

Selenium in Tuna

White Versus Light and Water Versus Oil Packing

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Canned tuna fish is one of the most commonly consumed types of seafood in the United States as well as a prominent source of dietary selenium. Thirty-two samples of canned tuna were analyzed using fluorescence spectrophotometry to determine whether type of tuna or packaging liquid significantly affects selenium concentrations. Light tuna fish showed a slightly greater overall mean concentration of selenium (149.5 ppb) than white style tuna fish (145.1 ppb). Tuna packed in water, regardless of type of tuna, had higher overall mean concentrations of selenium (156.9 ppb) than tuna packed in soybean oil (137.6 ppb). These results based on category, however, were not statistically significant. In order to get a sense of the average selenium content in a typical can of tuna in the marketplace, the data were collapsed across categories, giving a calculated 12.5 micrograms per can.

Introduction

Selenium is a trace mineral in human diets essential for the proper functioning of the immune system. It is incorporated into selenoproteins and selenium-dependent enzymes, which facilitate antioxidant defense, muscle function, and thyroid hormone production (1). Selenium is also essential for the synthesis of the selenoprotein glutathione peroxidase (2). Furthermore, epidemiological studies spanning fifty years indicate an anticarcinogenic effect of selenium against many forms of cancer (3).

Despite its importance as a micronutrient, selenium intake must be monitored because a narrow window separates selenium deficiency from toxicity. According to the Institute of Medicine, the recommended dietary intake of selenium is 55 micrograms per day for adults, and the tolerable upper intake level is 400 micrograms per day (4). Deficiency can make the body more susceptible to disease whereas high blood levels of selenium can cause symptoms of selenosis, such as hair loss, abnormal functioning of the nervous system, and gastrointestinal upsets (2).

Fish is one of the primary sources of dietary selenium in the United States. The most commonly consumed fish in the United States is canned tuna, according to a report by the National Oceanic and Atmospheric Administration, making it a particularly relevant source of selenium to study (5). Knowledge of the selenium content of different brands and species of canned tuna may therefore assist in achieving a diet that avoids either extreme in selenium intake.

An additional motivation for considering the selenium content of canned tuna is that tuna bioaccumulates the toxic heavy metal mercury, and selenium reduces vulnerability to mercury toxicity in humans (6). The mechanism of selenium's protective effect on methyl mercury is unknown, but its effect on inorganic mercury toxicity is likely due to *in vivo* formation of mercuric selenide (7).

These considerations of selenium's role in the overall health of humans motivated our project to quantify the amount of selenium in leading brands of canned tuna fish using fluorescence spectrophotometry. Because many types of canned tuna are available to consumers, our project examines variations in selenium among different species of tuna readily available in grocery stores, and between the packaging of tuna in water versus oil.

Materials and Methods

Source of canned tuna samples

The types of canned tuna used in this experiment were Bumblebee Chunk Light Tuna in Water, Bumblebee Albacore Tuna in Water, Bumblebee Albacore Tuna in Oil, Chicken of the Sea Chunk Light Tuna in Oil, and StarKist Chunk Light Tuna in Water. Albacore tuna is also referred to as white tuna, and light tuna is a combination of skipjack and yellowfin species. The oil packing used was soybean oil.

Chemicals and other materials

All chemicals and materials were provided by the Department of Chemistry at Dartmouth College, and included concentrated nitric acid, concentrated hydrochloric acid, cyclohexane, 2,3-DAN (2,3-diaminonaphthalene), selenium dioxide, filter paper, hydroxylammonium chloride, pipettes, amber bottles, ethylenediaminetetraacetic acid (EDTA), separatory funnels, screw-cap Erlenmeyer flasks, pH meter, 1100W Sharp Carousel microwave, micropipets, volumetric flasks, graduated cylinders, glass funnels, cuvettes, hot plate, fluorimeter (Shimadzu RF-1501 Spectrofluorophotometer), and high pressure Teflon microwave bomb.

The procedure for this work was adapted from a recently published paper by Sheffield and Nahir (8).

Preparing a standard curve

A stock solution of Se(IV) was prepared by pipetting 1 mL of selenium dioxide into a 50 mL volumetric flask and diluting with nanopure water to yield a 14 ppm selenium solution. A 1.4 ppm solution was prepared by adding 1 mL of the 14 ppm selenium solution to a 125 mL Erlenmeyer flask and treating it with 10 mL of 2.5 % hydroxylammonium chloride, 0.1% EDTA (EDTA solution), and 20 mL of water. The pH was adjusted to 2 ± 0.2 using HCl. Then 5 mL of 0.1 % 2,3-diaminonaphthalene solution (DAN) were added to the solution. After heating in a 50 °C water bath for 30 minutes, 10 mL of cyclohexane were added, which diluted the concentration of the stock solution to 1.4 ppm. Because 0.5 mL of 14 ppm stock solution were added to the Erlenmeyer flask before the EDTA solution was added, the

dilution yielded a 0.7 ppm solution. Serial dilutions were performed to derive concentrations of 0.35, 0.175, 0.0875, and 0.0091 ppm. Measurements were taken on the fluorimeter at these six concentrations to create a standard curve.

