

# Commercial squids: Characterization, assessment of potential health benefits/risks and discrimination based on mineral, lipid and vitamin E concentrations

A. Torrinha, F. Gomes, M. Oliveira, R. Cruz, E. Mendes, C. Delerue-Matos, S. Casal, S. Morais

## ABSTRACT

The most consumed squid species worldwide were characterized regarding their concentrations of minerals, fatty acids, cholesterol and vitamin E. Interspecific comparisons were assessed among species and geographical origin. The health benefits derived from squid consumption were assessed based on daily minerals intake and on nutritional lipid quality indexes. Squids contribute significantly to daily intake of several macro (Na, K, Mg and P) and micronutrients (Cu, Zn and Ni). Despite their low fat concentration, they are rich in long-chain omega-3 fatty acids, particularly docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids, with highly favorable n-3/n-6 ratios (from 5.7 to 17.7), reducing the significance of their high cholesterol concentration (140–549 mg/100 g ww). Assessment of potential health risks based on minerals intake, non-carcinogenic and carcinogenic risks indicated that *Loligo gahi* (from Atlantic Ocean), *Loligo opalescens* (from Pacific Ocean) and *Loligo duvaucelii* (from Indic Ocean) should be eaten with moderation due to the high concentrations of Cu and/or Cd. Canonical discriminant analysis identified the major fatty acids (C14:0, C18:0, C18:1, C18:3n-3, C20:4n-6 and C22:5n-6), P, K, Cu and vitamin E as chemical discriminators for the selected species. These elements and compounds exhibited the potential to prove authenticity of the commercially relevant squid species.

**Keywords:** Squids Minerals, Polyunsaturated fatty acids Cholesterol, Health risks and benefits Chemometric discrimination

## 1. Introduction

Cephalopods are interesting marine organisms due to their nutritional, commercial and ecological importance. Mediterranean countries are top consumers of cephalopods being only equaled or surpassed by some Asian countries (FAO, 2012). Diets that include seafood on a regular basis may represent some health benefits regarding nutritionally relevant compounds such as minerals, omega-3 fatty acids and vitamin E. However, cephalopods contamination with aquatic pollutants, namely toxic metals has been reported as a consequence of environmental pollution (Galitsopoulou et al., 2009; Lourenço et al., 2009; Manso et al., 2007; Prafulla et al., 2001; Seixas et al., 2005; Storelli et al., 2010). In addition, cephalopods have poor metabolic capacity (Gomes et al., 2013; Semedo et al., 2012) which facilitates

bioaccumulation. Consequently, characterization of metals, fatty acids, vitamin E, and cholesterol levels in cephalopods is relevant from the nutritional and safety points of view, enabling a more balanced intake with potential health benefits.

Among cephalopods, squids represent an important food source, with *Loligo duvaucelii*, *Loligo gahi*, *Loligo reynaudii*, *Loligo opalescens* and *Loligo vulgaris* as the major squid species in terms of world trade and landing (FAO, 2010, 2011). However, there is quite limited information on the macro- and micronutrients, as well as toxic elements except for mercury (Cardoso et al., 2012), of other commercially important squid species than *L. vulgaris* (Lourenço et al., 2009; Manso et al., 2007; Olmedo et al., 2013; Storelli et al., 2010). Recently Cardoso et al. (2012) assessed the risks of methyl-mercury intake through cephalopods consumption in Portugal. Authors found that squid presents no health concern with respect to methyl-mercury. Moreover, the majority of the few studies available are related with environmental monitoring studies while in terms of food safety only the edible tissues should be analyzed (Falandysz, 1989; Galitsopoulou et al., 2009; Lourenço et al., 2009; Manso et al., 2007; Prafulla et al., 2001; Seixas et al., 2005).

