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Research Note

A Limited Survey of Heavy Metal Concentrations in Fresh and Frozen Cuttlefish Ink and Mantle Used As Food

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ABSTRACT

Cuttlefish ink is consumed as a delicacy worldwide. The current study is the first assessment of heavy metal concentrations in cuttlefish ink versus mantle under different storage methods. A total of 212 samples (64 of fresh mantle, 42 of frozen mantle, 64 of fresh ink, and 42 of frozen ink) were analyzed for the detection of the following heavy metals: arsenic (As), chromium (Cr), iron (Fe), lead (Pb), mercury (Hg), and cadmium (Cd). The median As concentrations were 12.9 mg/kg for fresh mantle, 8.63 mg/kg for frozen mantle, 10.8 mg/kg for frozen ink, and 0.41 mg/kg for fresh ink. The median Cr concentrations were 0.06 mg/kg for fresh mantle and frozen ink, 0.03 mg/kg for frozen mantle, and below the limit of quantification (LOQ) for fresh ink. The median Fe concentrations were 4.08 mg/kg for frozen ink, 1.51 mg/kg for fresh mantle, 0.73 mg/kg for frozen mantle, and below the LOQ for fresh ink. The median Pb concentrations of almost all samples were below the LOQ; only two frozen ink, one fresh ink, one frozen mantle, and one fresh mantle sample exceeded the limit stipulated by the European Union. The Hg concentrations were statistically similar among the four categories of samples; the median Hg concentrations were below the LOQ, and the maximum concentrations were found in frozen ink, at 1.62 mg/kg. The median Cd concentrations were 0.69 mg/kg for frozen ink and 0.11 mg/kg for frozen mantle, fresh mantle and fresh ink concentrations were below the LOQ, and in 11.3% of the tested samples, Cd concentrations were higher than the European Union limit. The probability of samples having a Cd concentration above the legal limit was 35.75 times higher in frozen than in fresh products. Fresh ink had significantly lower concentrations of As, Cr, Fe, and Cd, but the concentrations of Hg and Pb were not significantly different from those of other products. Frozen ink had significantly higher concentrations of Cd, Cr, and Fe, but concentrations of As were lower than those in fresh mantle, pointing out a possible role for the freezing process and for different fishing zones as risk factors for heavy metal contamination.

Key words: Consumer health; Cuttlefish ink, Food quality, Heavy metal detection

Fish and fish products are important protein sources in the human diet. According to the Italian food pyramid *(20),* fish should be eaten at least two or three times per week because fish contain high concentrations of n-3 fatty acids, which help to prevent cardiovascular pathology. According to the Food and Agriculture Organization of the United Nations (FAO) world fishery status *(16),* in 2013 a total of 19.7 kg of fish per person was available, and 0.5 kg was composed of cephalopods. In 2012, Storelli et al. *(33)* reported 70 g as the average weekly consumption of cephalopods in Italy.

Cephalopods are quickly growing animals with a short life span that is highly influenced by environmental variability. Among the many cephalopod species, those that are consumed by humans include species of octopus, squid, and cuttlefish. Cephalopod demand and consumption slightly increased in recent years. The countries that consume and import the most cephalopods are Spain, Italy, and Japan.

In 2014, 46% (67 million tons) of fish was chilled, live or fresh, and 30% (44 million tons) was placed on the market in a frozen state. Because of the high perishability of fish, freezing is important to maintain product quality for the designated market *(16)*.

Sepiida is an order of the Cephalopoda subclass Coleoidea. All the species in this subclass have an ink sac and produce ink. In cuttlefish, the ink is always present, from hatchling to adulthood *(2)*. Cuttlefish use ink as a primary defense against predators by quick ejection, creating a cloud or pseudomorph that distracts the predator, allowing the escape of the cuttlefish *(6)*. Each ink sac contains 1 g of melanin, which is 15% of the total weight of the ink, and proteins, which are 5 to 8% of the ink weight *(7)*. Cuttlefish ink has been studied for its antitumor, antioxidant, antimicrobial, antifungal, antihepatotoxic, and antiretroviral properties *(15, 27, 29)*.

Ink is a traditional delicacy in Italy, Spain, and Japan and is used in many countries as a food coloring agent *(7, 26)*. The melanin in ink has been chemically evaluated for antioxidants features and the ability to accept electrons *(17,*

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22, 24, 28, 30, 34, 35). Nevertheless, very little is known about the total chemical composition of ink as a food, and there is a knowledge gap about the concentrations of heavy metal residues in ink *(7)*.

Because cuttlefish are predators at the top of the food chain, these animals can accumulate heavy metal residues and other contaminants in their tissues *(1)*. In the present study, the following heavy metals were evaluated: arsenic (As), total chromium (Cr), lead (Pb), mercury (Hg), cadmium (Cd), and iron (Fe).