Preparations of reagents and solutions: DAN

To make 0.1 % DAN solution, 25 mg of 2,3-diaminonaphthalene were transferred to a 125 mL separatory funnel. 25 mL of 0.1 M HCl were added, and the flask was capped and shaken for about 10 minutes until a fairly homogenous, cloudy solution formed. Next, 10 mL of cyclohexane was added, and the flask was shaken for one minute. The aqueous layer was removed into a clean beaker, the organic layer was discarded, and the separatory funnel was cleaned with nanopure water. This extraction was repeated, and the aqueous layer was collected in an amber bottle through gravity filtration.

Preparations of reagents and solutions: EDTA Solution

To make the EDTA solution, 2.5 g of hydroxylammonium chloride and 0.1 g of EDTA were added to a 100 mL volumetric flask. Nanopure water was added, and the contents were mixed to create a homogenous solution.

Digestion of samples and derivatization of selenium

Each can of tuna was opened and drained. Samples of tuna were taken from the center of the can and blotted with filter paper to remove excess liquid. A 0.5 g sample of tuna was placed into the Teflon cup of a microwave bomb apparatus containing 2.5 mL of concentrated nitric acid. The bomb was placed into an 1100W microwave oven and irradiated for 25 seconds at 40% power. After heating, the bomb was placed in an ice bath for 25 minutes. To reduce any Se(VI) to Se(IV), the solution was transferred into a 125 mL Erlenmeyer flask using a Pasteur pipet, and 4 mL of HCl was added and heated on a hot plate. After heating, 10 mL of EDTA solution and 20 mL of water were added to the flask. The pH of the solution was adjusted to 2 ± 0.2 by adding concentrated ammonium hydroxide and HCl as needed. Then, 5 mL of 0.1% DAN were added to the flask and placed into a 50 °C water bath for 30 minutes. After warming, 20 mL of nanopure water were added to the solution and 10 mL of cyclohexane were added to extract the fluorescent selenium compound, piaszelenol. Roughly 3 mL of the extracted solution were placed into a 1 cm square cuvette for fluorimetric analysis.

Fluorimetric analysis

A three dimensional scan was performed on each standard and sample using a fluorimeter. The excitation frequency was set to 378 nm, and the emission range was between 450 and 650 nm with an expected selenium peak at 518 nm.

Results

Determination of standard curve

Because fluorimeters lack a means of calibration

based on an internal standard, a standard curve was created to give a basis from which the concentration of a sample could be calculated from the measured intensity. Eleven standards of various concentrations were prepared, ranging from 9.1 ppb to 1400 ppb Se(IV). In addition, three blank samples were prepared in the same manner as the standards but omitting the addition of Se(IV) to establish a baseline. These data were first used in a linear regression that used intensity to predict concentration, $F(1,12)=3570$, $p < 0.001$. While this linear relationship was strong ($R^2=.997$), the original Sheffield procedure reported a quadratic standard curve because of the self-absorption that occurs at higher concentrations (8). Therefore, the data was fitted with a quadratic curve, $F(2,11)=19422$, $p < 0.001$, which showed a better fit ($R^2=1.00$). The standard curve is shown in Fig. 1, and the equation of the curve was $Concentration = -4.484 + 2.229(Intensity) + 0.001(Intensity)^2$.

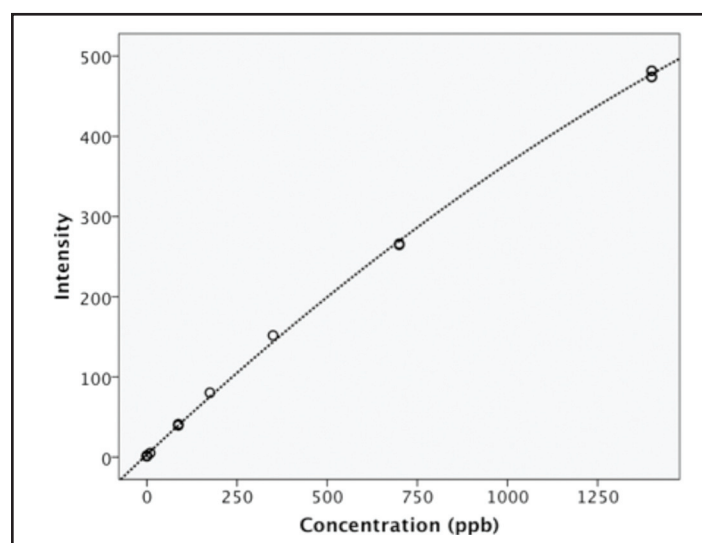


Fig. 1: Standard curve.

