OP 2.1

Microwave-assisted enzymatic hydrolysis: evaluation of its capabilities for Se-speciation

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Selenium (Se) is an essential nutrient to humans, animals and micro-organisms, playing an important role in maintaining a healthy immune system, fertility and thyroid metabolism. However, nutritional bioavailability and toxicity are dependent upon its chemical form and concentration. The accurate determination of Se species requires extraction methods that exhibit high extraction efficiencies, and are capable to preserve the identity of the Se-compounds.

Enzymatic hydrolysis, utilising protease XIV or proteinase K, has been the most widely used technique to release protein-bound compound such as selenomethionine (SeMet) in a number of food/supplement matrices (1). Efficiency of SeMet extraction with proteolytic enzymes has been found critically dependent upon incubation or extraction time and sample to enzyme ratio. Successful enzymatic approaches involve the use of multiple consecutive steps, with duration of at least 18 hours each (1). Such tedious multi-step approaches have often resulted in transformation and/or interconversion of target Se species (e.g. SeMet).

More recently, the combination of enzymatic digestion with probe sonication has been reported as a promising tool for extraction of Se species from food and supplements, overcoming the main drawback of the traditional enzymatic treatments (e.g. incubation and bath sonication) such as long sample treatment times (2). It has proven to be a powerful system in order to speed the extraction of Se species from yeast, oyster and mussel tissues, but, of course, the extraction efficiency was found to be critically dependent on the extraction conditions. Preliminary work in our laboratory has shown, for the first time, the promising capabilities of enzymatic extraction assisted by microwave energy for enhancing the enzyme activity without varying the nature of target dietary Se species. To the best of the authors's knowledge, the use of enzymes with microwave energy for Se speciation studies has never been reported.

This work aims at evaluating the potential of microwave-assisted enzymatic hydrolysis, in comparison with conventional enzymatic hybridization, for the extraction of SeMet from food samples, followed by HPLC-ICP-MS analysis. The influence of different parameters such as extraction time, enzyme type, solvent volume and microwave power on the extraction efficiency of SeMet was investigated. The use of enzymatic extraction assisted by microwave energy led to SeMet extraction efficiencies, which are similar to those obtained by conventional multi-step enzymatic hydrolysis. However, with the newly developed approach, the extraction time was found substantially shortened. In addition to this, the simplicity and robustness of the method offers a straightforward approach that can be applied in future to the investigation of Se species distribution in other complex matrix samples

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