



Detoxification of tuna from mercury through L-cysteine: a speciation-based study

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ABSTRACT

Mercury (Hg) contamination in tuna, especially as methylmercury (MeHg), remains a persistent food safety concern. This study assessed the effectiveness of L-cysteine in reducing total Hg (tHg) and MeHg in canned tuna and fresh fillets. Samples were treated with a 1.2% (w/w) cysteine solution and analysed by ICP-MS for tHg and frontal chromatography ICP-MS for MeHg. Untreated canned tuna contained 0.066–0.225 mg kg⁻¹ tHg, whereas fresh fillets reached 1.036 mg kg⁻¹. Cysteine treatment significantly reduced Hg levels, with mean removal of 48% in canned tuna and 64% in fillets. MeHg decreased proportionally with tHg, indicating cysteine binds both Hg and MeHg without species selectivity. Removal was more consistent in fillets than canned tuna, likely due to processing effects on protein binding. Overall, cysteine treatment represents a simple, low-cost, food-safe intervention that lowers Hg exposure and reduces concentrations below regulatory limits, providing additional protection for vulnerable consumers (pregnant women and infants).

1. Introduction

Fish is an extremely nutritious food source and is often referred to as a natural “superfood” due to its richness in essential nutrients. However, contamination with toxic elements, especially heavy metals, continues to raise concerns for food safety and public health (Custódio et al., 2020; Polak-Juszczak, 2023). Heavy metals such as cadmium, lead, arsenic and mercury are natural components of the earth's crust; they are not degradable, persist in the environment, and accumulate in living organisms, resulting in long-lasting ecological and health effects (Jomova et al., 2025; Rahman & Singh, 2019).

Among these, mercury (Hg) – especially in its organic form methylmercury (MeHg) – is one of the most dangerous neurotoxins in aquatic food. Although Hg occurs naturally, elevated levels mainly originate from anthropogenic activities, such as coal burning, mining, and artisanal and small-scale gold mining, which is currently recognized as the largest anthropogenic source of mercury emissions (Esdaille & Chalker, 2018). Once released into aquatic systems, inorganic Hg can be transformed by microorganisms into MeHg, which bioaccumulates and biomagnifies through the food chain, reaching its highest levels in long-lived predatory fish (Pei et al., 2022). Tuna, shark, swordfish, blue marlin, tilefish and king mackerel are among the species at greatest risk

of contamination.

Tuna is of particular importance. It is one of the most commonly consumed top predator fish species worldwide and, therefore, one of the main sources of MeHg in food. Bigeye tuna (*Thunnus obesus*), prized for its use in sushi and sashimi, has some of the highest levels of MeHg (range 0.143–2.222 mg kg⁻¹ wet weight) (Chen et al., 2011). It inhabits tropical and subtropical seas, but is not found in the Mediterranean.

Seafood consumption is the primary route of human exposure to MeHg, with the central nervous system (CNS) being the main target. Once ingested, MeHg readily crosses both the blood-brain barrier and the placental barrier, posing a particular risk to developing fetuses and infants (Li et al., 2021). Chronic exposure is associated with neurological and developmental disorders, including cognitive deficits, motor dysfunction and behavioural changes. Because of its strong affinity for thiol groups (–SH), MeHg readily forms the stable CH₃Hg–S–Cys complex, promoting tissue penetration and increasing its toxic potential. Developing nervous systems are particularly vulnerable, leading to neurodegeneration, loss of neuronal and glial cells and long-term deficits in memory, learning and motor skills (Fang et al., 2023; Li et al., 2021).

These risks are well recognized: Hg remains on the World Health Organisation (WHO) list of chemicals of public health concern (World

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Health Organization (WHO), 2021). Accordingly, regulatory agencies, including the Joint FAO/WHO Expert Committee on Food Additives (JECFA), have established provisional tolerable weekly intakes (PTWIs) of $1.6 \mu\text{g kg}^{-1}$ body weight for MeHg and $4 \mu\text{g kg}^{-1}$ body weight for inorganic Hg. These thresholds were subsequently confirmed by the EFSA Panel on Contaminants in the Food Chain (CONTAM) (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2012). Nevertheless, the issue remains controversial. Some experts consider the existing limits overly strict, while others argue they remain insufficient. Surveys consistently reveal wide variability in mercury concentrations in canned tuna, even within the same brand (Consumer Reports, 2022; Rumiantseva et al., 2024). Historical incidents, such as the major FDA recall in 1971, underscore the persistence of the problem despite decades of regulatory intervention (Bashiri Dezfouli et al., 2018). Moreover, global monitoring indicates that mercury concentrations in tuna have remained largely unchanged over the past fifty years (Médieu et al., 2024), even as tuna consumption has nearly quadrupled, from 500 to 600 thousand tons annually in the 1970s to 1.9 million tons in 2018 (Kawamoto, 2022).

