

Hyphenated sample preparation procedure for measuring phytoestrogens in milk, cereals, and baby-food by liquid-chromatography tandem mass spectrometry with subsequent determination of their estrogenic activity using transcriptomic assay

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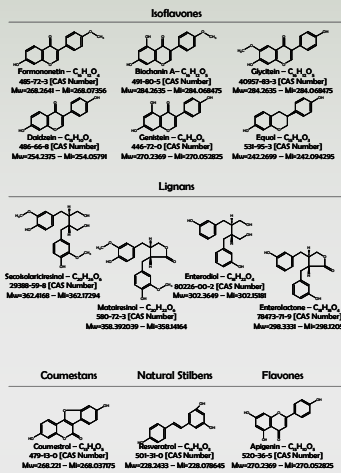
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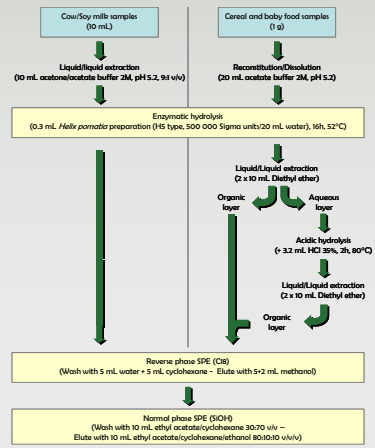
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Introduction

Phytoestrogens are natural substances originated from various vegetal species, which can be divided in different sub-families according to their chemical structure. Isoflavones are mainly found in soy-based products but also in other *Leguminosae* such as clover. Lignans are typically retrieved in cereals and other *Gramineae* species. Coumestans appears in particular in alfalfa and clover. Natural stilbens such as resveratrol are mainly present in the skin of grapes as well as in other fruits. Phytoestrogens, especially isoflavones, have been extensively studied for many years in a scope of revealing some beneficial properties on human health, including for instance reduced post-menopausal symptoms and risks of cardiovascular diseases. Their involvement in the prevention or development of cancers was also widely discussed, although no definitive conclusions were established on this topic, especially at the epidemiological scale. The estrogenic potency of some phytoestrogens has also led to their classification as endocrine disrupting chemicals (EDCs). Thus, various experimental studies performed on animal models have demonstrated some side-effects consecutive to an exposure to phytoestrogens during the perinatal period, i.e. in utero or just after birth. Then, a need for a specific exposure assessment of human foetus and new-borns to phytoestrogens may appear. In the frame of the BIOCOPI project, the biological impact of phytoestrogens was assessed at the genomic level through a transcriptomic approach. One crucial issue during this development was related to the sample preparation procedure. Indeed, this work implicated several major analytical constraints including (1) apply a sufficient purification procedure in order to avoid any cytotoxicity problem with final extracts applied onto the cellular cultures used for the transcriptomic assays, (2) keep a limited level of selectivity in order to collect an extended range of phytoestrogens with distinct physico-chemical properties in the same fraction, (3) limit as far as possible the presence of other estrogenic substances in the final extracts that could interfere with phytoestrogens in term of transcriptomic expression profile, (4) fit with the current analytical standards in terms of unambiguous identification and accurate quantification of the target phytoestrogens.

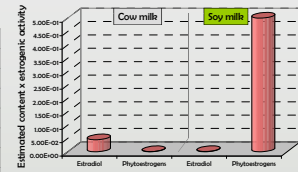
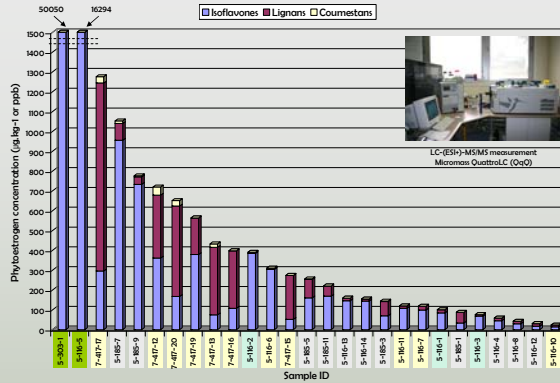


Sample preparation



Results

Sample ID	Sample Type
S-185-01	Raw (Farm) bovine milk
S-185-03	Raw (Farm) bovine milk
S-185-05	Raw (Farm) bovine milk
S-185-07	Raw (Farm) bovine milk
S-185-09	Raw (Farm) bovine milk
S-185-11	Raw (Farm) bovine milk
S-185-13	Raw (Farm) bovine milk
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In spite of the significantly lower biological activity of phytoestrogens compared to natural estrogens hormones (around 10⁵ fold), such extreme concentration levels found in soy-based products lead to a significant contribution in terms of global "estrogenic load" induced by these products.

More detailed results regarding this work will be presented during the following dissemination event:

Analysis of estrogenic endocrine disruptors in food: state-of-the-art and future trends
"From specific spectrometric approaches to emerging global transcriptomic approaches"

Workshop organized in the frame of the BIOCOPI research project (EU, 6th FP, FP) by
School for Advanced Residue Analysis in Food (SARAF)

9th - 11th December 2008

Laboratoire d'Etude des Résidus et Contaminants dans les Aliments (LABERCA)
École Nationale Vétérinaire (ENVA), Nantes, France

An Integrated Project funded by the European Commission under the Food Quality and Safety Priority Thematic Area.
Contract Number: **FOOD-CT-2005-006988**
Project Co-ordinator: **Prof. Chris Elliott**, Queen's University Belfast

