Impact of fish growing conditions and cooking methods on selenium species in swordfish and salmon fillets

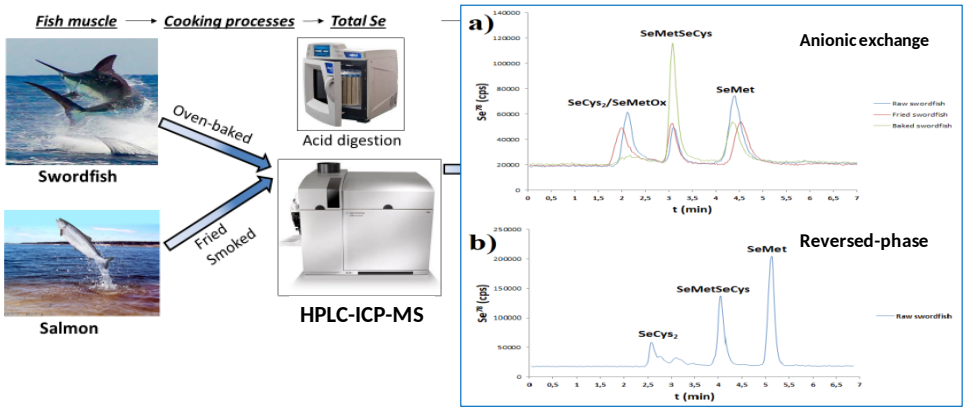
David VICENTE-ZURDO, Beatriz GÓMEZ-GÓMEZ, María Teresa PÉREZ-CORONA and Yolanda MADRID*

Departamento de Química Analítica, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, 28040 Madrid, Spain

*Corresponding Author:
Prof. Yolanda Madrid Albarran
Dept. of Analytical Chemistry
Faculty of Chemistry
Universidad Complutense de Madrid
E-28040 Madrid, Spain
Phone: ++0034913945145
Fax: ++0034913944329

HIGHLIGHTS

- Se concentration in fishes ranged within 0.39- 0.68 mg Kg^{-1}
- Lowest selenium concentration was found in salmon from aquaculture
- Se was present as organic selenium (93% total selenium) in all tested fishes
- Selenium species profile was different depending on the type and fish origin
- Cooking do not originate new species but alter concentration of the existing ones


Fish muscle
Cooking processes
Total Se
Swordfish
Salmon
Oven-baked
Fried
Smoked
Acid digestion
HPLC-ICP-MS
a)
Anionic exchange
SeMetSeCys
SeCys2/SeMetOx
SeMet
Raw swordfish
Fried swordfish
Baked swordfish
t (min)
b)
Reversed-phase
SeMet
SeMetSeCys
SeCys2
Raw swordfish
t (min)

Impact of fish growing conditions and cooking methods on selenium species in swordfish and salmon fillets

Abstract

The study evaluates the effect of fish growing conditions (captured fisheries or aquaculture) and cooking methods (fried, oven-baked and smoked) on Se-species distribution in fish fillets from highly consumed fishes (salmon and swordfish). For this purpose, samples of fish fillets from 10 individual fishes for each fish species were analyzed. Selenium speciation was achieved by HPLC-ICP-MS. Selenium in fillet samples was mainly present as organic selenium (around 93% of selenium content). Selenomethionine (SeMet) and selenocystine ($SeCys_2$) were the main species found in salmon regardless of its growing conditions (farmed or wild), however $SeCys_2$ was found at a higher concentration in wild salmon fillets. Concerning swordfish, SeMet, $SeCys_2$ and selenomethylselenocysteine (SeMetSeCys) were detected and quantified. New selenium species were not produced when fillets were submitted to cooking processes, however differences in selenium species distribution were observed for some fishes and/or treatments applied. $SeCys_2$ in salmon and SeMetSeCys in swordfish were significantly modified by the cooking treatments applied. Baking led to a notable increase of SeMetSeCys in swordfish (68% of the total selenium) in comparison with the amount of SeMetSeCys found in raw fish (17% of total selenium) whereas a notably decrease of $SeCys_2$ occurred when salmon was submitted to the different cooking techniques. In contrast, the analysis of smoked salmon provided a selenium species distribution that was similar to that of raw farmed salmon

Keywords: Selenium speciation; HPLC-ICP-MS; fish growing conditions; cooking process; species distribution; extraction optimization.

1. Introduction

The consumption of fish is well implemented all around the world. As indicated in the report "The state of world fisheries and Aquaculture from the Food and Aquaculture Organization of the United Nations (FAO, 2016)", fish provides 6.7% of all the proteins consumed by humans as well as offering a source of omega-3- fatty acids, vitamins A and D and essential elements such as Ca, Se, Zn, Fe and I.

Farmed fish is an important source of seafood accounting for 44% of total production in 2014. Atlantic salmon (*Salmon salar)* is one of the leading farmed marine species with an average increase of 11.2% during the period from 2000 to 2010 (Bostock et al, 2010). Selenium is one of the most important essential elements among those provided by fish consumption. Selenium is an essential nutrient since it is indispensable for the normal activity of the human body (Ermakov and Jovanović, 2010). Selenium deficiency could induce the Keshan disease, an endemic cardiomyopathy, and the Kashin-Beck disease, an endemic osteoarthropathy (Yang et al., 1988). Supplements containing selenium are usually administered with the aim of preventing diseases cause by selenium deficiency, (Kubachka et al., 2017). However, when selenium is supplied in excess there is a high risk of toxicity, which is call "selenosis" (Raisbeck, 2000). Consequently, selenium is considered as one of the elements with the narrowest gaps between deficiency and toxicity (WHO, 1996). According to the latest report of the European Food Safety Agency (EFSA, 2014), the estimate selenium supply for adolescents from 15 years and adults per day is 70 μg selenium; 85 μg selenium is allocated to breast feeding mothers and the estimated values for children (from 7 months) and younger adolescents (up to 14 years) ranged from 15 to 55 μg selenium per day. Selenium demand appears, therefore, dependent on the individual state of nutrition and health of individuals as well as the chemical form in which is supplied. Organic selenium species ($SeCys_2$, SeMet and SeMetSeCys) are three times more effective in alleviating deficiency symptoms than inorganic ones (Se(IV) and Se(VI)), and in general, inorganic selenium is

more toxic than organic forms (Ganther & Lawrence, 1997). Meanwhile, selenate is more toxic than selenite (Nagy et al., 2015).

The main source of selenium in humans is the diet, especially the seafood. The presence of selenium in seafood is due to its occurrence in the aquatic environment. Selenium biomagnifies through the food chain leading to the presence of a large amount of selenium in bigger fishes like predatory ones (Copat et al., 2014; Økelsrud, Lydersen, & Fjeld, 2016). Marine animals have more selenoproteins (around 30-37 selenoproteins) than terrestrial animals. Fish have several species-specific selenoproteins (fish 15 kDa selenoprotein-like protein (Fep15), selenoprotein J and selenoprotein L) which biological functions are still unclear (Zhang & Gladyshev, 2008).