This widespread exposure underscores the importance of understanding mercury's interactions at the molecular scale. The strong affinity of MeHg for thiol groups ($-\text{SH}$) enables stable complexes, particularly with cysteine ($\text{CH}_3\text{Hg-S-Cys}$) (Bernabeu de Maria et al., 2023; Starý & Kratzer, 1988; Strachowski et al., 2024). This conjugate is structurally similar to methionine and utilizes neutral amino acid transporters to cross cell membranes and promote distribution and persistence in tissues (Nong et al., 2020). The "Rabenstein reaction" (the formation of complexes between MeHg and thiol-containing compounds such as cysteine or glutathione) further increases the mobility of MeHg by exchanging bonds with sulfur-containing ligands, facilitating passage through biological barriers. In addition, MeHg inhibits important selenium-dependent antioxidant enzymes such as glutathione peroxidase (GPx) and thioredoxin reductase (TrxR), thereby weakening cellular defences, increasing reactive oxygen species (ROS) and increasing oxidative stress (Rahman & Singh, 2019).

In fish tissues, Hg is strongly bound to proteins, particularly to sulfur-containing amino acids, which makes it difficult to remove. Heat treatment, such as canning, further enhances this binding by coagulating proteins, making detoxification more difficult. Therefore, Hg removal can be achieved using chelating agents, although other strategies may also be effective (Bhat et al., 2021; Hajeb & Jinap, 2009; Madabeni et al., 2020).

Active food packaging introduces functional compounds that enhance food safety, for example by releasing antimicrobial agents or scavenging oxygen and moisture. Unlike conventional packaging, these systems intentionally interact with the food or its surrounding environment while preserving material integrity and ensuring food safety.

Recent studies by Chalmers University of Technology (Sweden) (Strachowski et al., 2024) have shown that the addition of cysteine to the packaging liquid of canned tuna can reduce the Hg content by up to 35%. Cysteine, which is already approved as a food additive, can be integrated into industrial processes without major adjustments. As a chelating agent, it forms stable complexes with Hg and facilitates its transfer into the liquid phase. The coordination of Hg (II) with cysteine, in particular the stable linear Cys-Hg-Cys complex, is well documented, with stability constants ranging from 10^{15} to 10^{42} (Starý & Kratzer, 1988).

Further advances include the combination of cysteine with thiolated silica adsorbents (Strachowski et al., 2025). Silica modified with $-\text{SH}$ groups immobilized in container linings can selectively absorb MeHg from aqueous matrices such as tuna in sauce, milk or rice porridge. The main technical challenge is to maintain the accessibility of the thiol groups within the polymer matrix while preserving porosity. Mesoporous silica microparticles offer a promising balance of cost, efficiency and compatibility with industrial coatings.

Although strategies based on cysteine and thiolated silica are still

under development and not yet widely used, initial results indicate their great potential to reduce Hg levels in packaged seafood. Legal thresholds remain relatively high. Under Commission Regulation (EU) 2023/915, the maximum permitted levels for Hg (wet weight) are set at 0.50 mg kg^{-1} for most fish, crustaceans, and bivalve molluscs; 1.0 mg kg^{-1} for high-Hg species such as tuna and other large predatory fish; and 0.30 mg kg^{-1} for low-Hg species as well as for cephalopods without viscera. These limits may slow down practical implementation. Nevertheless, offering products with Hg levels well below the legal limits could provide a competitive advantage in consumer markets.

Ultimately, the effectiveness of these approaches will depend not only on technological innovation, but also on their integration with monitoring systems, species selection practices, and regulatory frameworks as part of a broader, coordinated strategy to reduce Hg risks in aquatic foods.

Within this context, the present study aimed to evaluate the approach recently proposed at Chalmers University of Technology (Strachowski et al., 2024, 2025), extending its application to both canned tuna and fresh tuna fillets. Particular attention was devoted to the initial step of the process, namely the treatment of tuna matrices with cysteine. The novelty of this work lies in the combined assessment of the effects of cysteine treatment on both inorganic Hg and MeHg across two widely consumed tuna products, supported by both, complementary Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Frontal Chromatography-ICP-MS (FC-ICP-MS) analyses. By investigating Hg speciation in these distinct matrices, the study provides a more comprehensive evaluation of the potential of cysteine-based treatments to mitigate Hg-related risks in seafood.

