Poster Abstract

Detection and quantification of phyto-oestrogens in different matrices : development of a protocol based on UPLC-MSMS method

ROMNEE Jean-Michel[a] *– GUILLEAUME Lorraine*[b] *– GEERTS Pascal*[c] *– FROIDMONT Eric*[b]

[a]**CRAW – *Valorisation of Agricultural Products* Department**
M. Henseval Building – Chaussée de Namur, 24 – B-5030 GEMBLOUX
Tel : ++ 32 (0) 81 62 03 50 - Fax : ++ 32 (0) 81 62 03 88
[b]**CRAW – *Production and Sectors* Department**
[c]**CRAW – *Life sciences* Department**

romnee@cra.wallonie.be - http://cra.wallonie.be

Introduction

The main objective of this work describes the development of a simple analytical protocol making it possible to follow several phyto-oestrogens during their metabolization by a cow. The methodology associates an ultrasonic extraction with an ultra liquid chromatography, coupled to a tandem quadrupole mass spectrometer.

This analytical protocol could be applicable to the plant (food) and to the end product (milk, for example), but also to the blood plasma, the urine and the faeces.

Material and methods

The phyto-oestrogens extraction is done in an ultrasonic bath (2 * 30 minutes at 70°C), in 80 % methanolic solution. The extract obtained after filtration of the solution on paper filter is filtered on 0.22 µm before injection in chromatography. The separation of the molecules of interest is carried out by liquid chromatography (UPLC® Acquity – Waters). The detection and the quantification are being ensured by a mass spectrometer (Quatro Premier XE - Micromass). The column used is a HSS T3 1,8 µm column (Waters). The mobile phase is a gradient based on the water - acetonitril - formic acid mixture, for a run of 15,5 minutes.

Results

To date, 17 molecules are separated: 6 phyto-oestrogens in glycosyled form, 8 in a-glycon form and 3 bacterial metabolites (equol, enterolacton and enterodiol). In order to keep a simple elution gradient, the use of the mass spectrometry makes it possible to solve the bad separation between the prunetin and the biochanin A. The limits of detection on solution are about 1 $\mu g \cdot L^{-1}$ and the calibration curves obtained between 1 ppb and 1.000 ppb present a R^2 higher than 0,995.

The extraction, initially developed for plants, was successfully applied to spiked milk samples and juice rumen samples. These liquid samples were freeze-dried before being treated like the vegetable powders

Conclusions

The developed analytical protocol (ultrasonic extraction and UPLC-MSMS) makes it possible to follow the phyto-oestrogens present in plants throughout their metabolization (in the rumen) and to evaluate their transfer in a product like milk. The method will be supplemented by the addition of new molecules (chromatographic development) and by the adaptation of the extraction protocol to blood plasma, to urine and faeces.

Research carried out within the framework of the Moermann project “PhytoHealth”