QUALITATIVE ANALYSIS OF PHYTOESTROGENS BASED ON A SPECTRAL LIBRARY DEVELOPMENT USING LC-ESI-MS/MS

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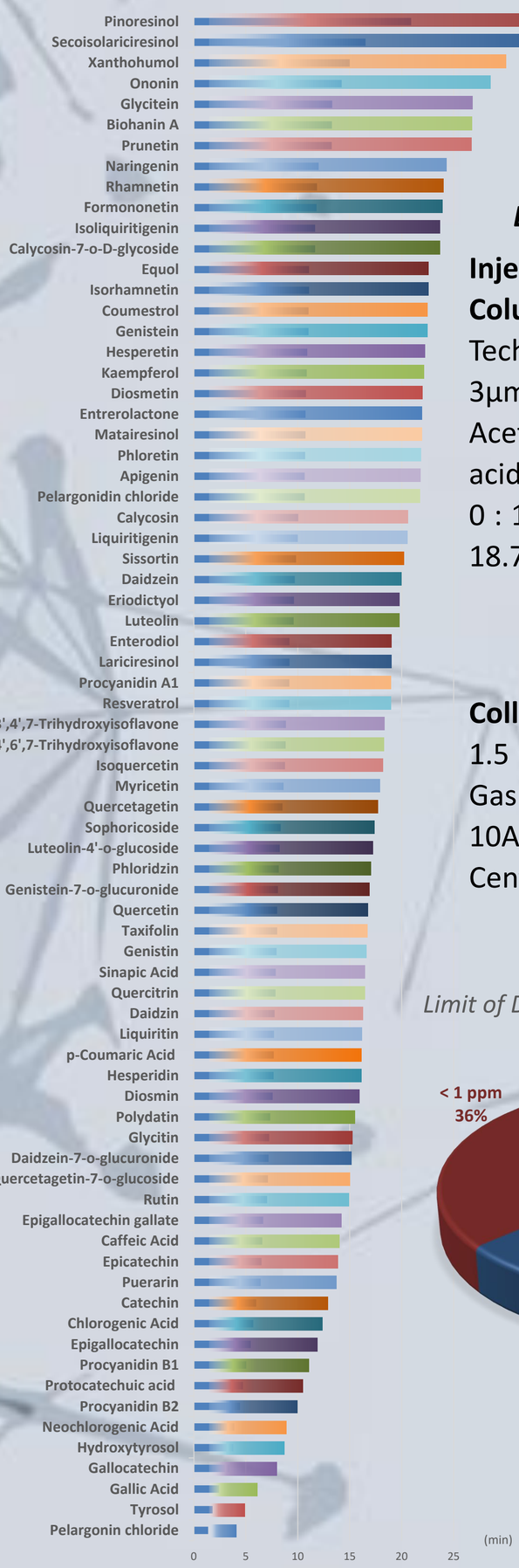
INTRODUCTION

Phytoestrogens comprise a class polyphenolic compounds that naturally occur in plants such as soya, fruits, and vegetables. The principal phytoestrogens are classified into isoflavonoids, flavonoids, stilbenes and lignans. As it is implied by their name, they bind to estrogen receptors, displaying endocrine agonist and antagonist activities. In addition, their presence is also related with a reduced risk of developing cardiovascular diseases, various forms of cancers, obesity, menopausal symptoms, etc. Study herein refers to the construction of a database containing the LC/MS and MS/MS spectra for 74 phytoestrogens belonging to various classes. The database efficiency is revealed in the context of screening and characterizing the phytoestrogens contained in samples from different fish feeds and their plant-based raw materials.