2. Experimental section

2.1. Standards, reagents, and certified reference materials

Pure nitric acid and hydrochloric acid obtained by sub boiling distillation from commercial HNO_3 (Carlo Erba, 65%) and HCl (Carlo Erba, 37%), were used for sample digestion and solution acidification. Thiourea (Carlo Erba, 99% pure) and hydrobromic acid (Carlo Erba, 48%) were purchased from Carlo Erba Reagents, Italy and used for the extraction and analysis of MeHg. Ultrapure water for the dilutions described here was prepared using a Sartorius Arium mini-UV Lab Water System.

Calibration of tHg was performed with a monoelemental Hg stock solution (TraceCert®, Sigma-Aldrich, 10 mg L^{-1}), while a MeHg chloride standard solution (Alfa Aesar, 1000 mg L^{-1}) was used to prepare the MeHg calibration solutions. A lutetium (Lu) standard solution (TraceCERT®, Sigma-Aldrich, 1000 mg L^{-1}) was added as an internal standard for MeHg determination. Quality assurance for the determination of tHg and MeHg was ensured by regular measurements of two certified reference materials of tuna muscle (BCR-463 and ERM®-464CE).

2.2. Sample preparation and extraction tests with L-cysteine solution

Six canned tuna (*Thunnus albacares*) samples in water and four fresh *T. albacares* fillet samples from different manufacturers were analysed. Aliquots of approximately 20 g of drained tuna were taken from each sample, weighed with an analytical balance, and transferred to inert containers (glass or polypropylene) that had been pre-cleaned to avoid contamination. The containers were tightly closed to prevent oxidation and evaporation. From the six initial canned tuna samples (labelled T1-T6), 12 subsamples were obtained (two per manufacturer): i) six were analysed in their unaltered state (without treatment), and ii) six were treated with an L-cysteine solution (ITW Reagents, 99%) and analysed after treatment. Analogous subsamples were produced for the fresh tuna fillet samples (labelled TF1-TF4).

The treatment was carried out with an aqueous L-cysteine solution prepared according to the protocol of Strachowski et al. (2024). L-

cysteine powder was dissolved in ultrapure water without pH modifiers or additional chelating agents. The optimal concentration for Hg extraction from tuna was 1.2% w/w. The treatment was carried out using a mass-to-volume ratio of 1:4 (20 g of tuna, 80 mL of cysteine solution). The containers were sealed and gently shaken to ensure contact between the solution and the muscle tissue. The samples were incubated for 4 h at 4 °C in the dark, shaking or rotating them regularly. Incubation in the dark prevented photo-induced oxidation of cysteine. At the end of the treatment, the solution was carefully separated and collected. The tuna samples were rinsed with ultrapure water (Milli-Q) and then prepared for Hg analysis.

Samples were first freeze-dried and then subjected to microwave-assisted acid digestion. For freeze-drying, representative aliquots (~5 g) of both untreated and treated tuna were collected, placed in tubes with perforated lids and frozen overnight at -80 °C. Water was removed using a VirTis Sentry freeze dryer, which operates by sublimating water under low pressure and temperature to yield stable, dry samples. The freeze-dried material was then homogenized and ground into a fine powder using a ceramic knife on a Teflon pad to avoid metal contamination. These powdered samples were stored at 4 °C and subsequently used for total Hg and MeHg determination, as described in the following sections.

2.3. Total mercury determination

Total Hg was determined by microwave-assisted acid digestion followed by mass spectrometric analysis using inductively coupled plasma mass spectrometry (ICP-MS).

Samples were digested with an ETHOS One device (Milestone) equipped with polytetrafluoroethylene (PTFE) vessels. Custom-made PTFE inserts allowed three sealed quartz tubes to be placed in each vessel (Spanu et al., 2020). Approximately 10 mg of each sample was weighed and placed into pre-labelled quartz digestion tubes. Each tube received 0.5 mL HCl and 0.5 mL HNO₃ and was loosely capped. The tubes were placed in the microwave carousel, and samples were digested at 150 °C using a 50-min program. After cooling, the digests were transferred to pre-weighed high-density polyethylene (HDPE) containers and diluted to a final mass of 15 g. All steps were carried out under clean laboratory conditions. The diluted samples were analysed with a Thermo Scientific iCAP Q ICP-MS using a helium collision cell in KED mode (detailed conditions in Table S1). Total Hg was quantified by external calibration, and all concentrations are reported on a wet weight basis. The ICP-MS method exhibited an instrumental limit of detection (LOD) of 7.5 ng kg⁻¹, corresponding to a method LOD and limit of quantification (LOQ) of 2.8 µg kg⁻¹ and 8.4 µg kg⁻¹, respectively, on a wet weight basis, assuming a moisture content of 75%. Analysis of the certified reference materials BCR-463 (certified value: 2.85 ± 0.15 mg kg⁻¹) and ERM®-464CE (certified value: 5.24 ± 0.10 mg kg⁻¹) resulted in recoveries of 99.6 ± 4.7% and 101.2 ± 2.5% (n = 3), respectively. For freeze-dried samples, values were corrected for moisture content, which was determined gravimetrically with a five-digit analytical balance before and after lyophilization (mean moisture content: 72.7 ± 4.5% for pristine samples and 79.7 ± 3.8% for cysteine-treated samples).

