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THERE IS A NEED FOR SPECIATION ANALYSIS OF SELENIUM IN FISH FEED AND FISH TISSUE

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Background

Selenium (Se) is an element that is both essential and toxic for living organisms. The element can be present in organic and inorganic forms, and the bioavailability and toxicity of Se are affected by its chemical form, or Se species. Seafood and marine raw materials have natural high levels of Se. Hence, in traditional salmon feeds where there is a high inclusion of marine raw materials, such as fish meal, the Se levels are high. The Se levels typically range from 0.2 to 9.0 mg Se kg^{-1} feed [1, 2]. The Se levels in fish feeds are dependent on the Se levels in the feed ingredients, and replacement of marine-based ingredients with plant-based ingredients lowers the Se levels in the final feeds. In turn, this may cause a need for supplementation of Se to assure a robust farmed salmon. Hence, the substitution of feed ingredients and supplementation of feeds with *e.g.* Se-containing yeast or inorganic Se salts also affect the Se species pattern in the final feeds and possible in tissues of farmed Atlantic salmon. To study the bioavailability and toxicity of different Se feed additives, there is a need for speciation analysis of both feeds and of fish.

→ Aim of this study was develop analytical method for determination of Se species in feed ingredients, fish feeds and in fish tissue.

Introduction

For Se speciation analysis High Pressure Liquid Chromatography (HPLC) coupled to Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is commonly used. A range of natural occurring Se species exist in environmental samples, both inorganic Se species (selenite and selenate) and organic Se species (*e.g.* selenomethionine; SeMet), Selenomethylselenocysteine; SeMetSeCys and selenocysteine; SeCys). In fish tissue SeMet is normally the major Se species detected [3, 4]. Since SeMet and SeCys are bound to proteins, Se was extracted from sample matrix using enzymatic hydrolysis using a non-specific protease. The extraction recovery (%) of Se in the soluble extracts was determined by measurement of total Se in the extracts by ICP-MS, and comparing the results with total Se concentrations in the samples (Fig 1). For the Se speciation analysis two chromatographic separation modes were used to achieve the optimal chromatographic separation of the Se species; an anion-exchange HPLC-ICP-MS method was applied for the determination of the inorganic Se species, whereas a cation-exchange HPLC-ICPMS method was applied for determination of organic Se species. Fig 2 shows fish feed supplemented with a high level of inorganic Se analysed by anion-exchange HPLC-ICPMS, and a feed supplemented with high level organic Se-containing yeast analysed by cation-exchange HPLC-ICPMS.

Outline of Se speciation analyses

Approximately 0.2 g of freeze dried sample was mixed with 2.5mL of 1mM ammonium phosphate, pH 7, containing 20 mg protease type XIV from Streptomyces griseus (Sigma Aldrich). The samples were left on water bath at 37°C and shaking at 100/min for 24h. The samples were cooled, centrifuged (3500rpm, 10min) and filtered with 0.45µm, membrane filters (Meck Millipore) and Amicon (0.5mL, 10kDa cut-off, Merck Life Science AS) prior to Se speciation analysis with HPLC-ICPMS.

Table 1. Instrumental settings for ICP-MS and HPLC (1260, both Agilent Technologies) in Se speciation analysis.

RF power	1520 W
Carrier gas flow	0.9 L min^{-1}
Plasma gas flow	0.20 L min^{-1}
H_2 gas flow (collision-reaction cell)	4 mL min^{-1}
Spray chamber temperature	2 °C
Interface cones	Nickel
Nebulizer	Babington
Integration time	0.1 sec
Isotope monitored	78, 80, 82
HPLC settings – Anion-exchange HPLC	
Column	Hamilton PRP-X100 (4.6 x 150mm, 5µm)
Injection volume	25µL
Flow rate	1 mL min^{-1}
Mobile phase	A: 0.5mM ammonium acetate, pH 5.2. B: 100mM ammonium acetate, pH 5.2
Gradient	0-3min: 95% A, 5% B, 3-5min: 5-100% B, 5-13min: 100% B, 13.1 – 20min: 95% A, 5% B.
HPLC settings – Cation-exchange HPLC	
Column	Ionosphere 5C (3 x 100mm, 5µm)
Injection volume	25 µL
Flow rate	1 mL min^{-1}
Mobile phase	A: 0.5mM Pyridine, pH 3. B: 10mM Pyridine, pH 3
Separation mode	0-2min: 90% A, 10% B, 2-4min: 10-100% B, 4-10min: 100% B, 10.1-15min: 90% A, 10% B.

Results & Discussion

Table 2. Se levels in experimental diets; mean ± SD, *n* = 3.