Liquid Chromatography Conditions

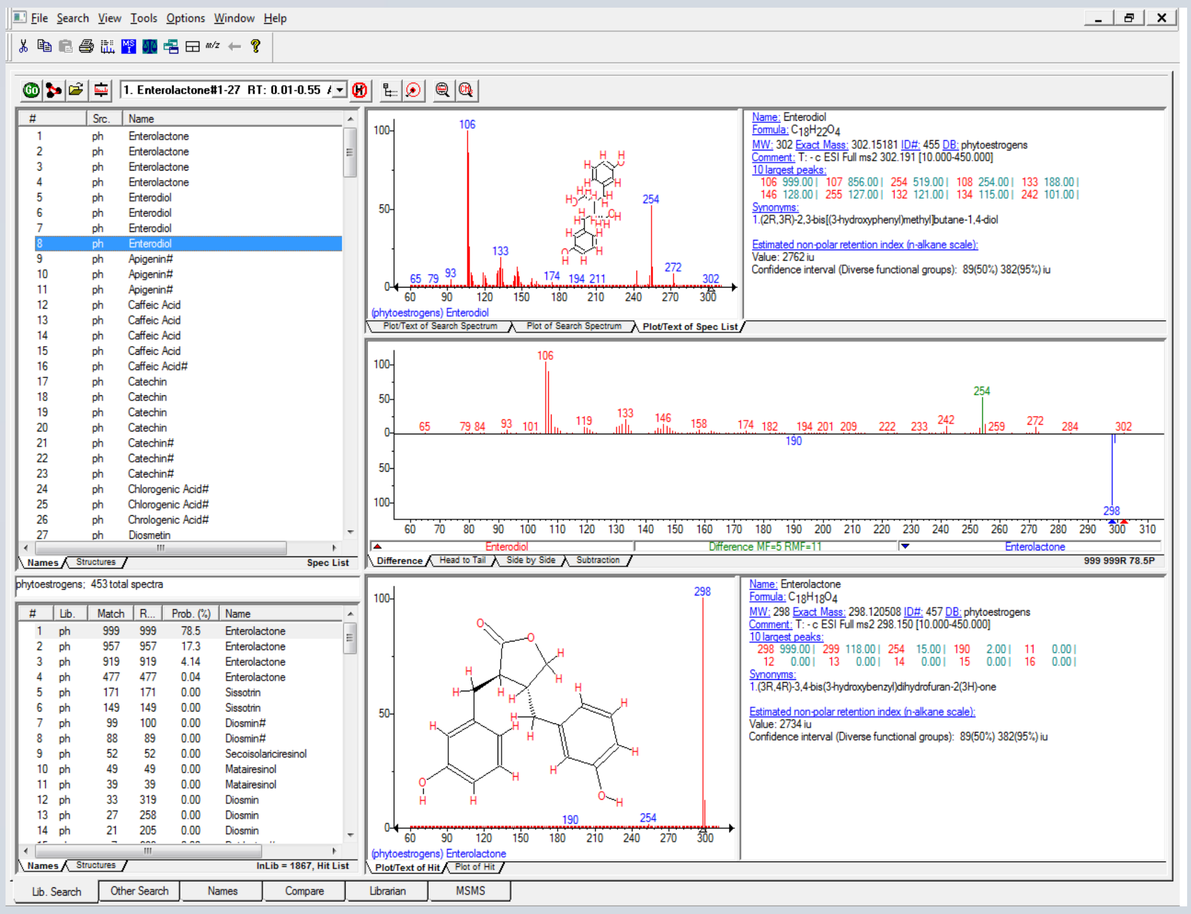
Injection Vol. 10 μL, **Tray Temp.** 20°C, **Column Temp.** 30°C, **Column** Fortis Technologies Ltd., C18 (150x2.1mm, 3μm), **Flow Rate** 300 μL/min **Sol A** Acetonitrile, **Sol B** Water 0.1% formic acid, **Gradient Program:** t(min): A%, B% : 0 : 10, 90 → 2 : 10, 90 → 16.7 : 100, 0 → 18.7 : 100, 0 → 18.8 : 10, 90 → 22 : 10, 90

Mass Conditions

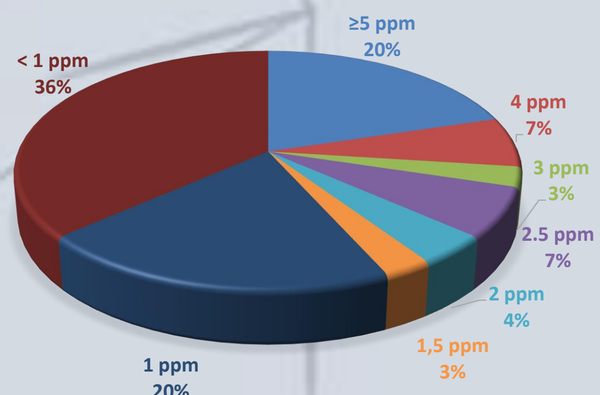
Collision Energy 15 V, **Collision Gas Press.** 1.5 mTorr, **Capillary Temp.** 300°C, Sheath Gas Press. 35Arb, **Auxiliary Gas Press.** 10Arb, Spray Voltage 3,500 V, **Data Type** Centroid