Quartz tubes and HDPE containers were thoroughly decontaminated. The tubes were first rinsed with Milli-Q water and acetone, then hot-soaked in a 4% (w/w) Nalgene L900 detergent solution for 24 h. They were then treated with a 1:10 dilution of aqua regia with heating for 6 h. The cleaned tubes were stored in sealed containers filled with diluted aqua regia until use. HDPE containers were decontaminated using a three-step procedure: one week of soaking in 0.4% (w/w) Nalgene L900 detergent, followed by 2% (w/w) HNO₃ and HCl (pH 2) with thorough rinsing in ultrapure water between each step.

2.4. MeHg determination

MeHg was quantified using the frontal chromatography ICP-MS (FC-

ICP-MS) method described by Spanu et al. (2024). Hg species were extracted from tuna samples with a solution containing 0.5 M HCl, 3.3 mM thiourea, and 0.037 M HBr. Approximately 20 mg of each sample was placed in a 10 mL polypropylene test tube, sonicated with 5 mL of extraction solution for 15 min at room temperature, and then filtered (0.22 µm). The extracts were analysed on a Thermo Scientific iCAP Q ICP-MS equipped with a short anion-exchange column (AmberChrom® 1 × 2, 2.5 mm × 20 mm) positioned upstream of the nebulizer for selective separation. To minimize Hg memory effects, a 0.19 M thiourea solution was introduced post-column. Signals of ²⁰²Hg and ¹⁷⁵Lu (1 µg kg⁻¹, internal standard) were detected using a helium collision cell in KED mode. The total analysis time per sample was approximately 5 min. Experimental details are summarized in Table S2. All MeHg concentrations are reported on a wet weight basis (please, see above). The FC-ICP-MS method exhibited a LOD and a LOQ of 1.4 µg kg⁻¹ and 4.2 µg kg⁻¹ on a wet weight basis, assuming 75% moisture content (Spanu et al., 2024). Analysis of BCR-463 (certified value 2.83 ± 0.15 mg kg⁻¹) and ERM®-464CE (certified value 5.12 ± 0.16 mg kg⁻¹) reference materials yielded recoveries of 105.6 ± 6.7% and 103.5 ± 5.5% (n = 3), respectively. Polypropylene test tubes were decontaminated following the three-step procedure described earlier.

3. Results and discussion

As reported in the literature (Strachowski et al., 2024), the efficiency of Hg removal depends mainly on cysteine concentration, solvent-to-matrix ratio, and contact time. Literature indicates that cysteine promotes Hg release up to a plateau (≈1.2% w/w), while extending extraction beyond a few hours does not significantly improve recovery. In this work, these established conditions were applied without further optimization, as the focus was on characterizing the chemical distribution of the extracted Hg species. Moreover, the ability to remove both tHg and MeHg was systematically assessed in canned tuna and tuna fillet samples.

3.1. Total mercury

The concentration of tHg in the untreated canned tuna samples ranged from 0.066 to 0.225 mg kg⁻¹, with a mean value of 0.143 ± 0.071 mg kg⁻¹ (Table S3). These values are consistent with those reported in the literature for canned tuna, considering possible deviations depending on species and catch area (Castellano-Baquero et al., 2025; Gerstenberger et al., 2010; Kumar, 2017). Notably, even the highest concentration measured (0.225 mg kg⁻¹, sample T5) is well below the maximum limit established by the European Commission Regulation (EU) 2023/915 of April 2023, which sets the allowable mercury content in fish at 1 mg kg⁻¹. In contrast, fresh tuna fillets show a much higher tHg content ranging from 0.139 to 1.036 mg kg⁻¹ with a mean value of 0.56 ± 0.40 mg kg⁻¹ (Table S4). Although higher Hg concentrations are expected in tuna fillets, this level represents a more critical scenario, underlining the higher exposure risk linked to the consumption of fresh tuna compared to canned products: the most contaminated tuna fillet sample (TF1) showed a peak Hg level slightly exceeding the maximum limit established by European regulation.

After cysteine treatment, tHg concentrations decreased systematically across all samples (Fig. 1), with average values of 0.074 ± 0.036 mg kg⁻¹ (mean removal efficiency 47.9 ± 11.2%) for canned tuna and 0.20 ± 0.13 mg kg⁻¹ (mean removal efficiency 63.5 ± 3.3%) for fresh tuna fillets.