Seafood polyunsaturated fatty acids (PUFA) are rich in long-chains of  $\omega$ -3 (Chow, 2008; Miliou et al., 2006; Navarro and Villanueva, 2000; Salman et al., 2007), with a recognized importance in the prevention of several of the modern health disorders (Russo, 2009). As a marine resource, squids are nutritionally valuable regarding their concentrations in docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), described as having hypotriacylglycerolemic, antiarrhythmic, antiatherogenic, antithrombotic and anti-inflammatory properties. Subsequently, EPA and DHA are considered as protective agents against coronary heart diseases (CHD) (Wijendran and Hayes, 2004).

Vitamin E is a lipid-soluble antioxidant agent known for its ability to protect sensitive molecules such as unsaturated fatty acids against oxidation, by its ability to neutralize free radicals and reduce lipid peroxidation (Pazos et al., 2005), with  $\alpha$ -tocopherol being the most bioactive component. In nature, it exists at high concentrations when  $\omega$ -3 PUFAs concentrations are also high (Chow, 2008).

Regarding cholesterol, and in comparison with other cephalopods, squids have a high concentration of this sterol in their composition (Oehlenschläger, 2006), which should not be neglected in nutritional studies due to its potential negative effect on blood cholesterol.

To the best of our knowledge, information about fatty acids composition, cholesterol and vitamin E concentrations in commercially available squids is scarce and even scarcer for squids other than the *L. vulgaris* species (Passi et al., 2002; Villanueva et al., 2009; Miliou et al., 2006; Navarro and Villanueva, 2000; Ozogul et al., 2008; Salman et al., 2007). Cholesterol concentration was previously described for *L. gahi* (García-Garrido et al., 2011; Valver-de et al., 2012) and *L. duvaucelii* (Mathew et al., 1999; Uddin et al., 2001), while vitamin E data were only found for *L. vulgaris* (Passi et al., 2002; Villanueva et al., 2009).

The present study aims to characterize and compare the most relevant commercial squid species in terms of their main essential elements (Ca, Mg, Na, K, P, Zn, Cu, Fe, Mn, Cr and Ni), toxic elements (Cd, Pb, As), fatty acids profile, cholesterol and vitamin E levels. The selected species are those commercially available worldwide, from the Indic, Atlantic and Pacific Oceans, and sold in the Portuguese market. As far as it is known, no previous study was published regarding a so extended nutritional characterization of squids. Another goal of this study was to assess the potential health benefits/risks for low and high squid consumer populations (FAO, 2012). This evaluation was based on the daily minerals intake, the nutritional lipid quality indexes, and on the non-carcinogenic target hazard quotient (THQ) and target carcinogenic risk (TR) established by the U.S. Environmental Protection Agency (US EPA, 2010). Furthermore, canonical discriminant analysis (CDA) was applied as pattern recognition technique to identify chemical descriptors for the selected species to allow authenticity control.

## 2. Material and methods

### 2.1. Reagents

A Milli-Q water purification system (Millipore, Molsheim, France) was used to obtain ultrapure water (resistivity of  $18.2 \text{ MO cm}^{-1}$ ) for quantitative analysis. Standard solutions of Ca (0.10–0.80 mg/L), Mg (0.05–0.35 mg/L), Zn (0.04–0.40 mg/L), Cu (2.50–22.5  $\mu\text{g/L}$ ), Fe (1.25–25.0  $\mu\text{g/L}$ ), Mn (2.50–30.0  $\mu\text{g/L}$ ), Cr (0.50–5.00  $\mu\text{g/L}$ ), Ni (2.00–20.0  $\mu\text{g/L}$ ), Cd (0.10–2.00  $\mu\text{g/L}$ ), Pb (1.00–20.0  $\mu\text{g/L}$ ) and As (10.0–60.0  $\mu\text{g/L}$ ) were prepared from their corresponding 1000 mg/L stock solutions (Panreac, Barcelona, Spain). K (0.10–1.40 mg/L), Na (0.10–1.20 mg/L) and P (2.00–28.0 mg/L) standard solutions were obtained by potassium chloride (99.5%, Riedel-de Haën, Seelze, Germany), sodium chloride (99.8%, Riedel-de Haën, Seelze, Germany) and potassium dihydrogen phosphate (99.5%, Riedel-de Haën, Seelze, Germany) dissolution in ultrapure water, respectively. Standards and samples were acidified with 1% (v/v) suprapur nitric acid (65%; Sigma–Aldrich, Steinheim, Germany). Cesium chloride (P99.5%; Sigma–Aldrich, Steinheim, Germany) was used as ionization chemical suppressor for the quantification by high-resolution contin-

