

# Characteristics of divalent minerals extracted from liver of Japanese common squid *Todarodes pacificus* under various experimental conditions

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**Abstract** In relation to the problem of how to utilize the waste from squid processing, we determined the proximate and mineral compositions of liver of the Japanese common squid *Todarodes pacificus*, and characterized the behavior of divalent minerals, focusing in particular on cadmium removal. The squid liver contained fat (44.0 g/100 g dry matter), protein (13.5 g/100 g dry matter), and ash (2.11 g/100 g dry matter). It also contained the macrominerals sodium, potassium, magnesium, and calcium, as well as the trace minerals iron, zinc, cadmium, and copper. Low- and high-pH treatments (pH 2 and 12) removed the cadmium entirely, but the trace minerals zinc, iron, and copper, as well as soluble protein, which are all important for proper functioning of the human organism, were also removed in large quantities. High percentages of soluble minerals and soluble protein were found in the low molecular weight (MW < 10,000) fraction. The solubility of zinc and copper were strongly related to the solubility of cadmium, but there was no relationship between the solubilities of magnesium and cadmium. On the other hand, while treatment with either 0.9 % NaCl or 100 mM phosphate buffer (pH

7.4) also removed the cadmium completely, it left some useful compounds (trace minerals and soluble protein) in the solid part, so these treatments may represent appropriate methods for removing cadmium from squid processing waste.

**Keywords** Cadmium · Liver of squid · Low molecular weight · Minerals · Solubility · Soluble protein

## Introduction

Cephalopods—including cuttlefish, squid, and octopuses—are important marine resources, as they are rich in taste and have few inedible parts [1]. They have become the preferred seafood in Japan, and are eaten raw (e.g., in sushi and sashimi), cooked (e.g., grilled and boiled), and as various processed products (e.g., as dried sheets and from cans). Although cephalopods have only a few inedible parts, waste parts from the processing of this seafood, such as hepatopancreas, liver, mantle, and digestive glands, have become a serious problem due to high concentrations of heavy metals in them, especially cadmium. The use of biomass and organic wastes as potential sources of materials and energy has recently increased due to the production of novel valuable compounds and the introduction of new environmental policies [2]. Squid waste contains important substances such as minerals [3–6], proteinase enzyme [7–9], and organic acids [3]. Therefore, there is a high possibility that valuable resources can be extracted from squid waste such as squid liver.

Cephalopods are well known to have the ability to accumulate not only the elements that are essential for their metabolism but also nonessential elements in high concentrations [10–13]. Cadmium is a toxic heavy metal that

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can accumulate in the internal organs of cephalopods, such as the midgut gland, liver, gills, and kidney. In the Japanese common squid *Todarodes pacificus*, the concentration of cadmium in liver was reported to be 15–33 mg/kg fresh weight [14], while another report mentioned that the cadmium concentrations in liver were 71.4–168 and 252–254 mg/kg dry weight in females and males, respectively [6]. The livers of the squid species *Loligo opalescens*, *Ommastrephes bartramii*, and *Symplectoteuthis oualaniensis* contained 85.0, 782, and 287 mg of cadmium/kg dry weight, respectively [15]. The cadmium content of squid liver depends on factors such as the condition of the environment or water as well as the sex, size, and age of the squid. However, as mentioned above, squid liver also contains some useful compounds. Therefore, there is the potential to take advantage of favorable constituents by eliminating components that are harmful, such as cadmium.

There have been many attempts to reduce the cadmium contents of intestinal organs of cephalopods via several treatments, including physical, microbiological, and chemical methods as well as combinations of them [2, 16–18]. However, the treatments can also affect other components that are beneficial to the human organism, especially minerals that have the same valence as cadmium, as well as protein and fat. Therefore, before applying any treatment to cephalopod processing waste, it is very important to know the chemical profile of the waste and the solubilities of minerals present in it, focusing in particular on the effective removal of cadmium. A large amount of solvent may be required to flush out cadmium, which may then become cadmium-containing waste solvent. Ghimire et al. [18] reported the removal of toxic minerals by passing the waste solvent through a gel. As a first step in the fractionation of useful compounds from a waste sample, we focused on the solid part of the sample. We will address the waste solvent in the next step of our research in this area.

Several studies have revealed that the contents, solubilities, and bioaccessibilities of minerals in cephalopod processing waste are influenced by several factors, such as the pH [19, 20], the presence of other compounds that act as a chelating agents with enhancing or an inhibiting actions [21, 22], and the treatment provided (such as heating or cooking) [19, 23, 24]. Moreover, trace minerals in cephalopods can also be bound with protein in low, intermediate, or high molecular weight fractions [5, 25]. In order to determine how to best utilize squid waste, we determined the proximate and mineral compositions of liver of the Japanese common squid *Todarodes pacificus* and evaluated the solubilities of divalent minerals and soluble protein under different conditions, focusing in particular on the removal of cadmium.

