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### **Research Article**

# Selenoneine and Total Selenium Concentrations in Canned Atlantic Bluefin Tuna

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#### Abstract

The concentrations of the selenium-containing imidazole selenoneine and total selenium were determined in canned Atlantic bluefin tuna produced in Tunisia. The selenoneine and total selenium concentrations of the canned tuna were 0.20 mg Se/kg (n = 4) and 1.2 mg/kg, respectively. In the muscle of this fish, a part of organic selenium was present as selenoneine. Selenoneine was stably present in the oily canned product. Therefore, the canned tuna products rich in organic selenium can be useful for selenium supplementation.

Keywords: Selenoneine, Selenium, Mercury, Food safety, Muscle, Fish, Seafood

#### Introduction

The selenium (Se)-containing compound selenoneine, 2-selenyl- $N_a$ , $N_a$ , $N_a$ -trimethyl-L-histidine, shown in Figure 1, is the major form of organic Se in the blood and other tissues of Pacific bluefin tuna (*Thunnus orientalis*) [1]. This compound contains an imidazole ring with a unique selenoketone group and has strong free-radical scavenging activity [1]. The dietary intake of

selenoneine from fish accumulated in the blood of a fish-eating population on remote islands in Kagoshima, which may enhance free-radical detoxification functions [2]. The dietary intake of selenoneine through the consumption of fish is thought to be important for enhancing antioxidant effects in tissues and cells [3]. Fish is recommended as a part of a healthy diet [4] and it is

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considered a key component of a cardioprotective diet [5]. Fish is also an important source of various nutrients, such as proteins, n-3 fatty acids, vitamin D, iodine, and Se [6]. Inuit living in the Arctic are exposed to high levels of both Se and Hg through their traditional diet rich in marine mammals and fish [7, 8]. The high Se and low Hg group had the lowest prevalence of cardiovascular outcomes, except for stroke, suggesting that Se protects against Hg in cardiovascular disease [7]. Dietary and blood Se are inversely associated with the prevalence of stroke among Inuit in Canada [7].

The beneficial and harmful effects of Se depend on its dose and chemical form. Miyata et al. determined that selenoneine attenuates hepatocellular injury and hepatic steatosis in a mouse model of non-alcoholic fatty liver disease [9]. Selenoneine also attenuated hepatic steatosis and hepatocellular injury in another mouse model [9]. Masuda et al. reported that an oral selenoneinecontaining tuna dark muscle extract significantly decreased tumor incidence and inhibited the accumulation of myeloidderived suppressor cells, while also inhibiting the downregulation of interferon- $\gamma$  production during carcinogenesis, suggesting that dietary selenoneine effectively reduces colorectal tumor progression [10]. Thus, selenoneine in seafood may be the most important dietary source of antioxidant-active Se. The selenoneine levels are high in the blood and other tissues of Pacific bluefin tuna [1,2].

This study determined the selenoneine and total Se concentrations of canned Atlantic bluefin tuna *T. thynnus* processed with olive oil. This contained the highest levels of selenoneine and total Se concentrations in the edible portion of various fish species commonly available in Japan.

#### **Materials and Methods**

The four different brands of canned products of Atlantic bluefin tuna produced in Tunisia were obtained from the IBC Corporation, Tokyo (Figure 2).

For chemical analysis, the contents of a canned food containing olive oil were homogenized. To measure total Se levels, each sample (0.1-0.2 g) was digested at  $100-200^{\circ}$ C in 1 mL of a 1:5

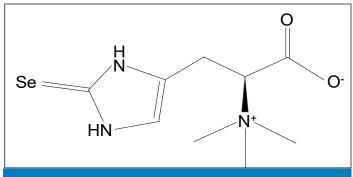


Figure 1: Chemical form of selenoneine

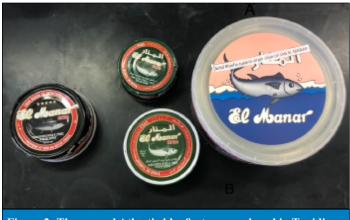


Figure 2: The canned Atlantic bluefin tuna produced in Tunidia

mixture of nitric acid and perchloric acid. The Se concentration was measured using a fluorometric 2,3-diaminonaphthalene assay [11]. For Se speciation analysis, chromatographic separation was performed using a 1100 Series high-performance liquid chromatography (HPLC) pump (Agilent, Santa Clara, CA, USA) in conjunction with an Ultrahydrogel 120 analytic column (7.8 × 250 mm; Nihon Waters, Tokyo, Japan) equilibrated with 0.1 M ammonium formate buffer containing 0.1% (w/v) IGEPAL CA-630 (Sigma-Aldrich Japan, Tokyo, Japan), according to a previously described method [1]. Each sample (0.1 g) was homogenized in 0.5 mL of water, and the supernatant was analyzed after two- to four-fold dilution in the mobile phase. The injection volume was fixed at 20 µL and the mobile phase delivered isocratically at a rate of 1 mL/min. Se was detected by online liquid chromatography inductively coupled plasma mass spectrometry (LC-ICP-MS) that monitored <sup>82</sup>Se levels. The plasma, auxiliary, and nebulization argon gas flow rates were 17, 1.3, and 1.02 L/min, respectively. The radiofrequency power was 1300 W.