uum source flame atomic absorption spectrometry (HR-CS-FAAS). The applied matrix modifiers for high-resolution continuum source graphite furnace atomic absorption spectrometry (HR-CS-GFAAS) determinations were 1% v/v  $\text{NH}_4\text{H}_2\text{PO}_4$  for Cd and Pb analyses, 1% v/v  $\text{Mg}(\text{NO}_3)_2$  for Cr and Fe, 0.05% v/v  $\text{Mg}(\text{NO}_3)_2$  for Ni and 0.1% v/v  $\text{Pd}(\text{NO}_3)_2/0.05\%$  v/v  $\text{Mg}(\text{NO}_3)_2$  for Mn and As. The color development reagent for P determination by spectrophotometry was prepared as described in 4500-P standard (Greenberg et al., 1992) by the addition of ammonium molybdate tetrahydrated (99.0%, Merck, Darmstadt, Germany) and ammonium metavanadate (99.0%, Merck, Darmstadt, Germany). All glassware and polyethylene materials were soaked in nitric acid (10% v/v) at least 24 h, thoroughly rinsed with ultrapure water and dried before use.

For cholesterol, vitamin E, fatty acids and total fat analysis, propan-2-ol, cyclohexane, and HPLC grade n-hexane were purchased from Merck (Darmstadt, Germany) while 1,4-dioxane was from Sigma–Aldrich (Steinheim, Germany). Methanol and potassium hydroxide were acquired from Panreac (Spain). Boron trifluoride in methanol (14%), butylated hydroxytoluene (BHT) and ascorbic acid were obtained from Sigma–Aldrich. The internal standard for fat estimation, triundecanoic acid, was also from Sigma–Aldrich (Steinheim, Germany), while the internal standard for vitamin E quantification, tocopherol, was obtained from Matreya (USA). Tocopherol and cholesterol standards were from Sigma–Aldrich (Steinheim, Germany) and the fatty acids methyl esters (FAMES) standard was from Supelco (Bellefonte, PA). All other chemicals were of analytical grade from diversified suppliers.

### 2.2. Sample collection and characterization

Squids from the different geographical origins available to Portuguese consumers were purchased randomly from the markets in NW region of Portugal during 2010 and 2011. The species collected were *L. duvaucelii* and *L. vulgaris* from the Indic Ocean, *L. reynaudii* and *L. gahi* from the Atlantic Ocean, and *L. opalescens* from the Pacific Ocean.

Sample collection, biometric characterization and preparation were performed in accordance with US EPA Guide No 823-B-00-07 and EC Regulation No. 333/2007 (European Commission, 2007). Specimens were carefully weighed, biometrically characterized (tentacle, mantle and total lengths) and manually eviscerated. The mean weight values were 24.5 g (*L. opalescens*) < 41.0 g (*L. gahi*) < 113.9 g (*L. vulgaris*) < 131.7 g (*L. duvaucelii*) < 185.7 g (*L. reynaudii*) while the mean total length order was 17.1 cm (*L. opalescens*) < 22.1 cm (*L. gahi*) < 27.2 cm (*L. vulgaris*) < 38.2 cm (*L. reynaudii*) < 45.8 cm (*L. duvaucelii*). These biometric parameters indicated that the species were all adults and have statistically significant different ( $p < 0.05$ ) weight (except between *L. duvaucelii* and *L. vulgaris*) and total length (except among *L. duvaucelii* and *L. reynaudii*).