Paired *t*-tests were performed separately for each pair of fresh and treated samples to evaluate differences in tHg concentrations before and after treatment. The analysis consistently yielded *p*-values <0.01, clearly demonstrating a statistically significant difference between the samples and confirming the robustness of the extraction protocol. Regarding removal efficiency, an independent two-sample *t*-test revealed statistically significant differences (*p* = 0.02) between canned

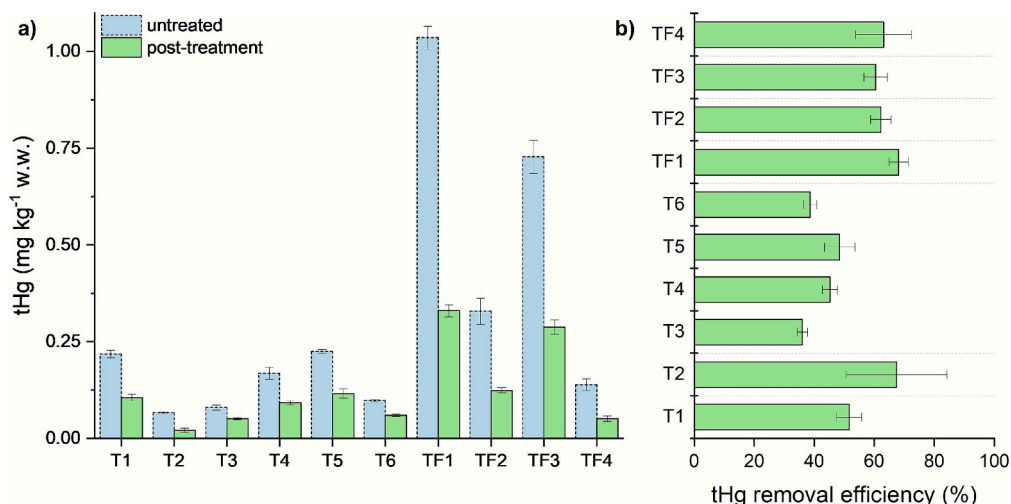


Fig. 1. (a) Total Hg (tHg) concentration in untreated (light blue bars with dashed borders) and cysteine-treated (green bars with solid borders) tuna samples. (b) Total Hg removal efficiencies for canned tuna (T1–T6 samples) and tuna fillets (TF1–TF4 samples). Error bars represent standard deviation ($n = 3$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

tuna ($n = 6$) and tuna fillet samples ($n = 4$), reflecting the impact of their different physical characteristics and industrial processing. These findings indicate that the two groups should be considered distinct (see Fig. S1).

Although incomplete, this reduction is quantitatively significant: in most contaminated samples, cysteine treatment lowered Hg levels from near-threshold concentrations to values well below the regulatory limit, thereby decreasing potential consumer exposure. The partial extraction of Hg is likely due to high-molecular-weight protein–Hg complexes that are poorly accessible to cysteine and only partly soluble in the aqueous solution, as suggested by the turbidity of the extracts. As shown in Table S5 and Fig. S2, this leads to tHg concentrations in L-cysteine about one order of magnitude lower than expected. A clearer mass balance is presented for sample TF1 as an illustrative example. The initial tHg concentration was 1.036 mg kg^{-1} , while the tHg remaining in the

muscle after treatment was 0.330 mg kg^{-1} . This difference indicates that approximately $14.1 \text{ } \mu\text{g}$ of Hg should have been released from 20 g of tuna into the cysteine solution. However, only $1.3 \text{ } \mu\text{g}$ was recovered, as ICP-MS analysis of the cysteine solution (total volume: 80 mL) showed a mercury concentration of $16.35 \text{ } \mu\text{g L}^{-1}$. The most plausible explanation is the loss of colloidal or insoluble Hg species, which are subsequently removed by filtration prior to ICP-MS, causing underestimation of the extracted Hg. However, it cannot be ruled out that rinse water used to wash the tuna samples after cysteine treatment contributed to this incomplete mass balance. Matrix effects during ICP-MS instead can be excluded, since spiking a cysteine solution ($0.22 \text{ } \mu\text{g kg}^{-1}$ Hg) with $1 \text{ } \mu\text{g kg}^{-1}$ gave $1.20 \text{ } \mu\text{g kg}^{-1}$.