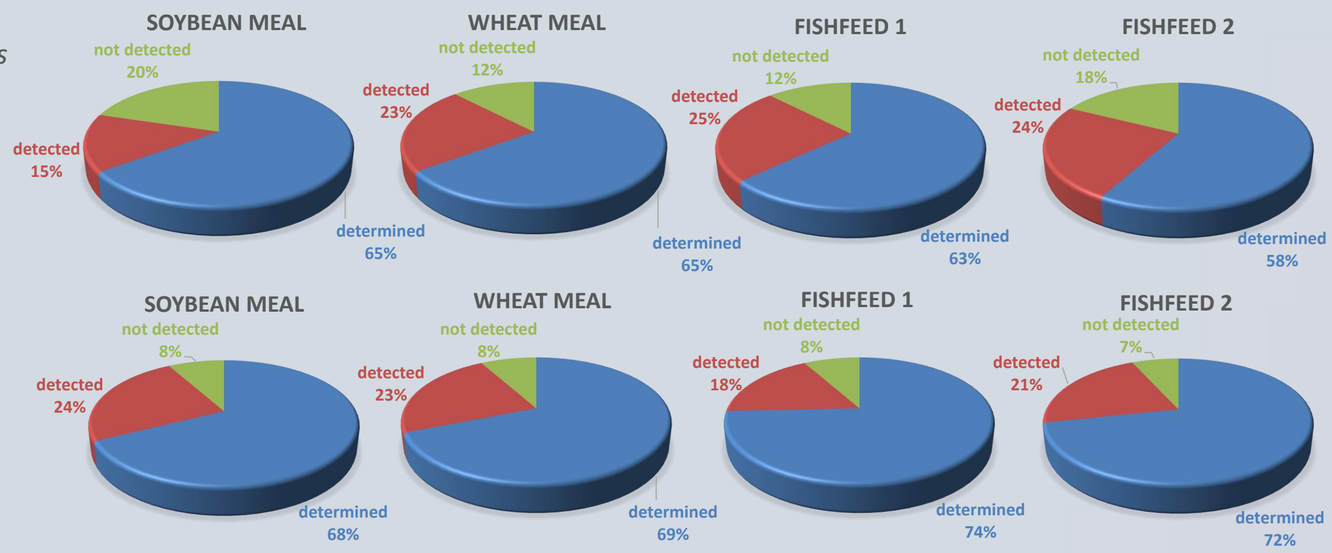
METHOD

Each individual analyte solution was infused directly into the ion source of the mass spectrometer and their respective fragmentation spectra were collected in both ionization modes. Most of phytoestrogens were well detected in negative ionization mode. Collision energies were varied from 0 to 45 eV for each precursor. Breakdown curves were illustrated using 3 average collision energies (0-15 eV, 15-30 eV and 30-45eV) for each analyte. Then, the 74 phytoestrogens were divided into 7 groups and detected according to the liquid chromatographic with tandem mass spectrometry (LC-MS/MS) conditions and the reverse phase analysis time was determined. The method was evaluated using four representative samples from two categories: (i) cereals (ii) fish feeds. The analyses of their extracts was performed utilizing the same ion source parameters and full scan acquisition in mass range from 20 to 800 amu. The method's capability to detect and identify the presence of each analyte was evaluated using samples fortified at two different concentration levels (high and low).



Limit of Detection (LOD) range of phytoestrogens





Detection (<70%) and determination (>70%) of phytoestrogens in fortified samples at concentration 2ppm and 10ppm.

CONCLUSION

- A new mass spectral library that include names, molecular formulae, and the respective MS/MS spectra of 74 phytoestrogens was constructed.
- An HPLC chromatographic method was optimized to allow their determination at low concentrations.

REFERENCES

1. *Inés Domínguez-López, Maria Yago-Aragón, Albert Salas-Huetos, Anna Tresserra-Rimbau, and Sara Hurtado-Barroso, A Review, Nutrients 2020, 12, 2456*
2. *Jéssica C. P. Petrine, Bruno Del Bianco-Borges, Phytotherapy Research.2021;35:180–19;*
3. *Thomas Bessaire, Marion Ernest, Nicolas Christinat, Benoit Carrères, Alexandre Panchaud, and Flavia Badoud, Food Additives & Contaminants: Part A, 2021.*

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