To remove the oil from the canned meat, two volumes of a dichloromethane/methanol mixture for lipid extraction from oily materials were added to 5 g of canned tuna meat, which was homogenized with a homogenizer (ULTRA-TURRAX, T-25, IKA, Staufen, Germany). After centrifugation at  $6000 \times g$  for 10 min at room temperature, the water-soluble fraction was collected. The remaining lower organic layer were re-extracted repeating the procedure, and the water-soluble fraction of the second extraction was collected. The water-soluble fraction obtained from the first and second extractions were mixed and vacuum-dried. The dried materials were dissolved in 5 mL of 0.1 M formate ammonium containing 0.1% (w/v) IGEPAL CA-630.

#### Results

The total selenium levels of the meats of canned Atlantic bluefin tuna were determined by the fluorescent method, and the selenoneine levels were determined by monitoring <sup>82</sup>Se levels by LC–ICP–MS using a gel permeation chromatography (GPC) column. This study showed that selenoneine and total Se levels in the canned Atlantic bluefin tuna meats were 0.20 mg Se/kg (1.20 mg/kg total Se; n = 4, 1.00–1.90 mg/kg) in this study (Table 1). In addition, the previous studies have also determined the total selenium levels of the muscle of Atlantic bluefin tuna [1, 2,12-15] (Table 1). However, selenoneine levels have never been reported in the Atlantic bluefin tuna.

Although the previous studies showed that the muscles of tuna and other marine fishes possessed selenoneine, processed products of marine fishes could not be determined because of the contamination of interfering materials contained in the processed products. Therefore, to remove the interfering materials and oils, we used the lipid extraction with the dichloromethane/methanol mixture for oily materials, and recovered water-soluble fractions containing selenoneine. After vacuum-drying, we measured selenoneine concentrations of the canned tuna meats by LC-ICP-MS with GPC column (Table 1).

Table 1: Selenoneine and total selenium concentrations in Atlantic bluefin tuna meat			
Sample Fishing location	Selenoneine	Total Se	Reference
	(mg Se/kg)	(mg/kg)	
Canned product			
T. thynnus Tunisia			
	0.2 (0.163–0.268, n=4)	1.2 (1.00–1.90, n=4)	This paper
Muscle			
T. thynnus Malta		$1.07\pm0.86^{\text{a})}$	12
T. thynnus Sardinia		$0.64\pm0.31^{\text{ a})}$	13
T. thynnus New Jersey		$0.43 \pm 0.038^{a}$	14
T. thynnus Mediterranean		0.58-2.3	15

#### Discussion

We used a mixture of dichloromethane/methanol for lipid extraction to remove the interfering materials and oils from oily meat products. This removes substances that inhibit the detection of Se compounds by LC-ICP-MS. This technique can be used for preprocessing to remove interfering substances contained in biological and food materials, such as blood, tissues, canned or dried products.

The previous studies showed that the muscles of swordfish and tuna contained high selenoneine levels (1.3 - 2.2 mg Se/kg tissue) [3], indicating that the predominant form of the organic Se was present as selenoneine [1-3]. In addition, canned Atlantic bluefin tuna meats of this study were also determined to be 0.2 mg Se/kg (Table 1), showing that selenoneine was stably present in the canned processed foods as well as fresh fish tissues. Selenoneine in the tissues and processed foods might be stable under processing conditions by heating at sterilizing temperature at 121 °C and stored during several years at room temperature. Therefore, the present analytical method can be applied to the determination of selenoneine in tuna and other processed products. We are planning to a survey of total selenium and selenoneine concentrations in marine products and the blood samples of fish-eating human population.

The selenoneine and total Se concentrations might be correlated with biological antioxidant functions in animal and humans. Therefore, knowledge of the Se concentrations in food, blood and other tissue might be important for evaluating biological antioxidant functions. Recent our study measured the oxidative– redox potential (ORP) in fish muscle using an ORP electrode and found that muscle ORP and ROS levels were closely correlated with the Se concentration in blood and muscles [16]. We conclude that dietary administration of selenoneine led to its accumulation in amberjack blood and muscles, resulting in reduced ORP and ROS levels in the muscles. Therefore, selenoneine concentrations in fish and marine products might be the important for biological antioxidant functions in both fish and human tissues.

### Acknowledgments

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