Various organic forms of As are found in food, and foods and beverages are the principal routes of human exposure to As *(14)*. Acute exposure to As could lead to abdominal pain, diarrhea, vomiting, and muscle weakness, whereas chronic exposure has led to skin problems and cancer *(1, 18)*. Maximum allowable concentrations of inorganic arsenic have been published for rice and rice-based products *(12)* and drinking water *(9)*. Authors in Italy reported the concentrations of arsenic found in cephalopods but not in ink alone *(4)*. Cr occurs naturally in the earth's crust and in the environment as the result of anthropogenic activities *(18)*. Limits for Cr have not been established by law, but according to the World Health Organization (WHO) and European Food Safety Authority (EFSA) Cr consumed as part of the diet should be limited to 250 μg/day *(13, 36)*. Cr concentrations in fish and cephalopods have been assessed by others *(19, 23)*.

Fe status in the blood is an important element for monitoring, especially in countries with endemic malaria *(25)* and for the role of Fe in the diet *(35)*. The Fe concentrations in *Sepia* (cuttlefish) and in melanin have been assessed by others *(23, 24, 28)*, but the total Fe concentration in ink as a food has not.

Pb is carried in the blood by erythrocytes and is accumulated in soft tissues (e.g., liver and kidneys) and bones of mammals; it also can pass through the placental barrier and can be present in milk during lactation. Pb has a half-life of 30 days in blood and 10 to 30 years in bones. According to European legislation, the limit for lead in cephalopods (without viscera) is 0.3 mg/kg *(10, 11)*. The Pb concentrations were evaluated by Cirillo et al. *(4)* in cephalopods, by Storelli et al. *(33)* specifically in cuttlefish, and by Sarzanini et al. *(28)* in the melanin of ink.

Fresh and frozen cephalopods were analyzed for total Hg concentrations by Cirillo et al. *(4)*. According to Cardoso et al. *(3)*, the consumption of cuttlefish and octopus should be limited to two 150-g meals per week because of the presence of methyl Hg. European legislation has set a limit on Hg in cephalopods and fishery products (e.g., ink) at 0.5 mg/kg *(10)*.

In cuttlefish, the hepatopancreas is the main organ of concentration and contains 98% of the total Cd in the body *(21, 31, 32)*. According to European legislation, the limit for Cd in the edible parts of cephalopods (animal without viscera) is 1 mg/kg *(10)*.

The aim of our project was to evaluate the presence of heavy metal residues in fresh and frozen cuttlefish and cuttlefish ink to better understand and compare the risks associated with high concentrations of heavy metals in fresh and frozen products. This is the first study of heavy metal concentrations in cuttlefish ink used as food.

MATERIALS AND METHODS

Cuttlefish mantle and ink samples. A total of 212 samples were gathered at the fish market in Chioggia, Italy, between April and June 2011 and 2012. The mantles and inks were collected from fresh and frozen *Sepia officinalis*. A total of 106 samples of cuttlefish mantles (42 frozen and 64 fresh) and 106 samples of cuttlefish ink (42 frozen and 64 fresh) were analyzed. Fresh animals were captured in the zone of Chioggia (area 37.2.1, FAO, Rome), and frozen products came from various areas around the world (Yemen, India, Senegal, and France), representing all the frozen products arriving in the Chioggia fish market. The mean weight of each cuttlefish was 0.2 kg. From a single cuttlefish, the mantle and ink were analyzed, representing one mantle sample and one ink sample.

Sample preparation. Samples were analyzed for detection of the heavy metals As, Cd, Cr, Hg, Pb, and Fe using inductively coupled plasma optical emission spectrometry (SPECTRO ARCOS EOP, Ametek, SPECTRO Analytical Instruments, Mahway, NJ). The protocol was modified from that of D'Ilio et al. *(8)* to include analysis of Fe and Hg. Cuttlefish mantle and ink were homogenized (T25 digital ULTRA TURRAX, IKA Labortechnik, Staufen, Germany), and then all samples were digested using the protocol of Conficoni et al. *(5)*. The acidified solution was filtered and diluted with deionized water and placed inside the spectrometer via auto sampling.

Heavy metal detection. The As, Cd, total Cr, Hg, Pb, and Fe concentrations were determined by spectrometry after sample preparation with a microwave digester, as reported by Conficoni et al. *(5)*. The limits of quantification were 0.005 mg/kg for As, 0.001 mg/kg for Cd, 0.001 mg/kg for total Cr, 0.005 mg/kg for Hg, 0.001 mg/kg for Pb, and 0.005 mg/kg for Fe.

