Speciation of zinc in fish feed by size exclusion chromatography coupled to inductively coupled plasma mass spectrometry – using fractional factorial design for method optimisation and mild extraction conditions

Zinc (Zn) is an element essential to all living organisms and it has an important role as a cofactor of several enzymes. In fish, Zn deficiency has been associated with impaired growth, cataracts, skeletal abnormalities and reduced activity of various Zn metalloenzymes. Fish meal and fish oil traditionally used in salmon feed preparation are being replaced by plant-based ingredients. Zinc additives are supplemented to salmon feed to ensure adequate Zn levels, promoting good health and welfare in Atlantic salmon (Salmo salar). The main objective of the present study was to evaluate Zn species found in an Atlantic salmon feed. This work describes a Zn extraction method that was optimized using a fractional factorial design (FFD), whereby the effect of six factors could be studied by performing only eight experiments. The effects of the type of extraction solution and its molar concentration, pH, presence of sodium dodecyl sulphate, temperature and extraction time on Zn extraction were investigated. Mild extraction conditions were chosen in order to keep the Zn species intact. Total Zn (soluble fractions and non-soluble fractions) was determined by inductively coupled plasma mass spectrometry (ICP-MS). The highest Zn recovery was obtained using 100mM Tris-HCl, pH8.5 at a temperature of 4°C for 24h where the total Zn in soluble fraction and non-soluble fraction was 9.9±0.2% and 98±6%, respectively. Zinc speciation analysis (on the soluble fractions) was further conducted by size exclusion inductively coupled plasma mass spectroscopy (SEC-ICP-MS). The SEC-ICP-MS method provided qualitative and semi-quantitative information regarding Zn species present in the soluble fractions of the feed. Four Zn-containing peaks were found, each with different molecular weights: Peak 1 (high molecular weight - ≥600kDa), peak 2 and peak 3 (medium molecular weight – 32 to 17kDa) were the least abundant (1–6%), while peak 4 (low molecular weight – 17 to 1.36kDa) was the most abundant (84–95%).

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