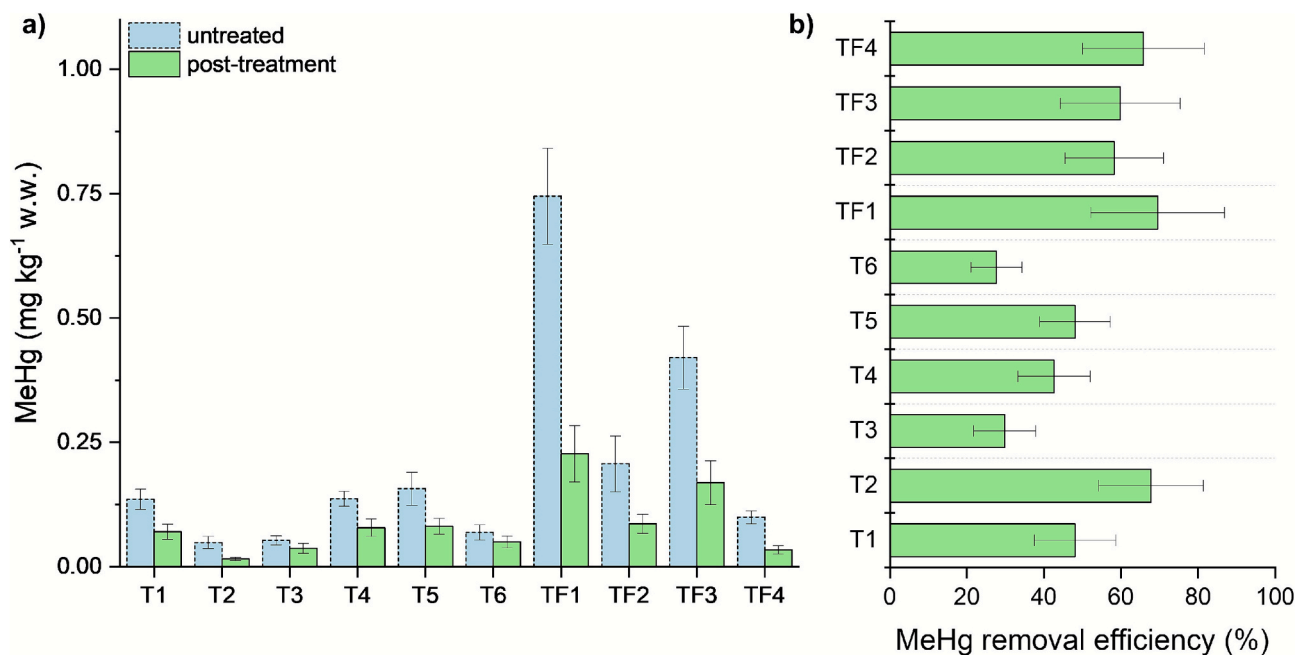


Fig. 2. (a) MeHg concentrations in untreated tuna samples (light blue bars with dashed borders) and cysteine-treated samples (green bars with solid borders). (b) MeHg removal efficiencies for canned tuna (samples T1–T6) and fresh tuna fillets (samples TF1–TF4). Error bars represent standard deviations ($n = 3$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Methylmercury

MeHg concentrations and their reduction after cysteine treatment showed similar patterns in canned tuna and fresh tuna fillets (Fig. 2a). In canned tuna (Table S6), untreated samples had relatively low MeHg levels ($0.100 \pm 0.048 \text{ mg kg}^{-1}$), which decreased to $0.056 \pm 0.026 \text{ mg kg}^{-1}$ after treatment. This corresponded to a mean removal efficiency of $44.0 \pm 14.6\%$, with substantial variability across samples (range 27.6–67.8%). In contrast, fresh tuna fillets (Table S7) had significantly higher baseline MeHg concentrations ($0.37 \pm 0.28 \text{ mg kg}^{-1}$), about four times those of canned tuna (*t*-test on untreated groups, $p < 0.01$). After cysteine treatment, MeHg decreased to $0.13 \pm 0.09 \text{ mg kg}^{-1}$, resulting in a mean removal efficiency of $63.3 \pm 5.2\%$. Significant differences (two sample *t*-test, $p = 0.04$) in the mean removal efficiency of MeHg were observed between canned tuna ($n = 6$) and tuna fillets ($n = 4$). Additionally, the dispersion of removal efficiencies differed markedly: canned tuna showed high heterogeneity, while fresh fillets had more consistent results (Fig. 2b). These findings indicate that cysteine treatment is generally more effective and more reproducible in reducing MeHg in fresh tuna fillets. The observed differences may be attributed to matrix-dependent factors. In fish muscle, MeHg is predominantly bound to thiol-containing proteins (e.g., cysteinyl residues), forming strong MeHg–S complexes (Bradley et al., 2017). Thermal and industrial processing, such as canning, can induce protein coagulation and structural rearrangements, thereby enhancing Hg trapping within the protein matrix (Bhat et al., 2021). These changes may alter the exposure, stability, and accessibility of MeHg-binding sites to added cysteine, ultimately influencing the extent of MeHg displacement.