In aquaculture, marine ingredients have been replaced with feeds containing fish oils, animal and plant proteins, minerals, and vitamins with the aim of fulfilling the nutritional requirements of the fish. Traditionally, diets for carnivorous fish such as salmon contain around 30-50% fish meal and oil. Concerning selenium, the European Union (Commission Regulation EU No 432/2012) has legislated that the level of total selenium added to aquaculture feeds must not exceed 0.5 mg kg^{-1} (dry mass). However, this level is based on feeding tests using inorganic Se (mainly sodium selenite) as supplement. It has been reported that supplementation of Atlantic salmon fed with organic (selenized-yeast) and inorganic (selenite) selenium provides differences in selenium accumulation. (Sele et al, 2018). The highest selenium levels was detected when supplying diets contained organic Se. Speciation analysis confirmed that SeMet was the major Se species in muscle of Atlantic salmon. Several studies have also evidenced that selenium supplementation as organic form up to 5 mg kg^{-1} has a beneficial effect on muscle development during fish larvae stage (Betancor et al., 2012) alleviates stress in fish cause by crowded conditions (Rider et al, 2009) and improves fish immune response (Pacitti et al, 2016). It is generally agreed that organic selenium is tolerated to higher concentration than inorganic selenium (Pedrero& Madrid, 2009).

In the last years, there is a growing trend in aquaculture in replacing fish meal and fish oils with plant-derived materials which it may not fulfil the requirements of selenium and other nutrients.

From the above it is clearly demonstrated that the level and chemical species of selenium in fish is dependent on the growing conditions of the fish (captures fisheries or from aquaculture).

Another factor that may impact selenium species distribution is food processing. As a rule, speciation studies are mostly performed in raw products, however fish is mainly consumed cooked. Several papers have shown that food processing can alter selenium species distribution and consequently its bioavailability (Schmidt et al., 2015; Pedrero et al. 2007). Most of the work performed so far has been focused on vegetable-based food products while the studies on seafood are scarce and limited to the effect of cooking processes on total selenium content (Martins, Alvito & Alameida, 2011)

The purpose of this work is to evaluate the effect of food processing (frying, oven-baking, smoked), type of fish (swordfish and salmon) and growing conditions (farmed and wild) on selenium content and selenium species distribution. The results obtained will be useful to obtain information on the nutritional or toxic impact of selenium when ingested from highly consumed fish, especially salmon.

2. Material and Methods

2.1. Reagents and standards

All solvents/chemicals used were of analytical grade. Water used throughout the experiments was ultrapure deionized water with resistance 18 MΩ·cm obtained from a Milli-Q water purification system unit (Millipore, Bedford, MA, USA). For total selenium analysis, samples were digested using nitric acid 60% from Scharlab (Spain) and hydrogen peroxide 33% from Panreac (Barcelona, Spain). Individual standards of selenium species were obtained by dissolving Na_2SeO_3, Na_2SeO_4, $_{DL}$-selenocystine ($SeCys_2$), $_{DL}$-selenomethionine (SeMet) and Se-methyl-$_{DL}$-selenocysteine

(SeMetSeCys) from Sigma-Aldrich (Steinheim, Germany) in deionised water. Working solutions were prepared daily by appropriately diluting the concentrate standards in 0.1% hydrochloric acid from Scharlab (Spain). For speciation analysis, mobile phase components included citric acid 99.5% from Sigma-Aldrich (Steinheim, Germany), trifluoroacetic acid (TFA) from Sigma-Aldrich (Steinheim, Germany) and HPLC-grade methanol from Scharlau (Walkerburn, Scotland). Enzymatic hydrolysis of samples was performed by using protease type XIV *Streptomices griseus* and tris(hydroxymethyl)aminomethane (TRIS) 99.8% both from Sigma-Aldrich.

2.2. Samples

Fish samples analyzed were swordfish (*Xiphias gladius*) and farmed and wild salmon (*Salmon salar*). Fish fillets (the edible part) were purchased at a local Spanish market and were immediately blended and stored at -18 ºC. With the aim of having representative results for the fish analyzed, fish fillets were obtained from 10 individuals of wild and farmed salmon and swordfish with lengths ranged between 60-100cm long for salmons and 1.2-2.5m long for swordfish. Fish fillets from 10 individual fishes for each fish species were individually analyzed and the results were expressed with the standard deviation that includes the inter- individual differences.

Each fish samples were analyzed raw (all samples), fried (swordfish, wild and farmed salmon) and oven-baked (swordfish). Frying was performed in a frying-pan with a minimum amount of olive oil at about 180 ºC (i.e. under conditions that represents normal household cooking practices). When finished, fish was removed from the pan and the superficial fat was eliminated. Swordfish was oven baked in an oven at 200 ºC for 45 minutes. Smoked salmon was acquired in a local supermarket. The results for total selenium determination were validated using a marine tissue reference material (Murst-ISS A2), certified for selenium (7.37 ± 0.91) mg Kg^{-1} from the Institute for Reference Materials and Measurements.

2.3. Instrumentation

A 1000W MSP microwave oven from CEM (NC, USA) equipped with temperature and pressure feedback controllers and 12 high-pressure vessels of 100 mL inner volume, operating at a maximum power of 1600W was used for microwave acid digestion.

An ultrasound probe Vibracell Sonics (Connecticut, USA) equipped with a 2-mm diameter titanium microtip fitted with a high-frequency generator of 130 W at a frequency of 20 kHz was used to extract the seleno-compounds.

An Agilent 7700x collision/reaction cell ICP-MS (using H_2 collision gas) from Agilent Technologies (Tokyo, Japan), equipped with a Meinhard nebulizer, a Scott spray chamber (double axial crossing) and a Peltier cooling system was employed for selenium specific detection. HPLC-ICP-MS measurements were carried out using a PU-2089 LC pump from JASCO Corporation (Tokyo, Japan) fitted with a six-port injection valve (model 7725i, Rheodyne, Rohner Park, CA, USA) with a 100-µL injection loop for the chromatographic separations. The m/z ^{74}Se, ^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se and ^{82}Se were monitored in all experiments, with ^{78}Se being the preferred isotope for reporting without reaction cell, and ^{80}Se when reaction cell was used.

Chromatographic separations were performed using two different columns: reversed-phase analytical column Zorbax Rx C8 (250 x 4.6 mm, 5 µm) (Agilent Technologies, USA), and an anionic exchange column Hamilton PRP-X100 (250 x 4.1 mm, 10 µm) (Hamilton, Switzerland). The outlet of the column was connected directly to the conical nebuliser of the ICP-MS with PEEK tubing. Operating parameters are compiled in Table 1.

2.4. Moisture content analysis

The moisture content of composite samples of swordfish, and wild and farmed salmon fillets was determined by weighing three replicates of about 2.5 g of fish samples and introduced in a lab stove at 100 ºC during 4 h. Then, the samples were cooled at room temperature in a desiccator and weighted. The process was repeated until constant weight.