## Materials and methods

### Chemicals and reagents

The chemicals and reagents used in this experiment (nitric acid, standard mineral solution, hydrochloric acid, sodium chloride, and sodium hydroxide) were of analytical grade and obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). A Bio-Rad protein assay kit was obtained from Bio-Rad (Rockford, IL, USA). Bovine serum albumin was obtained from Sigma–Aldrich Japan Co. (Tokyo, Japan).

### Collection of the squid and squid liver pretreatment

Japanese common squid *Todarodes pacificus* were captured off Kyonan (Tokyo Bay, Japan) in September 2008. Twelve individuals with mantle lengths of 20–30 cm and weights of 200–500 g were used in this experiment. Squid livers were taken from fresh squid. They were washed with tap water, wiped with paper towels, minced to obtain a homogenate using a food processor (MK-K75, Matsushita Electric, Co., Osaka, Japan), and stored at  $-30\text{ }^{\circ}\text{C}$  until use. All experiments were performed within a week after fresh liver processing.

### Proximate analysis

Squid liver (wet sample) was found to consist of moisture, ash, fat, and protein (nitrogen conversion factor of 6.25) using a method from the AOAC [26].

### Total mineral analysis

As discussed in our previous report [27], the samples (of squid liver) were treated with the wet ashing method using nitric acid. The destroyed samples were dissolved in 10 % hydrochloric acid and then analyzed using an atomic absorption spectrophotometer (model AA-660, Shimadzu Co., Kyoto, Japan). Sodium (Na, detection limit 0.005 ppm), potassium (K, detection limit 0.005 ppm), magnesium (Mg, detection limit 0.001 ppm), calcium (Ca, detection limit 0.04 ppm), iron (Fe, detection limit 0.06 ppm), zinc (Zn, detection limit 0.05 ppm), cadmium (Cd, detection limit 0.007 ppm), and copper (Cu, detection limit 0.04 ppm) were measured. Standard solutions were prepared in 10 % hydrochloric acid. All glassware and plastic bottles used were dipped in contaminon (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for at least 2 h and then rinsed with Milli-Q water to remove contaminants.

## Determination of soluble minerals and soluble protein at different pH values

Following the method described in our previous report [27], fresh squid liver samples (1 g) were blended in a tube with 30 ml of diluted hydrochloric acid at pH 2–5, or Milli-Q water, or sodium hydroxide solution at pH 7–12 at 5,000–10,000 rpm for 3 min using an Ultra-Turrax (T-25, Janke and Kunkel, IKA-Labortechnik GmbH Co., Staufen, Germany) to obtain the soluble fraction. The sample suspensions were centrifuged and the resulting supernatants were de-fatted using chloroform. The minerals and protein in the de-fatted layer were then measured. The minerals were measured using an atomic absorption spectrophotometer (Shimadzu AA-660). The solubility of each mineral was calculated using the equation: solubility (%) = (soluble mineral mg/g)/(total mineral mg/g) × 100. The soluble protein in the de-fatted layer was determined using the Bradford protein assay (protein assay kit, Bio-Rad, Rockford, IL, USA). Bovine serum albumin was used as a standard. The solubility of the protein was calculated using the formula: solubility (%) = (soluble protein g/100 g)/(total protein g/100 g) × 100.

## Determination of minerals and protein in the whole extract and in the low molecular weight fraction

The soluble minerals and soluble protein in both the whole extract and the low molecular weight fraction from squid liver were obtained as follows. Fresh squid liver samples (1 g) were blended in a tube with 30 ml of 0.9 % NaCl or 100 mM phosphate buffer (pH 7.4) at 5,000–10,000 rpm for 3 min using an Ultra-Turrax (T-25) to obtain the soluble fraction. Next, the samples were centrifuged at 5,500×g and 4 °C for 10 min (Kubota 6800, Kubota Corporation, Fujioka, Japan). The collected supernatants were de-fatted using chloroform to obtain the de-fatted layer. The de-fatted layer was then passed through an ultrafilter (Advantec Q0100 013D, Toyo Roshi Co. Ltd., Tokyo, Japan) with a molecular weight cut-off 10,000 to obtain the low molecular weight fraction. The minerals and protein in both the de-fatted layer and the low molecular weight fraction were measured. The methods used to determine the minerals and protein and to calculate their solubilities were the same as those mentioned above.