Diet	Information on feed diets	Se concentration (mg kg^{-1} dw)
Diet 1	Plant-based	0.35 ± 0.02
Diet 2	Supplemented- low inorganic Se	1.10 ± 0.03
Diet 3	Supplemented- low inorganic Se	15.0 ± 0.5
Diet 4	Supplemented- low organic Se	2.10 ± 0.05
Diet 5	Supplemented- low organic Se	15.0 ± 0.24
Diet 6	Marine-based	0.89 ± 0.03

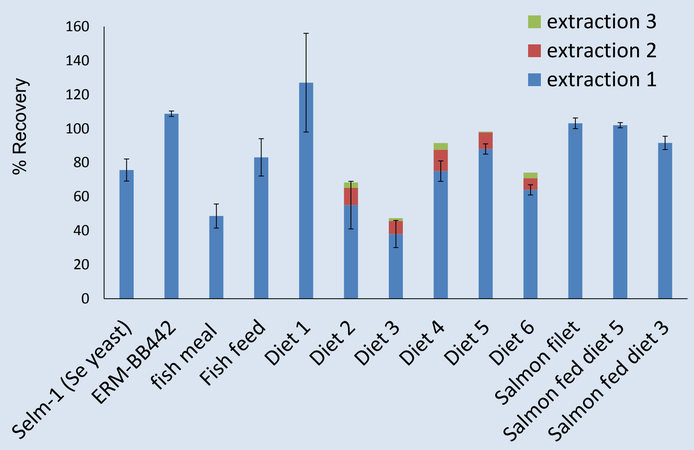

Fig 1. The extraction recovery (%) of Se in the protease extracts compared to total Se in samples (*n*=3), determined by ICP-MS. The extraction procedure was carried out three repetitive times for selected samples .

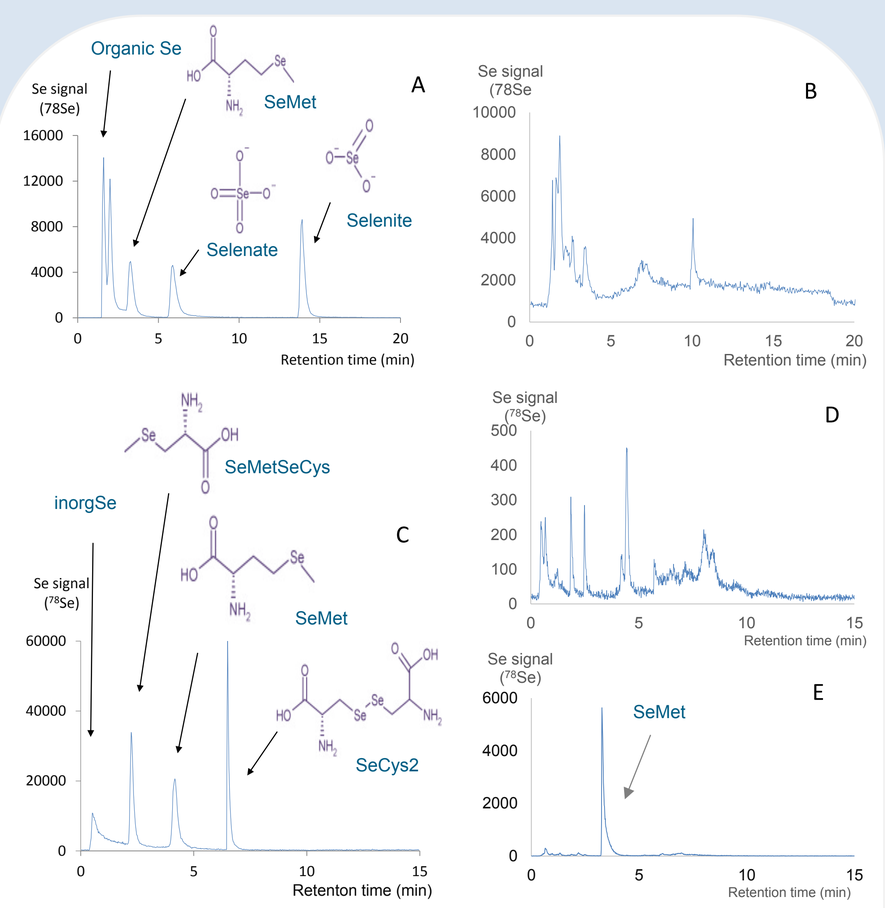

Fig 2. Anion-exchange HPLC-ICPMS of inorganic Se species of a standard mixture (100 µg L^{-1}; A) and diet 3 (B). Cation-exchange HPLC-ICPMS analysis of Se species in a standard mixture (C), fish feed diet 3 (E) and diet 5 (x4 dilution). The assigned SeMet peak in diet 5 was determined by spiking the sample with a standard mixtture.

Conclusion & future work

• The extraction efficiency of Se seems to depend on the type of sample and supplementation source. The extraction recovery is lower for the feeds supplemented with inorganic Se compared to feeds supplemented with organic Se yeast. The extraction recovery is 99 ± 12% (*n* = 17) for salmon muscle tissue.

• Two separate chromatographic methods have been developed for speciation analysis of inorganic and organic Se species in fish feeds and salmon muscle tissue. The methods needs yet to be fully validated.

• Speciation analysis of supplemented feeds shows that the feed supplemented with high inorganic Se contains several unknown Se species. Selenomethionine is the major Se species in the organic supplemented feed. Preliminary results shows that SeMet is the major Se species in the salmon tissues.

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