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Determination of selenium species in Zea mays samples by enzymatic extraction and HPLC-ICP-MS

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Abstract:
Selenium (Se) is an essential nutrient for animals and humans at very low dose, but is not a micronutrient for higher plants (Terry et al, 2000). The recommended dietary allowance of selenium is between 40 and 70 μg/person/day (World Health Organization, 1996). Especially several countries (UK, Brazil, France, and part of China, for example) suffer from deficiency in selenium intake. According to important consequences, sometimes lethal, caused by deficiency or excess of selenium, it is necessary to understand its biogeochemical cycle and in particular in the food chain transfer. Plants, especially crops which are one of the main sources of European dietary, play an essential role in the incorporation of selenium in the terrestrial food chain. The toxicity and bioavailability of Se depend not only on concentrations but also on their speciation in food. In fact, these Se-organic forms are more efficiently assimilated by animals and humans than inorganic form (Rayman, 2008). The plants absorb selenium by root system; it is then stocked in tissues or/and metabolized in selenoamino-acids and/or volatilized as methyl organic compounds. However, the accumulation rate of Se and its metabolic pathway depends not only on the plant species but also on the selenium form supplied (Li et al, 2008; Ximenez-Embun et al, 2004; Zayed et al, 1998).
In this context, the aim of the experiments realized in our laboratory is to evaluate the influence of selenium forms on accumulation, distribution and eventually toxicity of selenium in Zea mays and its transfer in water-soil-plant system. The determination of total Se concentration in different types of sample with acid digestion is well known. But, it is necessary to develop procedures of extractions that do not alter the Se speciation in samples. The procedures generally used on vegetable samples are water and enzymatic with protease XIV extractions (Mazej et al, 2008; Montes-Bayon et al, 2006; Ximenez-Embun et al, 2004). Enzymatic extractions efficiency varies between 55 and 120% and depends on the plant tissues (leaves, stems or roots) and Se form supplied. In this study, the different extraction methods of Se in certified reference material plant and Se-enriched Zea mays tissues are tested: 1.Water extraction; 2. Different mixture of enzymes extractions (protease XIV, lipase VII and cellulase) in Tris-HCl solution (pH =7). The samples and reagents were thoroughly homogenized, kept at 37°C for 16H, and then centrifuged at 4000 rpm for 30 min. The supernatant were removed and filtered through 0.45 μm and stocked in mercapto-ethanol (0.1%) solution to avoid oxidation. In each sample, total Se concentration is determined by ICP-MS as well as Se inorganic forms by HPLC-ICP-MS (with CCR) off line.
The first results show the mixture with protease+lipase is the most effective procedures. Efficiency extraction is not affected by types of plant tissues but strongly affected by Se form supplied. The Se concentrations in the plant samples supplied with selenite are lower than in the ones supplied with selenate.

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