Each sample for further analysis ( $n = 27$  for *L. duvaucelii* and *L. reynaudii*;  $n = 26$  for *L. vulgaris*;  $n = 59$  for *L. gahi*;  $n = 54$  for *L. opalescens*) consisted of an equal amount of the edible tissues (mantle and arms) of, at least, four individuals and had a minimum mass of 200 g. Homogenization was performed mechanically with a blender until a smooth paste was obtained. Samples were kept frozen in 50 mL polycarbonate containers at  $-20^\circ\text{C}$  until analysis. For each sample, three independent assays were performed. Moisture was determined by oven drying at  $100^\circ\text{C}$ .

### 2.3. Elemental analyses

Homogenized samples were accurately weighed (1.250–1.350 g) to Teflon vessels using a MS Analytical Balance (MS105, Mettler Toledo, Switzerland), and then dried in a microwave Mars-X 1500 W (Microwave Accelerated Reaction System for digestion and extraction, CEM Mathews, NC, USA) until three reproducible weight values were obtained. Ten milliliters mL of suprapur nitric acid (65%) was then added to each vessel, containing ca. 0.2 g (accurately weighed) of dry weight sample and the microwave digestion proceeded during different steps accordingly with Vieira et al. (2011). After digestion and cooling to ca.  $30^\circ\text{C}$ , samples were kept frozen in polycarbonate containers at  $-20^\circ\text{C}$  until analysis.

Ca, Mg, Na, K and Zn quantification were carried out using a High-Resolution Continuum Source Flame Atomic Absorption Spectrometer (Analytik Jena ContrAA 700 equipped with a xenon short-arc lamp XBO 301 (GLE, Berlin, Germany) with a nominal power of 300 W operating in a hot-spot mode as a continuum radiation source. Air–acetylene (Air Liquid, Portugal) oxidizing flame was used for atomization.

Cd, Pb, Fe, Mn, Cu, Cr, Ni and As quantification were analysed by using the same equipment but with the Graphite Furnace module equipped with an MPE60 auto-sampler (Analytik Jena, Germany) and argon as inert gas. Transversally coated graphite tubes with integrated platform were used. In order to obtain maximum absorbance and minimum background values, the operational parameters were optimized and are presented in Table 1S (Supplementary material). P measurements were performed in a dual beam UV visible spectrophotometer (Evolution 300, Thermo Scientific, USA) according to 4500-P standard at 420 nm (Greenberg et al., 1992). The analytical characteristics such as calibration data and detection limits were evaluated for each element (Table 2S, Supplementary material). Calibration curves showed good linearity over the entire range of concentrations with acceptable coefficients ( $r^2$ ) (Table 2S). Limits of detection (LODs) were defined and determined as the minimum detectable amounts of analyte with a signal-to-noise ratio of 3:1 (Miller and Miller, 2000). The attained LODs are sufficiently

low for cephalopods safety monitoring purposes considering the maximum heavy metal levels established in cephalopods (without viscera) by the European Commission Regulation (European Commission, 2006; 1.0 mg/kg wet weight (ww) for Cd and Pb). All measurements were performed, at least, in triplicate. Analytical blanks and standards were analysed daily and regularly along with samples to check instrument performance. Elemental concentrations were determined on wet (ww) and dry weight bases but to simplify the results, concentrations are discussed

in terms of ww.

$$HH = \frac{C18:1\omega-9 + C18:2\omega-6 + C20:4\omega-6 + C18:3\omega-3 + C20:5\omega-3 + C22:5\omega-3 + C22:6\omega-3}{C14:0 + C16:0} \quad (3)$$

#### 2.4. Fatty acids, total fat, cholesterol and vitamin E analysis

For lipid extraction and subsequent analysis, sample portions were lyophilized (Telstar Cryodos-80, Terrassa, Barcelona), preserved in the dark at 4 °C and finely ground before analysis. Lipids were extracted in accordance with Cruz et al. (2013). Briefly, an accurate sample portion (300 mg) was extracted with a ternary mixture of propan-2-ol, cyclohexane and 0.9% (m/v) KCl in the presence of the internal standards (tocol and triundecanoate), plus antioxidants (ascorbic acid and BHT). One third of the organic extract was used for vitamin E analysis by HPLC, after being taken to dryness under a gentle nitrogen stream and reconstituted in n-hexane. The remaining extract was hydrolyzed with KOH (0.5 mol/L in methanol) at 100 °C (10 min) and further methylated with BF<sub>3</sub> (30 min at 100 °C) to convert the acylglycerols and free fatty acids in the samples to volatile FAMES. The lipids were extracted with n-hexane and the solution was used initially for cholesterol quantification by HPLC, and thereafter for fatty acids analysis by GC-FID.