## Statistical analysis

The results are presented as the mean ± SD ( $n = 3–8$ ). ANOVA was used to check for significant differences ( $p < 0.05$ ) [28].

## Results

### Proximate and mineral compositions of the squid liver

Table 1 shows the proximate and mineral compositions of the squid liver. The squid liver sample had protein, fat, and ash contents of 7.69 g/100 g fresh weight, 25.0 g/100 g fresh weight, and 1.20 g/100 g fresh weight, respectively. After converting them to 100 g dry matter samples, the contents of protein, fat, and ash were found to be high: 13.5 g/100 g dry matter, 44.0 g/100 g dry matter, and 2.11 g/100 g dry matter, respectively. Potassium was found to be the major macromineral in squid liver (3.75 mg/g dry matter), followed by sodium (2.69 mg/g dry matter), magnesium (0.58 mg/g dry matter), and calcium (0.13 mg/g dry matter). The trace minerals present, in order of concentration (highest first), were iron, copper, zinc, and cadmium, with values of 162 µg/g dry matter, 133 µg/g dry matter, 66.3 µg/g dry matter, and 33.0 µg/g dry matter, respectively. Manganese was not detected (i.e., its content was less than 0.005 ppm, the detection limit of AAS) and phosphorus was not determined. When the values from the Food Composition Database (<http://fooddb.jp/>, accessed 20 October 2012) were converted to mg or µg/g dry matter, the edible part (muscle) of Japanese common squid was found to have sodium, potassium, magnesium, calcium, iron, zinc, and copper contents of 14.3 mg/g, 12.8 mg/g, 2.57 mg/g, 0.6 mg/g, 4.76 µg/g, 71.4 µg/g, and 16.1 µg/g, respectively. In comparison to these values, liver of Japanese common squid had much lower contents of sodium,

**Table 1** Proximate composition and mineral contents of liver of the Japanese common squid *Todarodes pacificus*

Composition	Values (g/100 g)	
	Fresh weight	Dry matter
Moisture	43.2 ± 3.84	
Crude protein	7.69 ± 0.51	13.5 ± 0.90
Total fat	25.0 ± 2.62	44.0 ± 4.61
Ash	1.20 ± 0.14	2.11 ± 0.25
Carbohydrate (by difference)	22.9 ± 4.52	40.3 ± 7.99
Mineral contents		
Sodium (mg/g dry matter)		2.69 ± 0.14
Potassium (mg/g dry matter)		3.75 ± 0.24
Magnesium (mg/g dry matter)		0.58 ± 0.05
Calcium (mg/g dry matter)		0.13 ± 0.01
Iron (µg/g dry matter)		162 ± 22.9
Zinc (µg/g dry matter)		66.3 ± 21.0
Cadmium (µg/g dry matter)		33.0 ± 4.70
Copper (µg/g dry matter)		133 ± 32.6

potassium, and magnesium, whereas it had much higher contents of iron and copper.

#### Solubilities of divalent minerals and protein at various pH values

In order to understand the behavior of divalent minerals and protein at different pH values, we evaluated their solubilities, as presented in Fig. 1. De-fatted squid liver was decomposed by wet ashing and the recoveries of the minerals were evaluated. The recovery of protein was measured by the Kjeldahl method [26]. Almost all (nearly 100 %) of the minerals and protein remained in the squid liver after de-fatting. For example, the content of cadmium in the de-oiled fraction was 33 µg/g dry matter, and nearly 100 % of it was dissolved at pH 12. Protein solubility was calculated to be 100 % based on a protein content of 13.5 g/100 g dry matter. The solubilities of divalent minerals (zinc, iron, and cadmium) showed the same pattern. They tended to be more soluble at low and high pH. There was no such pattern for the solubility of magnesium, which remained constant at ~60 % at all pH values. Low-pH treatment (pH 2) removed the cadmium entirely, while