2.5. *Total selenium analysis*

Total selenium content was measured by ICP-MS after acid digestion of the samples in a microwave oven. For this purpose, 5 mL of HNO_3 60%, 1 mL H_2O_2 33% and 1 mL Milli-Q H_2O were added to 1 g of homogenized of fish fillets sample and submitted to a microwave heating program consisted of a 20 min ramp to 130 °C and a 10 min hold time. The resulting extracts were diluted to 10 mL with Milli-Q water, filtered through a 0.22 μm filter and stored at -80 °C until analysis. Measurements were achieved by ICP-MS under the conditions showed in Table 1. The quantification was performed using external calibration method. Calibration range was prepared between 0 and 75 $\mu g \cdot L^{-1}$ from a Se(IV) individual standard of 1000 $mg \cdot L^{-1}$. All calibration curves provided r^2 values of >0.99. Detection (LOD= 0.0247 $mg \cdot kg^{-1}$) and quantification (LOQ= 0.0815 $mg \cdot kg^{-1}$) limits were calculated by analyzing 10 replicates of a blank solution and applying the 3SD and 10 SD criteria, respectively.

2.6. *Selenium speciation analysis*

Selenium species were determined in fish fillets samples from 10 individual fish for each fish species: swordfish (fresh, fried and oven-baked), farmed salmon (fresh and fried), wild salmon (fresh and fried) and smoked salmon. Measurements were performed by HPLC-ICP-MS prior enzymatic hydrolysis with protease type XIV.

Enzymatic hydrolysis was performed by treating 0.5 g of homogenized fish muscle samples with protease type XIV and 3 mL Tris-HCl 30 mM (pH= 7.5). Best extraction conditions were selected by applying an experimental design consisting of three factors with two levels each: extraction time (A, 2 and 6 min), ultrasound powder (B, 40 and 60 %) and protease amount (C , 20 and 40 mg). Response was evaluated by the percentage of extracted selenium**.** Eight experiments were performed (complete factorial design: 2^k, k=3) with three replicates per experiment. The resulting extracts were submitted to centrifugation during 25 min at 12633 and stored at -80 ºC until analysis. Finally, measurements of total selenium (for extraction yield) and selenium species in the extracts

were performed by ICP-MS and HPLC-ICP-MS, respectively under the conditions given in Table 1. For HPLC-ICP-MS measurements two different columns (Table 1) were used to avoid an erroneous identification of selenium species. Anionic exchange PRP-X100 column usually offers a better resolution for selenium species. Unfortunately, SeMet often transforms into Selenomethionine-Se-oxide that coelutes in the PRPX-100 column with $SeCys_2$. Therefore, a reversed-phase column Zorbax Rx C8 (250 x 4.6 mm, 5 μm) was also used for improving separation and identification of the extracted selenium species. If a species is identified under two different chromatographic conditions, its identity can be stated with more certainty. Identification of the selenium containing peaks was performed by spiking experiments and by comparing their retention time with those provided by organic selenium standards. Selenium species quantification was achieved by applying the standard addition method. The recovery of the column was calculated by comparing the total Se concentration of the extract measured by ICP-MS *versus* the concentration resulting of the sum of selenium compounds from speciation analysis of the same extract.

2.7 *Statistical Analysis.*

A one-factor analysis of variance was applied to detect possible differences in selenium and selenium species content between the different fish species and treatments studied. A significance level of $P < 0.05$ was adopted for all comparisons. The software package Statgraphics Centurion XVII version 17.01.0012, running under Windows10 was used for the statistical analysis.

3. Results and Discussion

3.1. Moisture content determination

The moisture content of swordfish and salmon (farmed, wild and smoked) was determined following the procedure detailed in Section 2.4. The results are detailed in Table 2.

3.2 Total selenium determination and selenium speciation. Effect of the type of fish and growing conditions

Total selenium content in fish fillets was determined by ICP-MS following the experimental procedure described in section 2.5. Table 2 includes total selenium contents found in fish fillets along with the standard deviation including the inter- individual differences. Validation of the results obtained was achieved by using a marine tissue reference material (Murst-ISS A2), certified for selenium (7.37 ± 0.91) mg Kg^{-1} from the Institute for Reference Materials and Measurements. Because at the 95% confidence level, no significant differences were detected between the certified value and the experimental one (7.41 ± 0.6 mg Kg^{-1} the method used was considered to be accurate for selenium determination.

The selenium amount in the different fishes varied between 0.39 and 0.68 $mg \cdot kg^{-1}$. Differences in selenium concentration were found between farmed and wild salmon. These differences can be attributed to the type of diet. The presence of selenium in marine fishes is due to its occurrence in marine environments. Selenium enters in marine environments, mainly as inorganic selenium (selenite, selenate) through erosion of rocks and soils and industrial activities and is biomagnified in food webs. Phytoplankton bioconcentrates selenium from 10^2 to 10^6 fold above dissolved concentration. Phytoplankton is also responsible of transforming inorganic selenium into Se-containing amino acids which is a crucial step in the accumulation and magnification of selenium. Nowadays is well established that selenium in big fishes is presented via food chain rather than accumulation from water (Schlenk et al, 2007).

In contrast, as it was mentioned in the introduction section, aquaculture employs manufacture feeds for supplying the nutrients required for the correct development of the farmed fish. The preparation of aquaculture feed requires a large amount of fish meal and oil to fulfil the needs of an increasingly demanded product such as farmed fish. In the last years interest has growth in developing alternative feeds more economically and environmentally friendly. In this line, the inclusion of

vegetable products combined with probiotic bacteria to improve the digestibility of vegetable ingredients seems to be an alternative to traditional feeds (Olmos Soto, Paniagua-Michel, Lopez & Ochoa, 2015). The source of selenium in farmed salmon is through the inclusion of fish meal in the feed. However, the alternative use of plant-derived materials with the aim of partially or totally eliminating animal products in feed formulations will reduce the levels of selenium and hence will require an additional supply of selenium to reach the Se levels found in marine ingredients. The current EU feed regulations on total selenium in fish feed has been established to be 0.5 $mg \cdot kg^{-1}$ feed.

A correct risk assessment of selenium consumption from fish requires also the determination of individual selenium species concentration which implies a first step of selenium species extraction. To achieve the best conditions for selenium species extraction, an experimental design as indicated in Material and Methods section 2.6 was applied. Eight experiments were performed (complete factorial design: 2^k, k=3) with three replicates per experiment. Once the experiments were performed, the factorial design allowed estimating both the individual influence of the factors and interaction effects on evaluated response. The resulting mathematical model adjusted response to a polynomial equation of the type:

Extraction efficiency = 48,1931 + 12,0188 x A + 9,04599 x B - 3,41005 x C + 5,55428 x A x B - 2,63074 x A x C - 0,903125 x B x C

with a R^2 of 0,977 where A is extraction time, B: ultrasonic powder and C: protease amount

The Pareto chart (Figure 1.a) showed that the most significant effects in selenium extraction, those which exceed the vertical line, were the extraction time, the ultrasonic powder and the interaction between these factors. The response surface (Figure 1.b) shown that the highest extraction efficiency was achieved when extraction time and ultrasonic powder acquired their maximum values. The highest percentage of selenium extraction was obtained with the conditions 7 (6 min, 60

% and 20 mg) and 8 (6 min, 60% and 40 mg). Condition 8 provides similar results to those obtained under condition 7, so the condition 7 was used in further experiment in order to save amount of protease XIV. The results, with an average recovery of 95–98% of total selenium, evidenced that selenium losses did not occur during sample treatment.