3.3. On the effect of Hg speciation on the removal efficiency

The distribution of MeHg relative to tHg was evaluated in both canned and fresh tuna before and after cysteine treatment (Fig. 3), which aimed to reduce Hg content. In canned tuna, MeHg/tHg ratios ranged from 62.1% to 81.0% in untreated samples, with a modest increase after treatment (66.7–84.9%). Fresh tuna showed similar variability in untreated MeHg/tHg ratios (57.7% to 71.9%), with mixed

responses to cysteine treatment: some samples showed slight increases, others minor decreases (MeHg fraction ranges from 58.7% to 69.7%). Paired *t*-tests indicated that differences in MeHg/tHg ratios before and after treatment were not significant for either canned or fresh tuna ($p > 0.05$). No statistically significant difference was observed between canned and fresh tuna in MeHg/tHg distribution after treatment. These findings suggest that cysteine treatment reduces tHg without selectively targeting any Hg species, and its effect on the relative abundance of MeHg is consistent across different tuna matrices. In other words, the treatment does not discriminate between inorganic and methylated Hg, and its impact is similar in both processed (canned) and unprocessed (fresh) tuna.

This negligible selectivity can be explained by the very high affinity of thiol groups for Hg, regardless of its coordination environment. Both inorganic Hg and MeHg readily form strong Hg–S bonds, as shown by literature values of stability constants ($\log \beta$) for cysteine complexes. Although thermodynamic data indicate that Hg(II) forms much more stable complexes with cysteine than MeHg ($\log \beta_2 \approx 40$ vs. $\log \beta_1 \approx 15.6$) (Starý & Kratzer, 1988), this pronounced difference does not result in selective removal in our experiments. Notably, both stability constants are extremely high, ensuring that cysteine effectively binds and removes both Hg species. This apparent lack of selectivity can therefore be attributed to the influence of the muscle matrix, where proteins, lipids, and other biomolecules may interact with both Hg species, modulating their bioavailability. Moreover, kinetic factors, including the rate of ligand exchange and the accessibility of binding sites, likely contribute to the similar extraction efficiencies observed for Hg(II) and MeHg.

As a final remark, any interconversion between MeHg and inorganic Hg during sample preparation and analysis was ruled out, as such processes could otherwise affect the interpretations discussed above. The analytical protocol was fully validated, and the use of complexing agents (chlorides, bromides, or thiourea) ensured the stability of the original Hg species. Similarly, the cysteine treatment does not promote methylation or demethylation and instead further stabilizes mercury species. Overall, these conditions effectively prevent species transformation throughout the analytical workflow.

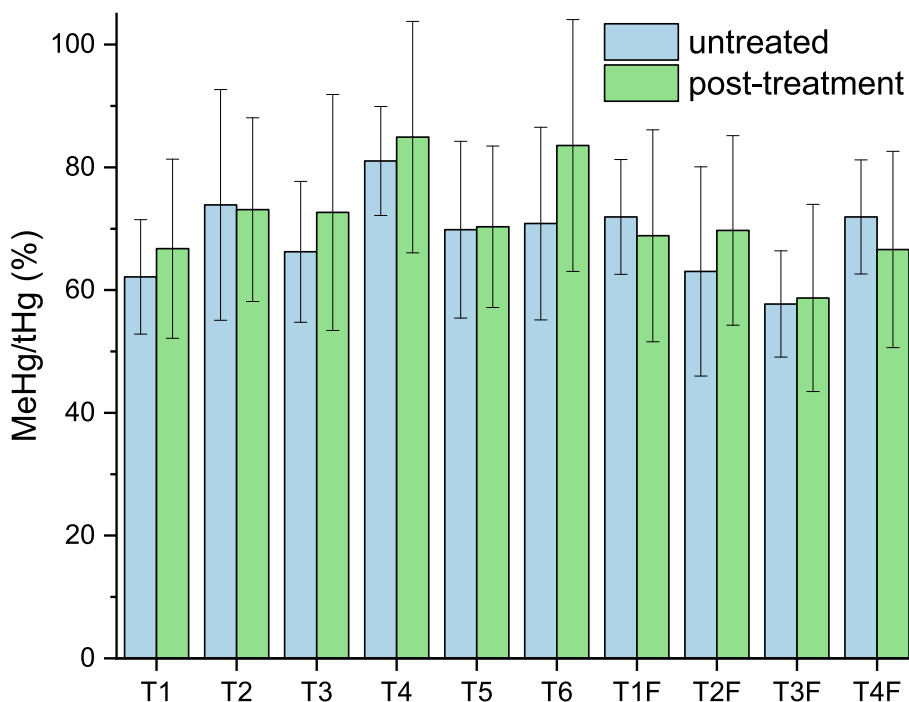


Fig. 3. MeHg/tHg ratios before and after the cysteine-based treatment for all tuna samples. Error bars represent one standard deviation ($n = 3$), with standard deviations estimated by error propagation from the individual replicates.