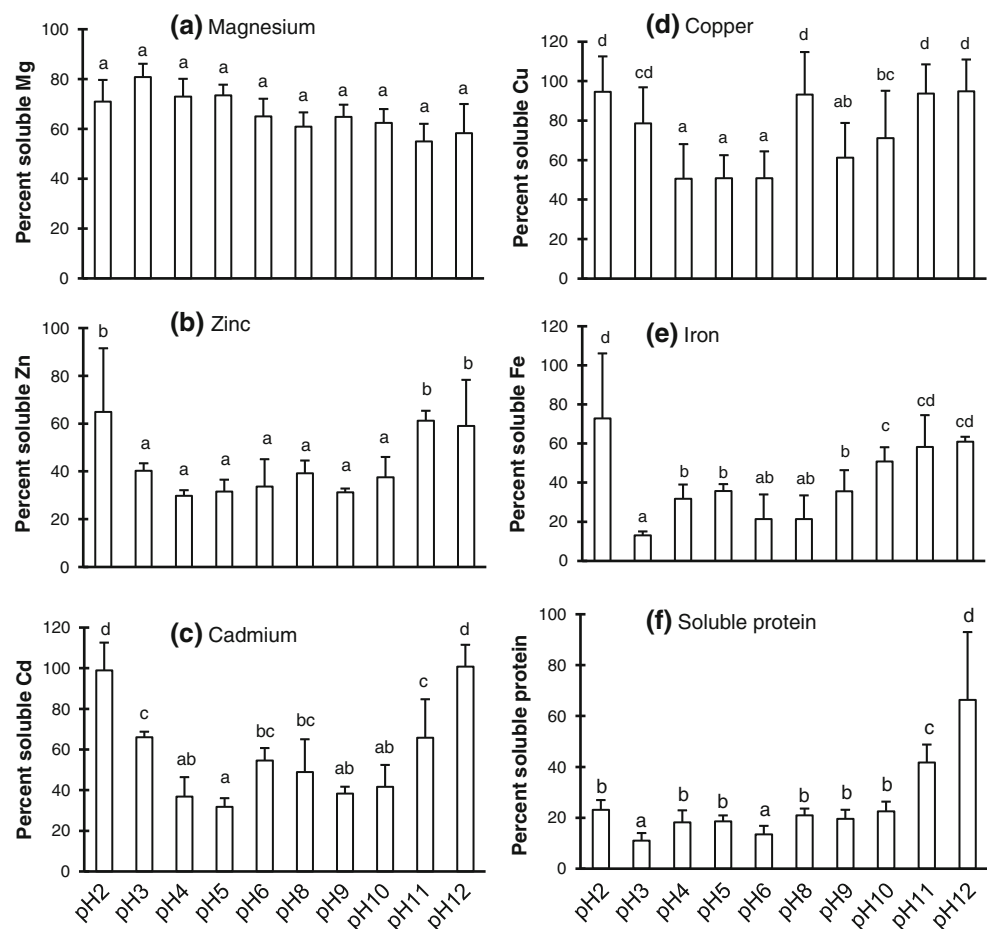
~80 % of the protein remained. However, important trace minerals (zinc, iron, and copper) were also removed in large quantities. In the high-pH treatment (pH 12), all of the cadmium was removed and <40 % of the protein was left. Moreover, high-pH treatment resulted in the removal of >60 % of the trace minerals copper, zinc, and iron.

The solubilities of divalent minerals (copper, iron, zinc, and cadmium), especially under alkaline conditions, tended to be positively correlated with the solubility of protein. This indicates that these minerals may bind to water-soluble protein, forming protein–mineral complexes.

#### Correlations of the solubilities of divalent minerals with the solubilities of cadmium and protein

Since there is competition among divalent minerals and protein in terms of solubility, it is important to evaluate the correlations among their solubilities. The correlations of the solubilities of divalent minerals with the solubility of cadmium (Fig. 2) and with the solubility of protein (Fig. 3) at different pH values are depicted in the figures. Cadmium solubility showed strong relationships with the solubilities of zinc and copper ( $r = 0.7640$  and  $r = 0.6528$ , respectively),

**Fig. 1** The solubilities of magnesium (a), zinc (b), cadmium (c), copper (d), iron (e), and protein (f) extracted from the liver of Japanese common squid *Todarodes pacificus* at various pH values. Columns with different letters above them on the plot are significantly different ( $p < 0.05$ ; each column and bar above the column show the mean and SD, respectively;  $n = 3$ )



whereas iron and cadmium exhibited a moderately strong relationship ( $r = 0.4795$ ) and there was no relationship between magnesium and cadmium ( $r = 0.0574$ ). These results suggest that zinc, copper, iron, and cadmium show similar solubility behavior, as indicated by their similar solubility patterns (Fig. 1). A positive correlation between the solubilities of divalent minerals and the solubility of protein at different pH values (2–12) was observed, as shown in Fig. 3. The highest solubility correlations were found for the minerals cadmium ( $r = 0.5113$ ), iron ( $r = 0.5083$ ), and zinc ( $r = 0.4399$ ). There was a negative correlation between the solubilities of magnesium and soluble protein ( $r = -0.5015$ ). In the case of cadmium, the correlation became more positive under alkaline conditions ( $r = 0.6710$ ). These results indicate that iron, cadmium, and zinc are extracted with protein at all pH values; in particular, cadmium tends to be extracted with protein at high pH. Increasing the pH may change the protein conformation to one that is more soluble (Fig. 1). This solubilized protein is then more likely to form a complex with cadmium through mineral–protein binding, thus increasing the solubility of cadmium at higher pH.

