator release). Detection methods should be improved in order to demonstrate their validity and robustness of detection at low concentrations.

CONCLUSIONS

Observed protein profiles of mustard were different (sometimes significantly) between raw and heated. Relative intensities of some digested peptides were sometimes very different between raw and heated state. Optimization of extraction and purification steps has notably played a key role in improvement of analytical methods. Food processing and food matrix can affect the structure change and allergenic activity (IgE-binding capacity and mediator release). Detection methods should be improved in order to demonstrate their validity and robustness of detection at low concentrations. A comparative study on effect of novel thermal and a non-thermal process on mustard allergen proteins is proposed.

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