3.4. Broader implications

The findings of this study have clear implications for food safety in relation to tuna consumption. Cysteine treatment reduced Hg levels by up to 68%, demonstrating a practical intervention capable of lowering consumer exposure, particularly in samples with initial concentrations close to regulatory limits. This reduction is especially relevant for vulnerable populations, such as pregnant women and children, who are more susceptible to MeHg toxicity. Although tHg levels decrease, the proportional contribution of MeHg remains largely unchanged, indicating that the overall toxicological profile is improved but not fundamentally altered.

From a processing perspective, cysteine represents a simple, cost-effective approach and is already approved as a food additive, suggesting potential scalability within seafood processing facilities. The slightly lower removal efficiencies observed in canned tuna compared to fresh fillets are consistent with matrix effects, including the formation of stable protein-Hg complexes during thermal processing. These results highlight an important limitation: Hg tightly bound to muscle proteins is less accessible to cysteine, and complete detoxification may therefore require complementary strategies. Potential approaches include enzymatic pre-treatments, protein solubilization, or the use of functionalized packaging materials (e.g., thiol-functionalized silica coatings) to enhance Hg sequestration.

It should be noted that hypothetical extensions of these findings—such as population-level exposure reduction or market-wide mitigation of Hg risk—were not directly assessed in this study and would require further investigation.

Overall, L-cysteine emerges as an effective, simple, and economically viable agent for reducing Hg in tuna, representing a meaningful step toward safer seafood. While not a definitive solution for mercury contamination in large predatory fish, it provides a solid foundation for future strategies integrating processing, packaging, and regulatory considerations, with broader population- or market-level impacts remaining to be demonstrated.

4. Conclusions

This study provides experimental evidence that L-cysteine treatment can reduce both tHg and MeHg in canned tuna and fresh tuna fillets. We simultaneously evaluated the removal of tHg and MeHg—the most toxic and bio accumulative form of Hg, accounting for more than 70% of the Hg present in tuna—under conditions representative of real commercial products. This integrated approach enables a direct assessment of treatment efficiency in terms of potential consumer health risk reduction. The treatment proved more effective in fresh tuna fillets than in canned tuna, underscoring the influence of matrix characteristics and processing history on treatment performance, as reflected by the greater variability in removal efficiencies observed in processed canned products. From a regulatory perspective, the concentrations of Hg in the tested samples were below the maximum levels currently set by the European Food Safety Authority (0.5 mg kg⁻¹ for most fish and 1.0 mg kg⁻¹ for tuna and other top predatory species). However, the additional reduction achieved after cysteine treatment would further lower exposure, potentially bringing Hg levels down to 0.3–0.4 mg kg⁻¹ in samples initially close to the legal threshold. Such reductions are particularly relevant considering that sensitive consumers (e.g., pregnant women, infants) are advised to maintain intake well below the tolerable weekly intake of 1.6 µg kg⁻¹ body weight established by JECFA.

Overall, the study highlights two key strengths: (i) the ability to reduce MeHg, which has the highest toxicological relevance, and (ii) quantitative evidence that cysteine treatment can shift Hg concentrations from near-threshold values to well-below regulatory limits. These findings pave the way for the development of practical detoxification strategies that could complement monitoring and regulatory measures, enhancing consumer protection and market competitiveness for seafood

products.

However, these results should be regarded as preliminary. Before any consideration of process scale-up, it will be necessary to evaluate the impact of cysteine treatment on the sensory properties of tuna (e.g., flavour, odour, and texture) as well as on its nutritional quality. Furthermore, translating the approach from laboratory conditions to industrial application will require additional investigation and careful technological development. Among the most important aspects are the stability of cysteine in contact with packaging materials, safety and regulatory compliance, and verification of the treatment's effectiveness under real storage and distribution conditions. In this context, integration with complementary systems, such as thiolated silica resins with high affinity for heavy metals, could be a promising development to further improve process performance and strengthen industrial applicability.

CRedit authorship contribution statement

Davide Spanu: Writing – original draft, Methodology. **Davide Besana:** Visualization, Validation, Methodology. **Simona Rimoldi:** Writing – review & editing, Visualization, Data curation. **Sandro Recchia:** Writing – review & editing, Software, Formal analysis. **Geniana Terova:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization.

Consent for publication

Not applicable.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2026.148156>.

Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

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