Once extracted, selenium species from fish muscle extracts were separated and detected by using HPLC-ICP-MS. Separation was performed by means of using two chromatographic columns as explained in Section 2.6. Figures 2 and 3 show the chromatograms obtained for and individual swordfish (Figure 2) and farmed salmon and wild salmon (Figure 3), respectively. Similar chromatographic profiles for fish fillets were obtained among individuals of the same fish species. $SeCys_2$, SeMetSeCys and SeMet were identified in fresh swordfish (Figure 2a, blue line). A second chromatographic mechanism corroborates the data obtained from the anion-exchange column (Figure 2b) and the presence of $SeCys_2$. Concerning fresh salmon, $SeCys_2$ and SeMet was the Se-species detected regardless the growing conditions of salmon either from captures fisheries or from aquaculture production (Figure 3a blue and red lines). The application of a Zorbax Rx C8 (250 x 4.6 mm, 5 μm) column coupled to ICP-MS corroborates again the results obtained by anion-exchange column (data no shown) and the presence of $SeCys_2$.

SeMetSeCys was not found in any kind of salmon. SeMetSeCys is a non-proteinogenic selenoamino acid that is metabolized by lyase to methylselenol *in vivo* (Ganther & Lawrence, 1997) and it is considered as a chemoprotective agent. Results illustrates in Figures 2 and 3 evidence that selenium in the fish analyzed is mainly present as organic selenium species that are tolerated to higher concentration than inorganic selenium.

The amount of Se species in the different fishes along with the respective mass balance calculation is presented in Table 3. Quantification was performed by using both anion exchange and reversed-phased columns providing similar results. As it can be observed in Table 3, the predominant Se species in swordfish fillets were SeMetSeCys and SeMet, both in similar amounts, around 0.2

$mg \cdot kg^{-1}$, and in a lesser extent $SeCys_2$. The sum of species in this fish is approximately 90% of the total selenium found in the samples, suggesting no selenium losses during the analytical procedure employed.

In farmed and wild salmon fillets, SeMet and $SeCys_2$ are the predominant selenium species, however as illustrated in either Figure 3 or in Table 3, $SeCys_2$ is present at a higher concentration in wild salmon fillets. It is well known that selenium can be present as SeMet in proteins containing selenium and as SeCys in selenoproteins. The differences found in the selenium species content between the two types of salmons could be explained in differences in diet and environment during their cycle of life. Wild salmons are developed in natural environments being their main food supply aquatic organisms found in their living ecosystems. In contrast, farmed salmons are raised in artificial environments and fed with processed high-fat feed to fulfil the requirement of nutrients and to produce larger fish. Again the sum of selenium species is approximately 90% of the total selenium found in the samples.

3.3 Effect of cooking process on selenium species distribution

Fish are frequently consumed fried or baked. It is known that food processing can modified the species distribution. Therefore, the influence of cooking process over selenium species content was evaluated in swordfish and wild and farmed salmon. For this purpose, fish fillets were cooked following the procedures described in section 2.2. The results obtained for total selenium concentration after fish processing are compiled in Table 2. As it was expected the selenium content in the processes fishes is higher than in raw fishes because of a dehydration process, except in the case of smoked samples with selenium contents similar to those obtained for farmed salmon samples. Once cooked, the resulting samples were submitted to enzymatic hydrolysis by using the optimal conditions previously applied to raw samples. Similarly to raw fish samples, an extraction yield ranged from 97-101% was achieved for all processed fish fillets. The extracts were

subsequently analyzed by HPLC-ICP-MS. Figures 2 and 4 show the selenium species distribution in cooked swordfish (Figure 2, red and green lines), in cooked farmed salmon (Figure 4a) and in cooked wild salmon (Figure 4b). As it can be observed, new selenium species were not detected in the processed compared to the raw fish fillet samples, however differences in selenium species distribution were observed for some of the fishes and/or treatments applied. The percentage of each species respect to the total selenium found was used to make comparisons between the two groups: raw and cooked fishes. For this purpose, selenium species were quantified in cooked fish fillets. The results are compiled in Table 4. When swordfish is submitted to a baking process, significant differences ($P<0.05$) in selenium species between raw and processed fish is detected. A notable increase of SeMetSeCys was observed (68% of the total selenium found in the extract) in comparison with the amount of SeMetSeCys found in raw fish (17% of total selenium). This increment was accompanied with a notable decrease of the level of $SeCys_2$ and SeMet. In contrast, no significant differences ($P<0.05$) were found between the percentage of selenium species in fried fish fillets compared to the values obtained in raw swordfish. Similarly, no new species derived from the cooking process were observed in salmon, however a significantly decrease of $SeCys_2$ was detected. This decrease was accompanied by an increase of SeMet in both types of salmon. In contrast, the analysis of smoked salmon provides a selenium species distribution that is similar to that of raw farmed salmon ($P<0.05$).

The results obtained are in agreement with other studies reported in the literature on the effect of cooking on selenium species distribution, however most of them have been carried out in vegetable samples. For instance, Lu et al, 2018 detected losses of Se-species in cereal samples during cooking. $SeCyst_2$ and SeMetSeCys were reported to be completely lost during boiling. In this line, Pedrero et al, 2005 revealed a noticeable degradation of SeMetSeCys in boiled Broccoli fruit. Moreover, it has been reported (V. Funes-Collado et al, 2015) that the boiling of Se-enriched cabbage favours the increase of SeMet content. The main cause of this seleno aminoacids

transformation has been attributed to the high temperatures applied during cooking that may alter the chemical distribution of the selenium species. In this line Perez et al, 2018, had indicated that traditional cooking processes involving the application of high temperatures cause variation on the selenium proteins profile that it was attributed to both protein denaturalization and hydrolysis by activation of enzymes present in the sample. Data on the effect of cooking processes on selenium content and distribution in seafood samples are scarce. Martins, Alvito & Alameida, 2011 evaluated the effect of different cooking methods (grilling, frying and boiling) on selenium contents of six marine species commonly consumed in Portugal. No statistically significant differences were found for selenium levels in raw and cooked samples and in different marine species. Unfortunately, data on the effect of cooking on selenium species distribution are not given.

The results reported in the current study evidence that food processing has a notably influence of selenium species distribution and consequently in the bioaccesibility and nutritional value of the final food product. In comparison with raw fish fillets, no new selenium species were detected after processing but variations on the distribution of existing selenium species were detected.