Statistical analysis. The statistical analyses were all performed with SAS (SAS Institute, Cary, NC). All samples of fresh mantle or ink and frozen mantle or ink were evaluated with the Kruskal-Wallis test. Values were considered significantly different when the P value was <0.0001. The risk ratio, the 95% confidence interval, and chi-square test P value also were evaluated when Cd, Hg, and Pb concentrations were above the legal limits.

RESULTS

Table 1 lists the Pb, Cd, Hg, As, Cr, and Fe concentrations of cuttlefish products by product type (fresh versus frozen) and food type (mantle versus ink), with the following four categories: frozen mantle, fresh mantle, frozen ink, and fresh ink. Median values were compared using the Kruskal-Wallis method.

The As concentrations were higher in fresh mantle than in frozen mantle and frozen ink, and lowest in fresh ink (fresh mantle > frozen mantle and frozen ink > fresh ink). Cr concentrations were higher in fresh mantle and frozen ink than in frozen mantle and lowest in fresh ink (fresh mantle and frozen ink > frozen mantle > fresh ink). Fe concentrations in frozen ink were higher than those in fresh mantle, concentrations in both of these sample types were higher than those in frozen mantle, and concentrations were

TABLE 1. *Cuttlefish products analyzed and their heavy metal concentrations*[a]

	Mantle (mg/kg)		Ink (mg/kg)		
Metal	Frozen	Fresh	Frozen	Fresh	*P* value
Pb	<0.001 (<0.001–0.31)	<0.001 (<0.001–0.43)	<0.001 (<0.001–1.15)	<0.001 (<0.001–0.59)	0.36
Cd	0.11 (0–9.01) B	<0.001 (<0.001–0.27) C	0.69 (0–44.8) A	<0.001 (<0.001–0.59) C	<0.0001
Hg	<0.005 (<0.005–0.5)	<0.005 (<0.005–0.54)	<0.005 (<0.005–1.62)	<0.005 (<0.005–0.52)	0.32
As	8.63 (1.66–21.2) B	12.9 (0.37–46.8) A	10.8 (0.39–32.5) B	0.41 (0–39.8) C	<0.0001
Cr	0.03 (0–0.92) B	0.06 (0–0.58) A	0.06 (0–1.32) A	<0.001 (<0.001–0.9) C	<0.0001
Fe	0.73 (0.02–11.6) C	1.51 (0–13.7) B	4.08 (0–32.3) A	<0.005 (<0.005–4.01) D	<0.0001

[a] Values are medians (minimum and maximum in parentheses). *P* values were generated with a Kruskal-Wallis nonparametric analysis. Within a row in which *P* values are significant (<0.0001), letters indicate the highest (A) to the lowest (D) heavy metal concentrations.

lowest in fresh ink (frozen ink > fresh mantle > frozen mantle > fresh ink).

Concentrations of Pb and Hg in the various products did not differ significantly. The concentration of Cd was higher in frozen ink than in frozen mantle, and concentrations of both of these sample types were higher than those in fresh mantle and fresh ink (frozen ink > frozen mantle > fresh mantle and fresh ink).

Cd, Hg, and Pb were present at concentrations above the European Union (EU) legal limit stated by Regulation 1881/2006 and subsequent amendments *(10–12)* in 24, 6, and 5 samples of cuttlefish products, respectively. The risk ratio for the presence of samples with concentrations above the legal limits for Cd, Hg, and Pb also was determined (Table 2).

TABLE 2. *Prevalence of cuttlefish samples noncompliant with EU legal limits: ink versus mantle and frozen versus fresh*[a]

	% of noncompliant samples			
Metal	All ink samples	All mantle samples	*P* value	RR (95% CI)
Cd	16.04	6.6	0.0302	2.43 (1.05–5.61)
Hg	3.77	1.88	0.42	1.96 (0.37–10.50)
Pb	2.91	1.92	0.651	1.50 (0.26–8.80)
	All frozen samples	All fresh samples		
Cd	27.71	0.78	<0.001	35.75 (4.92–259.70)
Hg	4	2.3	0.598	1.52 (0.31–7.37)
Pb	3.70	2.44	0.650	1.50 (0.26–8.75)
	Frozen ink	Fresh ink		
Cd	39.02	1.54	<0.001	25.37 (3.49–184.11)
Hg	4.76	3.13	0.665	1.52 (0.22–10.40)
Pb	5.00	1.59	0.331	3.05 (0.29–32.56)
	Frozen mantle	Fresh mantle		
Cd	14.63	1.54	0.008	9.51 (1.19–76.19)
Hg	2.38	1.56	0.762	1.52 (0.10–23.70)
Pb	2.44	1.59	0.762	1.52 (0.10–23.70)

[a] EU limits: Cd, 1 mg/kg; Hg, 0.5 mg/kg; Pb, 0.3 mg/kg. *P* values are given for chi-square tests. RR, risk ratio; 95% CI, 95% confidence interval.