##### 2.4.1. HPLC chromatographic conditions for cholesterol and vitamin E

The liquid chromatograph consisted of a Jasco integrated system (Japan) equipped with an LC-NetII/ADC data unit, a refrigerated autosampler (AS-2057 Plus), a PU-980 Intelligent Pump, and a multiwavelength DAD (MD-910, recorded at 210 nm), connected in series to a FD (FP-2020 Plus;  $k_{exc} = 290$  nm and  $k_{em} = 330$  nm; gain, 10). The chromatographic separation was achieved on a Supelcosil™ LC-SI column (75 × 3.0 mm, 3 μm; Supelco, Bellefonte, PA) operating at constant room temperature (23 °C). A mixture of n-hexane and 1,4-dioxane (97.5:2.5, v/v) was used as eluent at a flow rate of 0.8 mL/min. Data were analyzed with the ChromNAV

Control Center – JASCO Chromatography Data Station (Japan). The compounds were identified by chromatographic comparisons with authentic standards, by co-elution, and by their UV spectra. Cholesterol was quantified at 210 nm, with calibration based on external standard solutions. Vitamin E was evaluated by the internal standard method based on the fluorescence data.

##### 2.4.2. Gas chromatographic conditions for fatty acids and total fat analysis

Gas chromatography was performed on a Chrompack CP 9001 chromatograph (Chrompack, Middelburg, the Netherlands) equipped with a split – splitless injector, a flame-ionization detector, and a Chrompack CP-9050 autosampler. The temperatures of the injector and detector were 250 and 270 °C, respectively. Separation was achieved on a 50 m × 0.25 mm i.d. CP-Sil 88 column (0.19 μm film; Chrompack-Varian), using temperature program from 140 °C to 220 °C. Helium was used as the carrier gas at an internal pressure of 120 kPa. Fatty acid identification (from C14:0 to C22:6x-3) was accomplished by comparing the relative retention times of FAMES of commercially available fatty acids methyl esters (Supelco 37-FAME mix). Fatty acids were initially quantified on a g/100 g fatty acids basis and then converted to mg/100 g ww using the fatty acid internal standard (C11:0) and moisture content. Saturated fatty acids (SFA) include 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, and 22:0, monounsaturated fatty acids (MUFA) include 16:1 isomers (x-7 plus x-9), 18:1 (x-7 and x-9), 20:1x-9, 22:1x-9 and 24:1x-9 while PUFA include 18:2x-6, 20:2x-6, 20:3x-6, 20:4x-6, 22:4x-6, 18:3x-3, 20:5x-3, 22:2x-6, 22:5x-3 and 22:6x-3, divided into omega-3 and omega-6 fatty acids, respectively. Total fatty acids content was estimated on the basis of the total fatty acid methyl ester area counts in comparison with the undecanoic methyl ester by converting the FAMES to their respective fatty acid equivalents using the appropriate conversion factors.

#### 2.5. Estimation of potential public health benefits and risks

The health benefits derived from squid consumption were assessed based on the daily minerals intake and on the quality of their lipids, particularly regarding the long-chain PUFA concentrations. Therefore, important nutritional lipid quality indexes such as total x-3/x-6, DHA + EPA sum, atherogenicity index (AI), thromb-

ogenicity index (TI) and hypocholesterolemic:hypercholesterolemic ratio (HH) were determined (Guimarães et al., 2013). AI, TI and HH indexes were calculated using the Eqs. (1)–(3), respectively:

$$AI = \frac{C12:0 + 4 \times C14:0 + C16:0}{\sum MUFA + \sum \omega-3 + \sum \omega-6} \quad (1)$$

$$TI = \frac{C14:0 + C16:0 + C18:0}{0.5 \times \sum MUFA + 0.5 \times \sum \omega-6 + 3 \times \sum \omega-3} \quad (2)$$