4. Conclusions

Selenium is a matter of concern from the nutritional and toxicological point of view due to its narrow concentration interval between beneficial and toxic effects. Selenium is a toxic element at low concentration which is a topic of relevance, especially in environmental chemistry. Conversely, selenium is an essential element in living systems since it takes part of selenoproteins. As it was already mentioned, organic selenium species are more tolerated and less toxic than inorganic ones. Consequently, a correct risk assessment of selenium consumption implies to measure not only the total selenium concentration but also the concentration of individual selenium species in the samples. Seafood is one of the main sources of selenium in humans. The exponentially growth of aquaculture fish consumption could alter the nutritional value and health benefits of fish products

compared to that provided from seafood obtained from captures fisheries. In the current work the concentration of selenium was lower in farmed salmon than in wild salmon. However, in both cases selenium was present as organic selenium. Interestingly, the concentration of $SeCys_2$ in wild salmon was much higher than in farmed specimens which it may reveal a different selenoproteins distribution as a result of differences in their respective diets. No inorganic selenium was detected in any of the fish analyzed.

The food processing on the selenium species distribution was also studied in all fish fillets. The cooking methods did not originate any new selenium species compared to the species occurring in raw fishes. However, differences in selenium species distribution were observed for some of the fish and/or treatments applied. $SeCys_2$ in salmon and SeMetSeCys in swordfish were significantly modified by the cooking treatments applied. Any of the cooking processes tested produced inorganic selenium species.

Acknowledgements

The authors thank the Spanish Commission of Science and Technology (CTQ2017-83569-C2-1-R) and the Comunidad of Madrid and European funding from FSE and FEDER programs for financial support (project S2018/BAA-4393, AVANSECAL-II-CM).

Conflict of interest

None Declare.

References

Betancor, M.B., Caballero, M.J., Terova, G., Saleh, R., Atalah, E., Benítez-Santana T., 2012. Selenium inclusion decreases oxidative stress indicators and muscle injuries in sea bass larvae fed high-DHA microdiets. Br. J. Nutr., 108, 2115–28.

Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L., Handisyde, N., Gatward,I., Corner, R., 2010. Aquaculture: global status and trends. Phil. Trans. R. Soc. B. 365, 2897–2912.

Copat, C., Vinceti, M., D'Agati, M.G., Arena, G., Mauceri, V., Grasso, A., Fallico, R., Sciacca, S., Ferrante, M., 2014. Mercury and selenium intake by seafood from the Ionian Sea: A risk evaluation. Ecotoxicol. Environ. Saf. 100, 87–92. https://doi.org/10.1016/j.ecoenv.2013.11.009

EFSA, 2014. Scientific Opinion of Dietary Reference Values for Selenium. EFSA Journal, 12(10), 3846-3913.

Ermakov, V., Jovanović, L., 2010. Selenium deficiency as a consequence of human activity and its correction. J. Geochemical Explor. 107, 193–199. https://doi.org/10.1016/j.gexplo.2010.07.004

FAO, Fisheries and Aquaculture, 2016. The state of world fisheries and Aquaculture from the Food and Aquaculture Organization of the United Nations.

Funes-Collado, V., Rubio, R., López-Sánchez J. F.,2015 Does boiling affect the bioaccessibility of selenium from cabbage?. Food Chemistry, 181: 304–309

Ganther, H.E., Lawrence, J.R., 1997. Chemical transformations of selenium in living organisms. Improved forms of selenium for cancer prevention. Tetrahedron, 53, 12299–12310.http://www.ncbi.nlm.nih.gov/pubmed/5900632

Kubachka, K.M., Hanley, T., Mantha, M., Wilson, R.A., Falconer, T.M., Kassa, Z., Oliveira, A., Landero, J., Caruso, J., 2017. Evaluation of selenium in dietary supplements using elemental speciation. Food Chem. 218, 313–320. https://doi.org/10.1016/j.foodchem.2016.08.086.

Lu, X., He, Z., Lin, Z., Zhu, Y., 3, Yuan, L., Liu Y., Xuebin, Y., 2018, Effects of Chinese Cooking Methods on the Content and Speciation of Selenium in Selenium Bio-Fortified Cereals and Soybeans. Nutrients:10, 317-327

Martins, C.T., Alvito, P.C., Almeida, C.M.M., 2011 Selenium content of Raw and Cooked Marine Species Consumed in Portugal. Food Analytical Methods,4(1), 77-83

Nagy, G., Benko, I., Kiraly, G., Voros, O., Tanczos, B., Sztrik, A., Takács, T., Pocsi, I., Prokisch, J., Banfalvi, G., 2015. Cellular and nephrotoxicity of selenium species. J. Trace Elem. Med. Biol. 30, 160–170. https://doi.org/10.1016/j.jtemb.2014.12.011

Økelsrud, A., Lydersen, E., Fjeld, E., 2016. Biomagnification of mercury and selenium in two lakes in southern Norway. Sci. Total Environ. 566–567, 596–607. https://doi.org/10.1016/j.scitotenv.2016.05.109

Olmos Soto, J., Paniagua-Michel J.J., Lopez L., Ochoa L., 2015. Functional Feeds in Aquaculture. In Handbook of Marine biotechnology Chapter Functional feeds in aquaculture Kim SK. (eds) Springer Handbook of Marine Biotechnology. Springer, Berlin, Heidelberg.

Pacitti, D., Lawan, M.M., Feldmann, J., Sweetman, J., Wang T., Martin S.A.M., Secombes, C.J., 2016. Impact of selenium supplementation on fish antiviral responses: a whole transcriptomic analysis in rainbow trout (*Oncorhynchus mykiss)* fed supranutritional levels of Sel-Plex®. BMC Genomic*s*, 17, 116-142.

Pedrero, Z., Elvira, D., Camara, C., Madrid, Y., 2007. Selenium transformation studies during Broccoli (*Brassica oleracea*) growing process by liquid chromatography–inductively coupled plasma mass spectrometry (LC–ICP-MS). Analytica Chimica Acta, 596, 251–256.

Pedrero, Z., Madrid, Y., 2009. Novel approaches for selenium speciation in foodstuffs and biological specimens: A review. Analytica Chimica Acta, 634, 135-152

Perez, M.B., Avila Maniero, M.A.; Londonio, A.;,Smichowski, P., Willoud, R.G.; 2018, Effects of common cooking heat treatments on selenium contentand speciation in garlic. Journal of Food Composition and Analysis, 70:54-62

Raisbeck, M.F., 2000. Selenosis. Vet. Clin. North Am. Food Anim. Pract. 16, 465–480. https://doi.org/10.1016/S0749-0720(15)30081-5

Rider, S.A., Davies, S.J., Jha, A.N., Fisher, A.A., Knight J, Sweetman JW., 2009. Supra-nutritional dietary intake of selenite and selenium yeast in normal and stressed rainbow trout

(Oncorhynchus mykiss): Implications on selenium status and health responses. Aquaculture, 295(3–4),282–91.