Solubilities of divalent minerals and protein in de-fatted extract and in the low molecular weight fraction

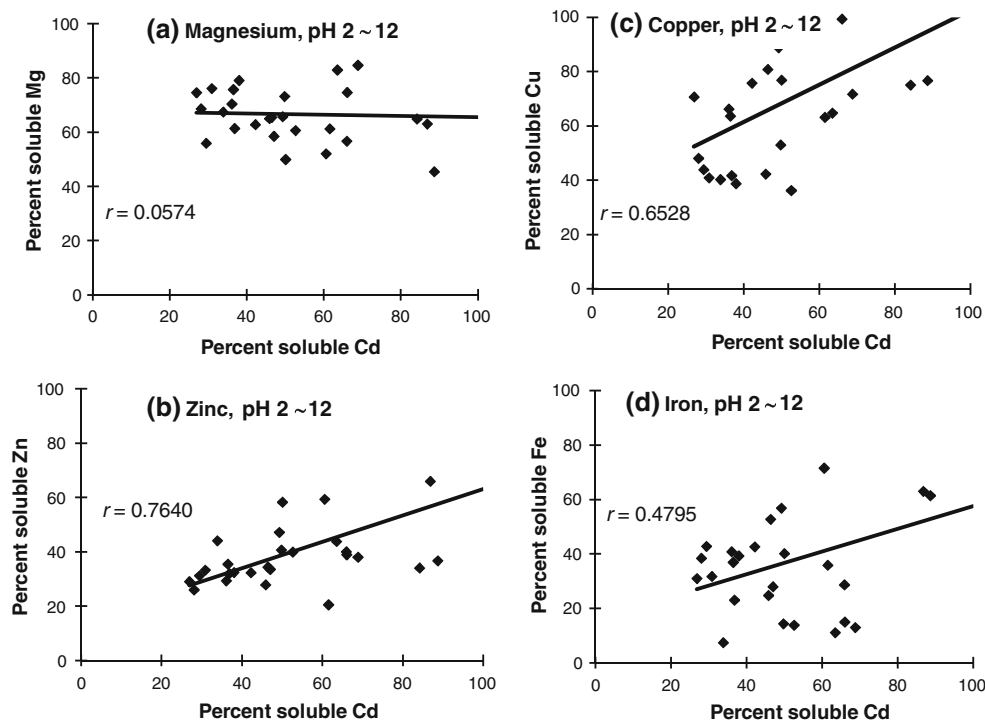
The divalent minerals and protein showed the same solubility patterns in 0.9 % NaCl and 100 mM phosphate

buffer (pH 7.4); see Fig. 4. Among the five minerals, cadmium was the most soluble in de-fatted extract and in the low molecular weight fraction in both 0.9 % NaCl and 100 mM phosphate buffer, followed by copper, magnesium, iron, and zinc. Protein was less soluble than the divalent minerals. All of the cadmium was in soluble form in both 0.9 % NaCl and 100 mM phosphate buffer. This means that it is possible to remove cadmium completely using 0.9 % NaCl or 100 mM phosphate buffer. After extraction with 0.9 % NaCl or 100 mM phosphate buffer, high percentages (25–70 %) of the zinc, copper, and iron were still present, and 75 % of the protein remained. After applying 0.9 % NaCl or 100 mM phosphate buffer, ~20 % of the protein was still present in the low molecular weight fraction.