DISCUSSION

Information regarding the heavy metal concentrations in various cuttlefish products, particularly ink used as human food, is lacking *(7)*. In this study, analyses were performed to obtain data for these products. The two categories of food (cuttlefish mantle and cuttlefish ink) were analyzed as both fresh and frozen products (Table 1).

The As concentrations were significantly different in the four categories of products. Fresh mantle had the highest concentration, followed by both frozen products (mantle and ink). Fresh ink had the lowest concentration of As.

Significantly higher concentrations of Cr were present in fresh mantle and frozen ink than the other cuttlefish products. The frozen mantle contained lower concentrations of Cr than did the fresh mantle and frozen ink, and the fresh ink had the lowest concentration of Cr. The Cr concentration in all samples was far lower than that established by the EFSA and the WHO as the maximum supplement per day *(13, 36)*.

The Fe concentration differed among the four types of product. However, all products contained lower Fe concentrations than those reported previously *(23, 24)*. Frozen ink had significantly higher Fe concentrations ($P < 0.0001$), followed by fresh mantle, and frozen mantle and fresh ink had the lowest concentrations (Table 2). Fe concentrations in the frozen products were significantly higher than those in the fresh products. Geographical factors and the freezing process may have played roles in the As, Cr, and Fe concentrations in these products.

Heavy metal limits in cephalopods without viscera have been stipulated by the EU *(10–12)* for Pb (0.3 mg/kg), Hg (0.5 mg/kg), and Cd (1 mg/kg), and the risk ratio was calculated in the present study for these elements (Table 2). An important aspect of the assessment was whether cuttlefish ink should be considered part of the viscera or an edible portion of the cuttlefish.

The Pb concentrations in the present study were lower than those reported previously, e.g., up to 0.689 mg/kg wet weight in fresh cephalopods and 0.463 mg/kg wet weight in frozen cephalopods *(4)* and a mean of 0.14 mg/kg in common cuttlefish *(33)*. The Pb concentration in the melanin present in cuttlefish ink was reported by Sarzanini et al. *(28)* as 0.18 mg/kg. In the present study, each type of food and storage method had similar results, with no significant differences ($P = 0.36$). The five samples with Pb concentrations above the EU legal limit *(11)* were evenly distributed between the food types: one sample of frozen mantle, one of fresh mantle, one of fresh ink, and two of frozen ink. Variability in Pb concentrations could also be explained by the geographical origin of the samples.

Hg concentrations were similar ($P = 0.32$) among the four categories of products. The median Hg concentration was below the LOQ, and the maximum concentration was found in one sample of frozen ink (1.62 mg/kg). Similar Hg concentrations in the products could be a sign of similar types of contamination of the foods, but no evidence of this type of contamination was found. Six of the 212 samples exceeded the EU legal limit for Hg (0.5 mg/kg). The calculated risk ratio for the various types of food and method of preservation revealed no significant differences between the type of food (ink versus mantle) or the method of preservation (fresh versus frozen). Within each food type, the differences in the risk ratio were not significant (Table 2). The Hg concentration was not significantly different among the products, and those samples with concentrations above the legal limit were similar and without specific contamination traits.

Significant differences in Cd concentrations ($P < 0.0001$) were found in frozen foods (Table 1). Frozen ink had Cd concentrations that were significantly higher than those in other products, and fresh products (food and ink) had the lowest Cd concentrations. During freezing, the Cd present in various cuttlefish organs could have migrated to the mantle and ink. Twenty-four samples had concentrations above the EU limit (1 mg/kg) *(10)*. The risk ratio (Table 2) was calculated for each type of product, and preservation method was evaluated within product type. The risk ratio was not significantly different for the total amount in ink compared with the mantle. The similarity between these two food types highlighted the effect of the storage method on Cd concentration. The risk ratio was significantly different ($P < 0.001$) between the two storage methods; frozen products were 35 times more likely to have Cd concentrations above the legal limit. Frozen ink was 25 times more likely than fresh ink to have Cd concentrations above the legal limit. The Cd concentration in frozen mantle was not significant ($P = 0.008$).

The present study is the first in which heavy metal concentrations were assessed in cuttlefish ink versus cuttlefish mantle held under different preservation conditions. The results suggest an effect of freezing on the concentration of Cd, which could be a risk for consumers. The concentrations of Cd, Hg, and Pb found were a concern with regard to EU regulations. The Hg and Pb concentrations, which were above the EU legal limit, were evenly distributed among food categories. Frozen products seemed to have higher Cd contamination. In conclusion, freezing could have important implications for heavy metal concentrations in certain products. Additional research is needed to determine whether geographical region of harvest, season of harvest and preservation method play a role in the differences in heavy metal concentrations in cuttlefish products.

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