Schlenk, D., Batley, G., King, C., Stauber, J., Adams, M., Simpson, S., Maher, W., Oris, J.T., 2007. Effects of light on microalgae concentrations and selenium uptake in bivalves exposed to selenium-amended sediments. Arch. Environ. Contam. Toxicol. 53: 365–370.

Schmidt, L., Bizzi, C.A., Duarte, F.A., Muller, E.I., Krupp, E., Feldmann, J., Flores, E.M.M., 2015. Evaluation of Hg species after culinary treatments of fish. Food Control 47, 413–419. https://doi.org/10.1016/j.foodcont.2014.07.040

Sele, V.; Ørnsrud, R., Sloth , J.J., Berntssen M.H.G., Amlunda, H., 2018. Selenium and selenium species in feeds and muscle tissue of Atlantic salmon. Journal of Trace Elements in Medicine and Biology 47 (2018) 124–133

WHO, 1996. Trace elements in human nutrition and health World Health Organization, World Health Organization.

Yang, G.-Q., Ge, K., Chen, J., Chen, X., 1988. Selenium-related endemic diseases and the daily selenium requirement of humans. World Rev Nutr Diet 55, 98–152.

Zhang, Y., Gladyshev, V.N., 2008. Trends in Selenium Utilization in Marine Microbial World Revealed through the Analysis of the Global Ocean Sampling (GOS) Project. PLoS Genet 4(6): e1000095

Figure Captions

Figure 1. a) Pareto chart with three factors (extraction time, power width and protease amount) and b) 3D response surface plot showing variation of the response (extraction efficiency) by using 20 mg of protease as a function of sonication amplitude and extraction time. Figures were obtained by using Statgraphics Centurion XVII.

Figure 2. a) HPLC-ICP-MS chromatograms by anion exchange (PRP-X100 column) of raw (blue line), fried (red line) and oven-baked (green line) swordfish. b) HPLC-ICP-MS chromatograms by reversed-phase (Zorbax C8 column) of raw swordfish.

Figure 3. a) HPLC-ICP-MS chromatograms by anion exchange (PRP-X100 column) of raw farmed (blue line), raw wild (red line) and smoked (green line) salmon.

Figure 4. HPLC-ICP-MS chromatograms by anion exchange (PRP-X100 column) of a) raw and fried farmed salmon and b) raw and fried wild salmon.

Table 1. Operating conditions for RP and AEX coupled to ICP-MS

Operating conditions	
ICP-MS parameters	
RF power (W)	1550
Plasma gas flow rate ($L \cdot min^{-1}$)	15.0
Ar auxiliary flow rate ($L \cdot min^{-1}$)	1
Carrier gas flow rate ($L \cdot min^{-1}$)	1
Nebulizer	Meinhard
Spray chamber	Scott
Acquisition mode	Continuous
Isotopes monitored	^{74}Se, ^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se, ^{82}Se
Replicates	3
Reaction gas	H_2
Reaction gas (mL $H_2 \cdot min^{-1}$)	4
RP chromatographic parameters	
Column	Zorbax C8 (250 x 4.6 mm, 5 µm)
Mobile phases	2% MeOH, 0.1% TFA (pH=2.20)
Mode	Isocratic
Flow rate ($L \cdot min^{-1}$)	1
Injection volume (µL)	100
Column	Hamilton PRP-X100 (250 x 4.1 mm, 10 µm)
Mobile phases	Ammonium citrate 10 mM, 2% MeOH (pH= 5.00)
Mode	Isocratic
Flow rate ($L \cdot min^{-1}$)	1
Injection volume (µL)	100

Table 2. Total selenium content found in fish samples by ICP-MS.

Samples	**Moisture (%)**	**Total Se fresh mass (mg kg-^{1})**	**Total Se dry mass (mg kg-^{1})**	**Total Se (mg kg-^{1})***
Swordfish	75.3± 1.9	0.68 ± 0.11	2.73±0.30	
Farmed salmon	70.9 ± 2.2	0.39 ± 0.03	1.30±0.02	
Wild salmon	67.6 ± 1.7	0.52 ± 0.07	1.58±0.05	
Smoked salmon	62.6 ± 1.3	0.58 ± 0.04	1.55±0.04	
Grilled swordfish				1.31 ± 0.25
Grilled farmed salmon				0.58 ± 0.03
Grilled wild salmon				0.75 ± 0.09
Baked swordfish				3.05 ± 0.25

Results expressed as the mean ± standard deviation (SD); n = 10 individual fishes for each fish species

* Total selenium was calculated in the product resulting after cooking.

Table 3. Selenium species concentration found in fish muscle after enzymatic hydrolysis and measurement by HPLC-ICP-MS

$[Se]_{Species}$ (mg·kg^{-1})			
Species	**Raw swordfish**	**Raw farmed salmon**	**Raw wild salmon**
$SeCys_2$	0.031 ± 0.004	0.19 ± 0.01	0.30 ± 0.06
SeMetSeCys	0.32 ± 0.01	*<LOD*	*<LOD*
Se(IV)	*<LOD*	*<LOD*	*<LOD*
SeMet	0.28 ± 0.01	0.16 ± 0.02	0.18 ± 0.03
Se(VI)	*<LOD*	*<LOD*	*<LOD*
$[Se]_{Sum\ of\ species}$ (mg·kg^{-1})	0.63 ± 0.05	0.35 ± 0.02	0.48 ± 0.06
R (%)	93 ± 6	89 ± 7	92 ± 9

R (%) calculated as sum of species related to total selenium found, expressed as fresh mass
Results expressed as the mean ± standard deviation (SD); n = 10 individual fishes for each fish species

Table 4. Percentage of selenium species in raw and cooked fish muscle

Processing	**$SeCys_2$ (%)**	**SeMetSeCys (%)**	**SeMet (%)**
Raw swordfish	37 ± 5	17 ± 7	46 ± 4
Fried swordfish	37 ± 4	21 ± 5	42 ± 6
Baked swordfish	8 ± 3	65 ± 6	27 ± 3
Raw wild salmon	70 ± 1	No detected	36 ± 1
Fried wild salmon	13± 3	No detected	90± 6
Raw farmed salmon	53 ± 6	No detected	47 ± 2
Fried farmed salmon	30 ± 5	No detected	71 ± 5
Smoked farmed salmon	51 ± 3	No detected	50 ± 3

Percentages were calculated with respect to the total selenium found, expressed as fresh mass
Results expressed as the mean ± standard deviation (SD); n = 10 individual fishes for each fish species