## Discussion

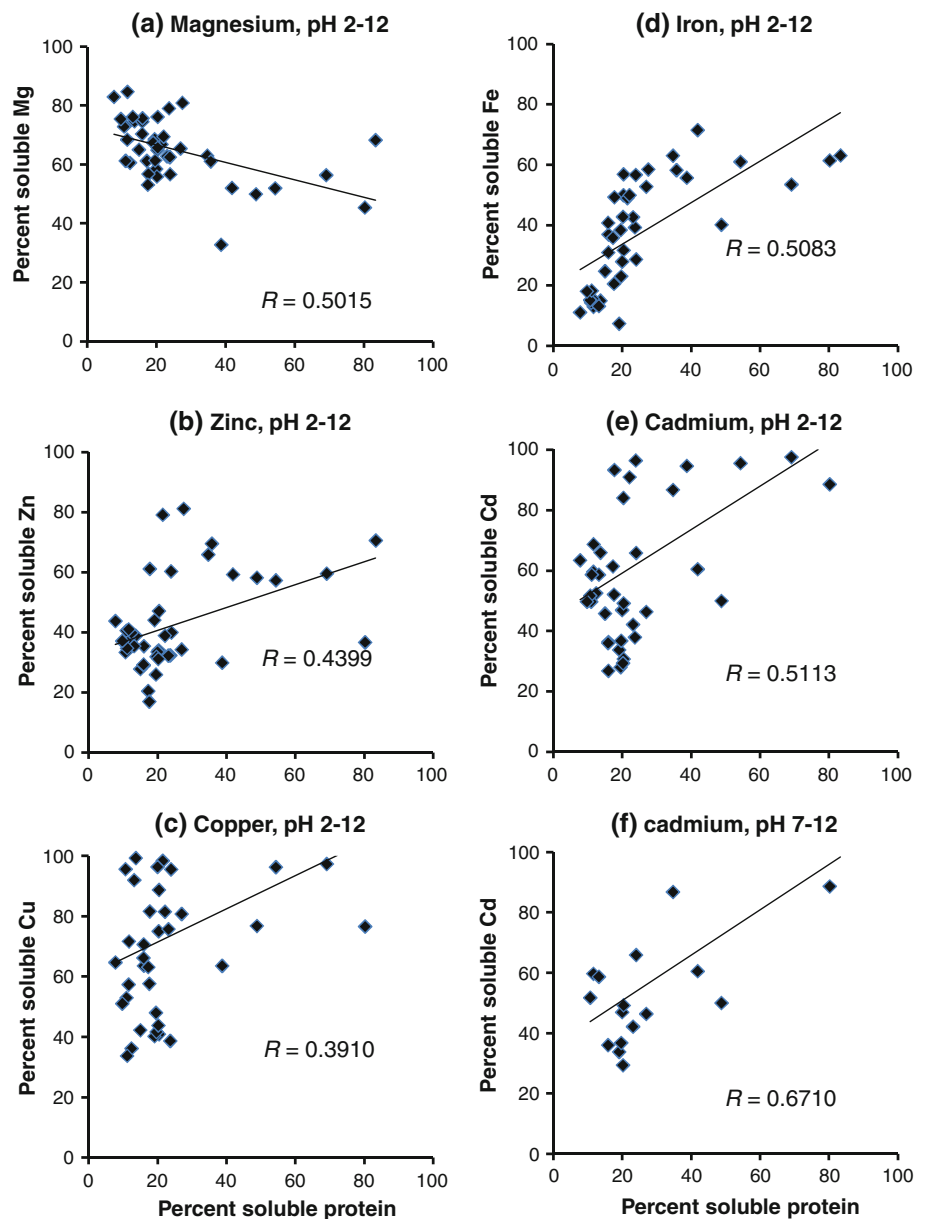
### Proximate and mineral compositions

Several experiments concerning the mineral content of squid liver have been conducted; however, they focused solely on trace minerals (they did not analyze the macro-mineral profile or the proximate composition). In the Japanese common squid *T. pacificus*, the trace minerals copper, zinc, and cadmium were present in liver at levels of



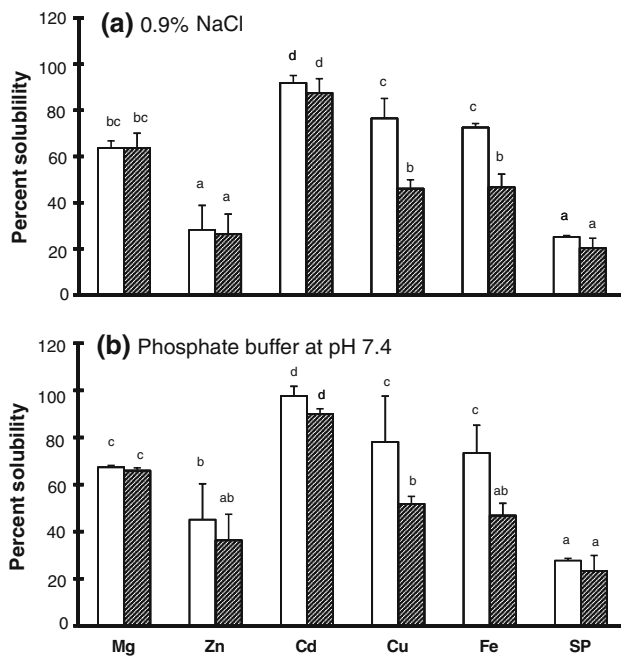
**Fig. 2** Correlations of the solubility of cadmium with the solubilities of other divalent minerals—magnesium (a), zinc (b), copper (c), and iron (d)—extracted from liver of Japanese common squid *Todarodes pacificus* ( $n = 33$ )