Experimental results

For experimental samples, it was necessary to correct for the mass of the samples before intensities could be compared. The value for each sample was divided by its mass to give the equation for the concentration as $(Concentration = -4.484 + 2.229(Intensity/Mass) + .001(Intensity/Mass)^2)$. It should be noted that the concentrations and intensities of the experimental tuna samples were low enough that self-absorption was not a concern; the maximum concentration was found to be 232 ppb, well below the point at which self-absorption becomes a factor.

Data from 32 samples of canned tuna were collected. The samples fell into one of four categories: white tuna in water, white tuna in oil, light tuna in water, and light tuna in oil. Light tuna fish had a slightly greater mean concentration of selenium (149.5 ppb) than white tuna fish (145.1 ppb). Tuna fish packed in water, regardless of the type of tuna fish, contained larger concentrations of selenium on average than tuna packed in soybean oil. The mean concentrations of each category are outlined in Table 1 and were calculated using a standard curve equation and a standardized intensity where the intensity was divided by the mass of the sample.

Type	Packing	Mean (ppb)	Std. Deviation	n
white	water	152.7	48.29	8
	oil	137.4	33.21	8
	Total	145.1	40.81	16
light	water	161.1	32.94	8
	oil	137.8	45.36	8
	Total	149.5	40.15	16
Total	water	156.9	40.17	16
	oil	137.6	38.40	16
	Total	147.3	39.89	32

Table 1. Descriptive Statistics. Dependent variable is the calculated concentration.

Statistical analyses

A 2X2 ANOVA was run analyzing type of tuna (white versus light) and kind of packing material (water versus oil), with eight samples in each cell. There was not a statistically significant effect of type of tuna, $F(1,28) = 0.093$, $p > 0.10$. There was also not a significant effect of packing material, $F(1,28) = 1.820$, $p > 0.10$, and there was not a significant interaction between the two factors, $F(1,28) = 0.079$, $p > 0.10$.

Discussion

Based on the overall mean of 32 samples across all categories, the average amount of selenium for the recommended 56g serving of tuna was $8.250 \pm 2.23 \mu\text{g}$. The amount of selenium in a 3-ounce can of tuna was $12.52 \pm 3.39 \mu\text{g}$. Selenium is toxic when consumed at rates greater than $400 \mu\text{g}$ per day, which would only be attained by eating 32 cans of tuna. The National Institutes of Health reported $63 \mu\text{g}$ of selenium in 3 ounces of tuna, a value that is higher than the experimental results (9). The experimental methods were different, and the higher result may stem from the use of dried samples, as opposed to samples that had merely been patted dry (10, 11).

The slightly higher mean concentration of tuna in water than tuna in oil was unexpected because the oil used to pack the tuna was soybean oil and soybeans are known to contain selenium. Thus, it was expected that tuna packed in oil would contain higher levels of selenium (12). This expectation was also confirmed by the nutritional information on the Bumblebee brand tuna cans, which claimed that tuna in water contains 50% of the recommended daily value of selenium while tuna in oil contains 60% of the daily value (13).

These discrepancies may be attributed to several confounds and limitations. To explore these possible confounds, various *post hoc* analyses were performed.

The first possible confound was that of brand. Bumblebee tuna was used for the majority of samples, but both Chicken of the Sea and StarKist samples were used as well. Bumblebee produces all four categories of tuna that were tested, but local grocery stores did not stock Bumblebee Light Tuna in Oil. As a result, Chicken of the Sea tuna was used for that category. The light tuna in water category also contained one sample that was Chicken of the Sea and two samples that were StarKist, in addition to the Bumblebee samples. A t-test com-

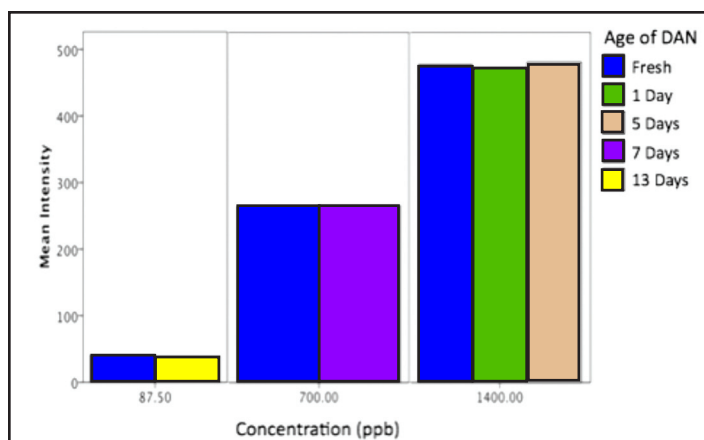


Fig. 2: Age of DAN.

paring Bumblebee tuna samples to the other brands did not reveal a significant difference between brands, $t(30) = 0.295$, $p > 0.10$. The three brands analyzed are all leading brands found in the marketplace regulated by the FDA, and as such, major variations in quality based on brand were not expected.