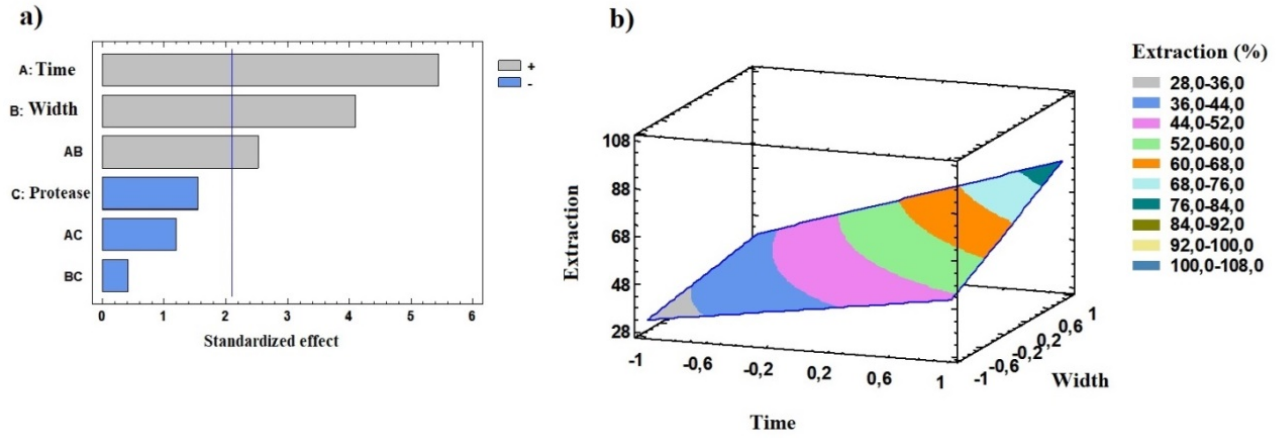

a)
A: Time
B: Width
AB
C: Protease
AC
BC
0
1
2
3
4
5
6
Standardized effect
+
-
b)
Extraction
108
88
68
48
28
-1
-0,6
-0,2
0,2
0,6
1
Time
Width
Extraction (%)
28,0-36,0
36,0-44,0
44,0-52,0
52,0-60,0
60,0-68,0
68,0-76,0
76,0-84,0
84,0-92,0
92,0-100,0
100,0-108,0

Figure 1.

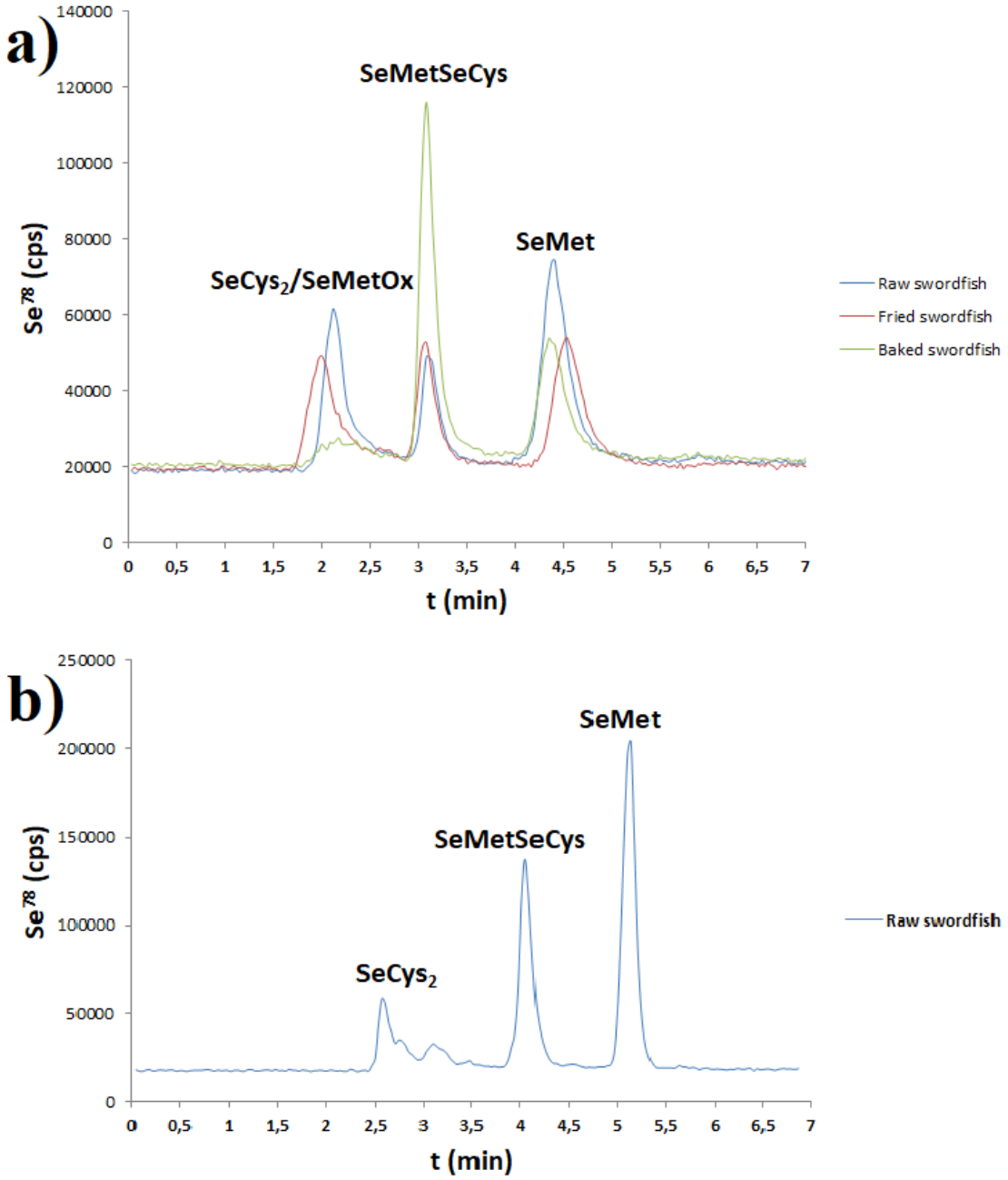

a)
SeMetSeCys
SeCys2/SeMetOx
SeMet
Se78 (cps)
t (min)
Raw swordfish
Fried swordfish
Baked swordfish
b)
SeMet
SeMetSeCys
SeCys2
Se78 (cps)
t (min)
Raw swordfish