**Fig. 3** Correlations of the solubility of protein with the solubilities of magnesium (a), zinc (b), copper (c), iron (d), cadmium at pH 2–12 (e), and cadmium at pH 7–12 (f), as extracted from liver of Japanese common squid *Todarodes pacificus* (a–e  $n = 33$ , f  $n = 18$ )



111–267, 31–89, and 15–33 mg/kg fresh weight, respectively [14]. Kim et al. [6] also measured the trace mineral contents in liver of the squid *T. pacificus* from offshore Korean waters. In squid with medium mantle lengths (20.5–23.2 cm), the contents of zinc, copper, and cadmium in liver were 221, 989, and 198  $\mu\text{g/g}$  dry matter, respectively. In squid with long mantle lengths (24.0–29.5 cm), the contents of zinc, copper, and cadmium in liver were significantly lower than those seen in squid with medium mantle lengths: 194, 793, and 155  $\mu\text{g/g}$  dry matter, respectively. In another squid species, *Nototodaros Gouldi*, Finger and Smith [4] reported that the liver contained copper (66.7 mg/kg fresh weight), zinc (152.6 mg/kg fresh weight), and cadmium (6.07 mg/kg fresh weight).

Furthermore, Craig and Overnell [5] analyzed the minerals in the liver of the squid *Loligo forbesi*. They reported that the liver contained iron, zinc, copper, and cadmium at levels of 52.2, 43.6, 110, and 1.5 mg/kg fresh weight, respectively. This wide range of mineral contents seen in livers in different studies may be related to factors such as species, geographical origin, seasonal variation, sex, age, size, and environmental and physiological variations. However, these experiments also show that squid liver, a seafood processing waste product, contains highly nutritional components that could be utilized further. Several studies have shown that squid processing waste also contains important substances such as minerals [3–6], proteinase enzyme [7–9], and organic acids [3].



**Fig. 4** The solubilities of magnesium, zinc, cadmium, copper, iron, and protein (SP) extracted from liver of Japanese common squid *Todarodes pacificus* using 0.9% NaCl (a) and 100 mM phosphate buffer, pH 7.4 (b). Open bars whole extract, filled bars low molecular weight fraction. Columns with different letters above them on the plot are significantly different ( $p < 0.05$ ; each column and bar above the column show the mean and SD, respectively;  $n = 3$ )

#### Solubilities of divalent minerals and protein at various pH values

The solvent/liver ratio affects the percentages of minerals and protein extracted. In our preliminary experiments, we tested various solvent/liver ratios from 2 to 100, and ultimately decided to use a solvent/liver ratio of 30. Ghimire et al. [18] used a ratio of 10, a similar ratio to that chosen by us.

The solubilities of divalent minerals (copper, iron, zinc, and cadmium) at high pH tended to be positively correlated with protein solubility. This indicates that mineral–protein complex formation may occur. Minerals can change their chemical form during and/or after processing or can interact with other compounds. As a consequence, their solubilities can increase or decrease depending on the treatment applied, which can influence their chemical form. Minerals in squid liver can change their chemical form depending on the pH employed, so they could interact with other compounds, such as proteins. Santoso et al. [19] reported that the solubility of magnesium and calcium from seaweed increased significantly after boiling it in acetic acid solution rather than boiling it in water or salt solution. It was also found that the presence of organic acid increases the solubilities of minerals [20, 29, 30]. Minerals in soluble form were also found in high concentrations in acidic foods such as yoghurt [31].

In our experiment, divalent minerals tended to be more soluble under both acidic and alkaline conditions. We presume that acid or alkali treatment can change the conformations of the minerals, making them more soluble. One possible way of improving mineral solubility is to bind the mineral with protein, establishing a soluble mineral–protein complex. In this case, the soluble protein of squid liver acts as a solubility enhancer. Clydesdale [22] defined an “enhancer” as a molecular species that binds with minerals to form compounds that are soluble and can be absorbed by mucosal cells. An enhancer may undergo cleavage to release the mineral in a soluble form, or its thermodynamic constants may lead to the transfer of the mineral to a mucosal acceptor.

