QUANTITATIVE ANALYSIS OF PHYTOESTROGENS USING LC-ESI-MS/MS

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Aquafeeds

Replacement Fishmeal with Plant Proteins
- Less costly
- Free of potential contaminants [1]

Mostly Soybean Meal Replacement
- High protein content
- Reasonable price
- Consistent quality [2]

Phytoestrogens
- Soybean meal is an abundant source of them
- Act as weak estrogen agonist or antagonist
- Controversial if they display beneficial or harmful effects on the fish development [3,4]

This has emerged the development of an analytical technique for the accurate determination of the *phytoestrogen content* in *fish feed* and *fish tissues* matrices.

Development of an analytical method for the simultaneous one pot determination of *68 phytoestrogens* by liquid chromatography tandem mass spectrometry (LC-MS/MS)

LC Conditions	Mass Conditions
Injection Volume: 10 µL	Capillary Temperature: 300°C
Tray Temperature: 25°C	Sheath & Axillary Gas: Nitrogen
Column Temperature: 35°C	Sheath Gas Pressure: 35 Arb Axillary Gas Pressure: 10 Arb
Column: Fortis Technologies Ltd., C18 (150x2.1mm, 3µm)	Electrospray Ionization (ESI)
Flow Rate: 280 µL/min	Spray Voltage: 3500 V
Solvent A: Acetonitrile Solvent B: Water 0.1% Formic Acid	Collision Gas (Ar) Pressure: 1.5 mTorr
Gradient Program: 0.0-2.0 min, A: 20%, 2.0-25.0 min, A: 20→51%, 25.0-30.0 min, A: 51→70%, 30.1-35.0 min, A: 20%	Selected Reaction Monitoring (SRM) mode

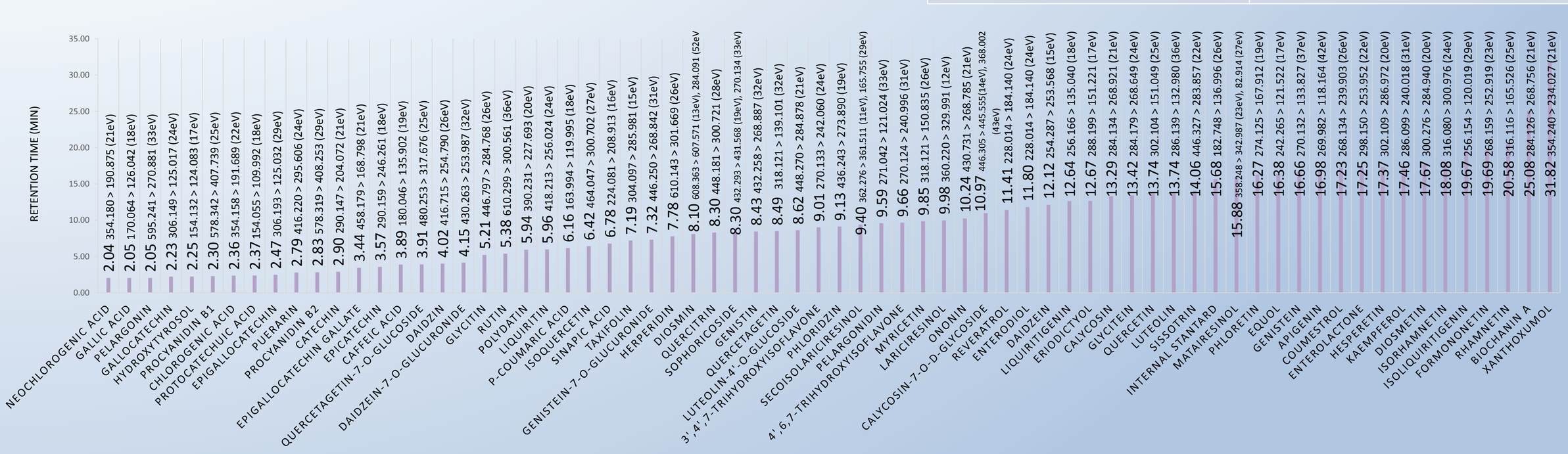

Figure 1. *Retention time and transition (Parent Mass > Product Mass (collision energy) applied for each phytoestrogen. All analytes were utilized in negative ion polarity expect for pelargonin, daidzin, glycitin, pelargonidin, ononin and calycosin-7-O-D-glucoside, which were utilized in positive ion polarity.*