Figure 2

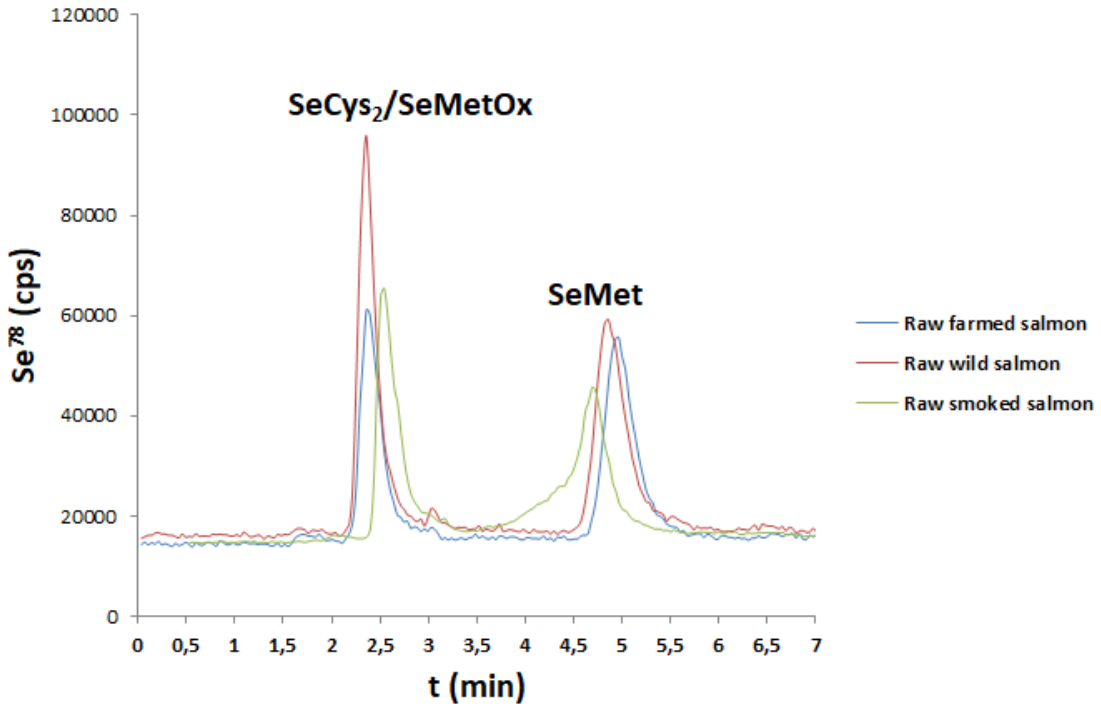
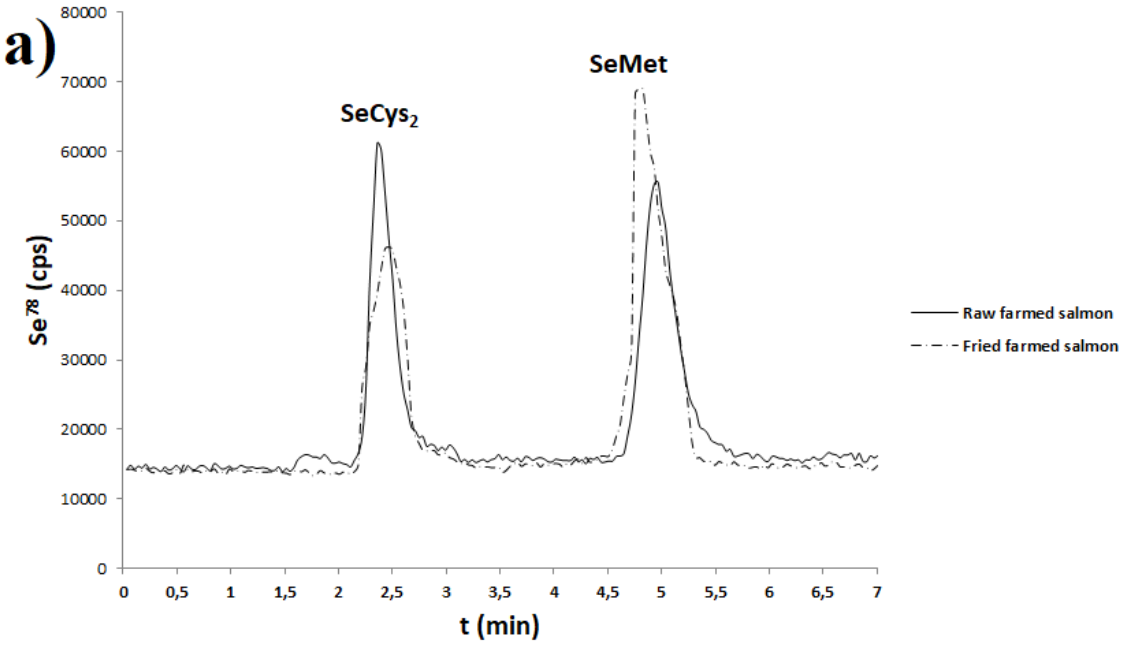
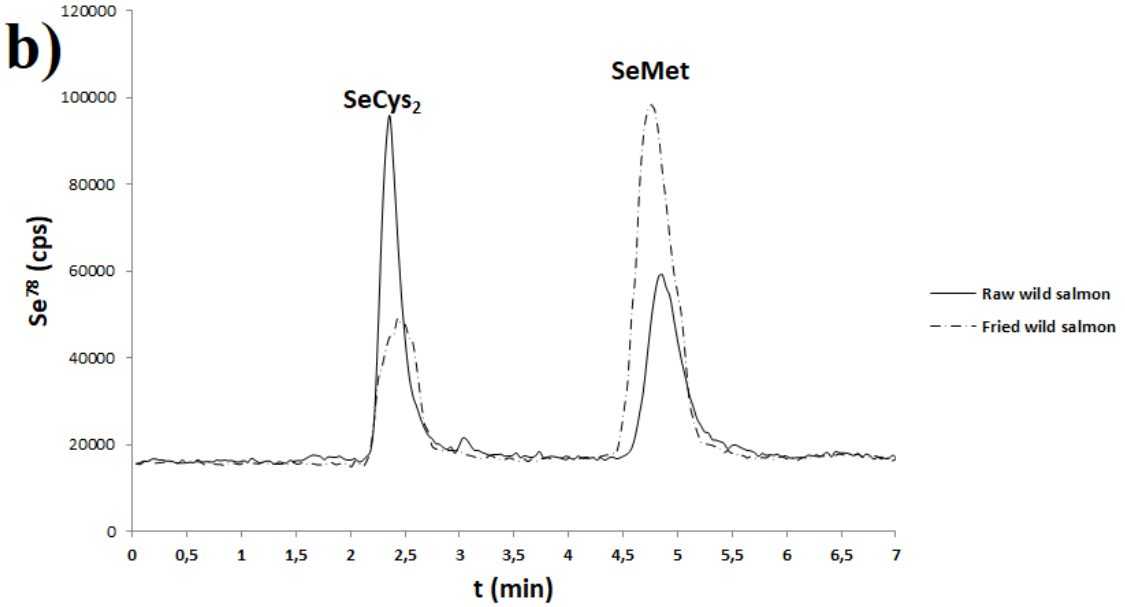

120000
100000
80000
60000
40000
20000
0
Se78 (cps)
SeCys2/SeMetOx
SeMet
0
0,5
1
1,5
2
2,5
3
3,5
4
4,5
5
5,5
6
6,5
7
t (min)
Raw farmed salmon
Raw wild salmon
Raw smoked salmon

Figure 3


a)
SeCys2
SeMet
Se78 (cps)
t (min)
Raw farmed salmon
Fried farmed salmon


b)
SeCys2
SeMet
Se78 (cps)
t (min)
Raw wild salmon
Fried wild salmon