#### Correlations of the solubilities of divalent minerals with the solubilities of cadmium and protein

Among the three trace minerals zinc, copper, and iron, zinc showed the highest correlation with cadmium, which may relate to its function in metallothioneins. Roesijadi [32] proposed a model for coupled metallothionein induction and the rescue of target ligands compromised by inappropriate metal binding. According to this hypothesis, the roles of cadmium and zinc are interchangeable. When nonessential metals such as cadmium, mercury, or silver enter a cell, there is inevitably competition between them and other minerals like copper and zinc for intracellular ligands, such as metalloproteins [33]. Therefore, cadmium can displace zinc as well as calcium [34]. Reeves and Chaneyb [35] reported that cadmium bioaccessibility was also related to the ingestion of other minerals such as calcium, zinc, and iron. Copper and zinc are essential trace metals that are required by a wide variety of metal-dependent enzymes. Cephalopods use the copper-containing protein hemocyanin as a respiratory pigment, so copper is required in large concentrations [36]. Therefore, copper and zinc are bound to either the particulate fraction or very low molecular weight species [5].

The correlations of the solubilities of divalent minerals with protein solubility at different pH values indicated that zinc, iron, and cadmium were extracted with protein at all pH values. However, at high pH, cadmium tended to be extracted with protein more than at low pH. As shown in Fig. 1, the percent of soluble Cd was almost the same as the percent of soluble protein at high pH. We assume that the proteins, in their metal-binding forms, dissolved in the liquid phase at high pH. Mineral-binding proteins known as metallothioneins are associated with the mechanism of metal detoxification in aquatic invertebrates [33, 37]. Trace minerals (copper, zinc, and cadmium) were found to bind strongly with squid protein, especially in the low molecular weight fraction [5]. Raimundo et al. [25] reported that the

strongly positive correlations among cadmium, zinc, copper, and low molecular weight protein point to the presence of metalloproteins with high affinities for these elements. Methallothioneins are low molecular weight proteins that can be induced by free cytosolic metal ions, especially cadmium, copper, zinc, and mercury, and are involved in defence against metal toxicity [4, 38, 39].

Solubilities of divalent minerals and protein in the de-fatted extract and the low molecular weight fraction

Although the use of a 1 % sodium chloride and boiling treatment can increase the solubilities of minerals from shellfish [40], this treatment decreases the solubilities of minerals from seaweeds [19]. This suggests that the solubilities of minerals depend on the treatment and the type of materials used. Aquatic organisms can remove nonessential metals such as cadmium, mercury, and silver using metal-binding sites of low molecular mass, such as those on metallothioneins, as part of the normal metabolism of the organism [33, 39]. Thus, almost all of the solubilized minerals from squid liver were found in the low molecular weight fraction (molecular weight = MW < 10,000). Low, intermediate, or high molecular weight proteins have been also found to act as potential binding sites for trace metals, including cadmium [4, 5]. In the present experiment, among the four trace minerals zinc, cadmium, copper, and iron, a significantly higher percentage of cadmium was found in the low molecular weight fraction. A similar result was also reported by Raimundo et al. [25]: cadmium extracted from the digestive gland of *Octopus vulgaris* exhibited a strong association with low molecular weight protein (LMW; 11,000–6,000 Da) and a weaker association with high molecular weight protein (HMW; 144,000–130,000 Da). Relatively high levels of magnesium were also found in low molecular weight fractions. This result is in accord with that of a study conducted by Suzuki et al. [40], in which a high percentage of the magnesium extracted from shellfish was found in the low molecular weight fraction. However, in seaweeds, high percentages of soluble minerals were found in the high molecular weight fraction (MW > 200,000) [19], because seaweeds contain high levels of dietary fiber and low levels of protein [41]. The dietary fiber in seaweed acts as an inhibitor of mineral solubilization (an inhibitor is a molecular species that forms an insoluble compound that cannot be absorbed or cleaved to release the mineral in a soluble form [22]).

According to the results of our study, liver of the Japanese common squid *Todarodes pacificus* contains high levels of nutritional compounds: fat (44.0 g/100 g dry matter) and protein (13.5 g/100 g dry matter). Low- and

high-pH (pH 2 and 12) treatments are able to remove cadmium entirely, although high percentages of soluble minerals and protein were found in the low molecular weight (MW < 10,000) fraction. The solubilities of zinc and copper were strongly related to the solubility of cadmium, but there was no relationship between the solubilities of magnesium and cadmium. In terms of methods for removing cadmium from squid liver, treatment with 0.9 % NaCl or with 100 mM phosphate buffer at pH 7.4 has several advantages: these treatments are more economical, environmentally friendly, and leave more useful compounds in the liver than treatment with hydrochloride acid or sodium hydroxide. When we filtered the solvent-treated liver, treatment with 0.9 % NaCl or 100 mM phosphate buffer at pH 7.4 gave 200–250 mg (dry basis) of de-oiled solid powder from 1 g of fresh liver. These two treatments may therefore be appropriate methods for removing cadmium from squid liver.

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