Study of a post-column synchronous fluorescence derivatization to analyze phytates in foods by chromatography

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Phytates (myo-inositol-1,2,3,4,5,6-hexakisphosphate or InsP6) is the storage form of about 80% of total phosphorus in foods. They are commonly referred to antinutritional compounds because they strongly chelate with divalent cations such as iron, potassium, calcium, magnesium, zinc and copper, impeding their absorption. Their nutritional effects and physiological functions are dependent on the number and the position of the phosphate groups on the myo-inositol ring ($InsP_{1\ to\ 6}$). However, the lack of analysis method accurate and reliable to quantify each form creates the need to develop new approaches.

In this study, a chromatographic methodology (HPLC) was developed and enhanced to separate and quantify the six forms of myo-inositol polyphosphates using synchronous fluorescence by post-column derivatization. Maximization of reagents content and assays were realized in static and dynamic step, and standards were bought pure from Larodan (USA). The HPLC (Agilent infinity II – Fluorescence/Diode Array Detectors) was improved with a supplementary pump for post-column derivatization through a knitted open tubular (KOT) reactor.

Results indicated that this innovative method crossing HPLC and synchronous fluorescence of iron-phenanthroline (0.0045 mol/L $FeCl_3 \bullet 6H_2O$: 0.0085 mol/L phenanthroline) with a post-column derivatization is promising and fast for phytates separation and quantification. Indeed, the quenching of phenanthroline caused by the presence of iron was canceled when InsP eluted through a separation by ion-pair reverse chromatography (44% 0.035M formic acid mixed with 56% methanol and 1% tetrabutylammonium hydroxide fixed at pH 4.3 adjusted with H_2SO_4 72%). It allowed an increase of the detection limits of InsP compare to conventional refractometer (RID). It seemed that using the same phase for InsP extraction could simplify the process route by avoiding unitary operations such as evaporation, purification or concentration compared to classical extraction. Methanol fraction contained in the solvent might be able to slow or stop enzymatic and non-enzymatic hydrolyses of InsP during extraction, stabilizing the extract environment too.

This innovative analytical approach to separate and quantify phytates by HPLC seemed robust and reliable. Additional assays need to be performed to determine the applicability of the method in multiple food environment and evaluate phytate subsequent impact on divalent minerals' bioavailability.