Results

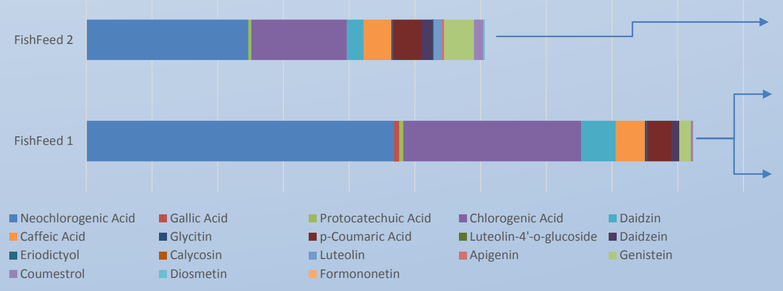

Figure 2. *Phytoestrogen content of fishfeed samples studied.*

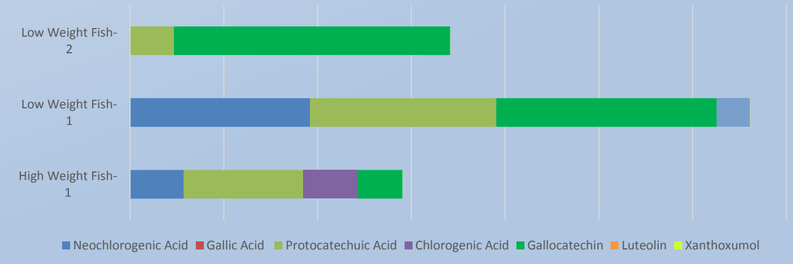

Figure 3. *Phytoestrogen content of fish tissues samples studied. Fish 1 fed with the fishfeed 1 and fish 2 fed with the fishfeed 2.*

The most abundant components in fishfeeds studied were neochlorogenic and chlorogenic acids. It Is interesting that neochlorogenic acid has been found only in the fish fed with the highest phytoestrogen content feed (Fishfeed 1) independent on their size, but chlorogenic acid has been found only in the high weight fish. Protocatechuic acid, gallocatechin and xanthohumol traces were represented in all fish tissue samples.

This study was part of the project (MIS: 5052097, CODE: Τ6ΥΒΠ-00536) entitled “Development and comparison of methods for detection and quantification of phytoestrogens in fish feed raw materials, fish feed and Mediterranean farmed fish species” that was co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation (EPAnEK-NSRF 2014-2020), under the call SPECIAL ACTIVITIES-AQUACULTURE.

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MANAGING AUTHORITY OF EPAnEK

EPAnEK 2014-2020
OPERATIONAL PROGRAMME
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ENTREPRENEURSHIP
INNOVATION

ΕΣΠΑ 2014-2020
ανάπτυξη - εργασία - αλληλεγγύη
Partnership Agreement 2014 - 2020

References

1. *The main ingredients of fish feed by The Fish Site, https://thefishsite.com/articles/the-main-ingredients-of-feed , accessed on 19/03/2022.*
2. *Nile Tilapia: Replacing Fish Meal With Soybean Meal And Yeast by The Fish Site, https://thefishsite.com/articles/nile-tilapia-replacing-fish-meal-with-soybean-meal-and-yeast, accessed on 19/03/2022.*
3. *Kolátorová L, Lapčík O, Stárka L. Phytoestrogens and the intestinal microbiome. Physiol Res. 2018 Nov 28;67(Suppl 3):S401-S408. doi: 10.33549/physiolres.934022. PMID: 30484667.*
4. *Paramita B., Camelia M. Phytoestrogens and breast cancer: In vitro anticancer activities of isoflavones, lignans, coumestans, stilbenes and their analogs and derivatives. Biomedicine & Pharmacotherapy. Volume 107: 2018. Pages 1648-1666, ISSN 0753-3322. https://doi.org/10.1016/j.biopha.2018.08.100.*