An additional confounding variable was the age of the 2,3-DAN solution used as a derivatizing agent. The original Sheffield procedure from which the procedure was adapted says of DAN: “for best results, it should be prepared immediately before analysis” (8). When making up the standard curve, standards were prepared using DAN of various ages and fluorimetric analyses revealed very little degradation in the quality of results obtained, even using 13-day-old DAN (Fig. 2). Based on these observations, DAN up to five days old was used in the analysis of experimental samples. A t-test comparing fresh DAN to day-old DAN (categories which account for 23 of our 32 samples) found no significant difference in results, $t(21) = 0.17$, $p > .50$.

DAN, therefore, need not be made up fresh every day, as long as it is used within one or two days and stored cold in a dark bottle.

Another issue encountered in the course of data collection was variation in coloration after digestion. The majority of tuna samples digested into a bright, emerald green solution, but a few digested samples appeared to be a thicker, yellow solution with an almost soupy consistency. This difference in coloration is surprising because the purpose of the nitric acid digestion is merely to dissolve the tuna, so it is unclear why a color change occurs. To add to the complexity, the green samples tended to give noticeably better results, with a clean expected selenium peak at 518 nm. The yellow samples, by contrast, tended to have a large peak at 484 nm in addition to the peak at 518 nm. The intensity at 484 nm per mass of sample is a significant predictor of intensity at 518nm per mass of the sample, $F(1,30) = 5.34$, $p = 0.03$. One possible explanation is that the yellow samples were only partially digested compared to the green samples, but this explanation cannot be confirmed because the tuna appeared dissolved in all cases and the procedure was consistent from sample to sample.

Possible confounding variables aside, a power analysis was run using G*Power to determine the number of samples needed to obtain a reasonably powerful (power = 0.8), statistically significant ($p = 0.05$) main



Image courtesy of Somara Maggl.

Selenium levels in canned tuna were determined to be independent of the liquid in which the tuna was packed.

effect of packing liquid that was hypothesized to exist. This analysis found that 123 samples would be needed to achieve statistically significant results. This corresponds well to a similar study that looked at methyl-mercury in canned tuna fish using samples from 168 cans of tuna fish, variations in methyl-mercury concentration of over 10% were found in a single can (14).

Conclusion

Selenium levels in canned tuna were determined to be independent of the liquid in which the tuna was packed. Although selenium concentrations were higher in those samples packed in water, the result is not statistically significant. Digestion by nitric acid led to extrapolated levels of selenium that did not match those listed on the nutrition labels. Regardless of the sample type, both white and light tuna do not approach toxic levels of selenium intake unless ingested at abnormally high levels. Thus, due to the role of selenium as an essential micronutrient, consuming selenium-containing foods such as tuna in moderation is important.

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References

1. M. P. Rayman, *Br. J. Nutr.* **92**, 557 (2004).
2. L. Koller, J. Exon, *Can J Vet Res.* **50**, 297 (1986).
3. M. Navarro-Alarcon, C. Cabrera-Vique, *Sci. Total Environ.* **400**, 115 (2008)
4. Dietary Reference Intakes: Vitamin C, Vitamin E, Selenium, and Carotenoids (National Academy Press, Washington, DC, 2000).
5. Press Release: shrimp overtakes canned tuna as top US seafood. (National Oceanic and Atmospheric Administration, 2002).
6. N. V. C. Ralston, L. J. Raymond, *Toxicology*, in press corrected proof.
7. D. Yang, Y. Chen, J. M. Gunn, N. Belzile, *Env. Rev.* **16**, 71 (2008).
8. M. C. Sheffield, T. M. Nahir, *J. Chem. Educ.* **79**, 1345 (2002).
9. Dietary Supplement Fact Sheet: Selenium, <http://ods.od.nih.gov/factsheets/selenium.asp>
10. U.S. Department of Agriculture, Agricultural Research Service. 2003. USDA National Nutrient Database for Standard Reference, Release 16. Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/main/site_main.htm?modecode=12-35-45-00
11. *JOAC* **63**, 485(1980).
12. G.N. Schrauzer, *J Am College of Nutr* **20**, 1 (2001)
13. BumbleBee Product BUMBLE BEE® - Nutrition and Size Information, http://www.bumblebee.com/Products/Individual/?Product_ID=13
14. J. Burger, M. Gochfeld, *Environ. Res.* **96**, 239 (2004).