(3)

The non-carcinogenic and carcinogenic risks through elemental intake were estimated in accordance with the methodology provided in the US EPA Region III Risk-based Concentration table (US EPA, 2010). One age-category P 21 years adult (70 kg) was used. The exposure duration (ED) value used in the intake calculations was 70 years. The non-carcinogenic risks for each individual metal through fish consumption were assessed by the THQ (US EPA, 1989): “the ratio of a single substance exposure level over a specified time period (e.g., subchronic) to a reference dose (RfD) for that substance derived from a similar exposure period”. THQ assumes a level of exposure (i.e., RfD) below which it is unlikely for even sensitive populations to experience adverse health effects. If the exposure level (E) exceeds this threshold (i.e., if THQ = E/RfD exceeds unity), there may be concern for potential non-carcinogenic effects. Higher THQ values mean a higher probability of experiencing long term non-carcinogenic effects.

For carcinogens, TR were estimated as the incremental probability of an individual to develop cancer, over a lifetime, as a result of exposure to that potential carcinogen (i.e., incremental or excess individual lifetime cancer risk; US EPA, 1989). Acceptable risk levels for carcinogens range from 10<sup>-4</sup> (risk of developing cancer over a human lifetime is 1 in 10,000) to 10<sup>-6</sup> (risk of developing cancer over a human lifetime is 1 in 1,000,000).

THQ and TR approaches (US EPA, 2010) were calculated using Eqs. (4) and (5), respectively:

$$THQ = (Efr \times ED \times IR \times C) / (RfD \times BW \times AT) \quad (4)$$

$$TR = (Efr \times ED \times IR \times C \times CSFo) / (BW \times AT) \quad (5)$$

where Efr is the exposure frequency (350 days per year); ED is the exposure duration; IR is the food ingestion rate (1.37 × 10<sup>-3</sup> and 1.12 × 10<sup>-2</sup> kg per day per person for World and for Portuguese population, respectively; FAO, 2012); C is the metal concentration in cephalopods (lg/g); RfD is the oral reference dose (0.001 lg/g/day for Cd, 0.004 lg/g/day for Pb, 0.0030 lg/g/day for Cr (VI), 0.04 lg/g/day for Cu, 0.3 lg/g/day for Zn, 0.02 lg/g/day for Ni, 0.7 lg/g/day for Fe and 0.02 lg/g/day for Mn; US EPA, 2010); BW is the body weight (kg) and AT is the number of days over which the exposure is averaged (ED years × 365 days/year for non-carcinogenic effects and 70 years × 365 days/year for carcinogenic effects); CSFo is the oral carcinogenic slope factor from the Integrated Risk Information System database (8.5 × 10<sup>-3</sup> (lg/g/day)<sup>-1</sup> for Pb) (US EPA, 2010). THQ and TR are dimensionless. Total THQ (TTHQ) for each species is the sum of individual metal THQ values.

#### 2.6. Statistical analysis

Statistical analysis was performed using the SPSS (IBM SPSS Statistics 20) and Statistica software (v. 7, StatSoft Inc., USA). Data were expressed as median and range and were grouped according to species and origins. The non-parametric tests Kruskal–Wallis and Mann–Whitney U-test using Bonferroni corrections for multiple comparisons were applied due to non-normal distribution of the data. Statistical significance was defined as *p* < 0.05 (*p*-value at 95% confidence level).

CDA was performed to evaluate which variables may be used as chemical descriptors between the five species and/or three origins.

### 3. Results

#### 3.1. Elements in edible tissues of squids

Median, 25% and 75%, percentiles minimum and maximum amounts of the 13 detected elements in the edible tissues of the selected squid species are presented in Fig. 1. Data are organized based on the essentiality and levels of the elements, i.e., as macro

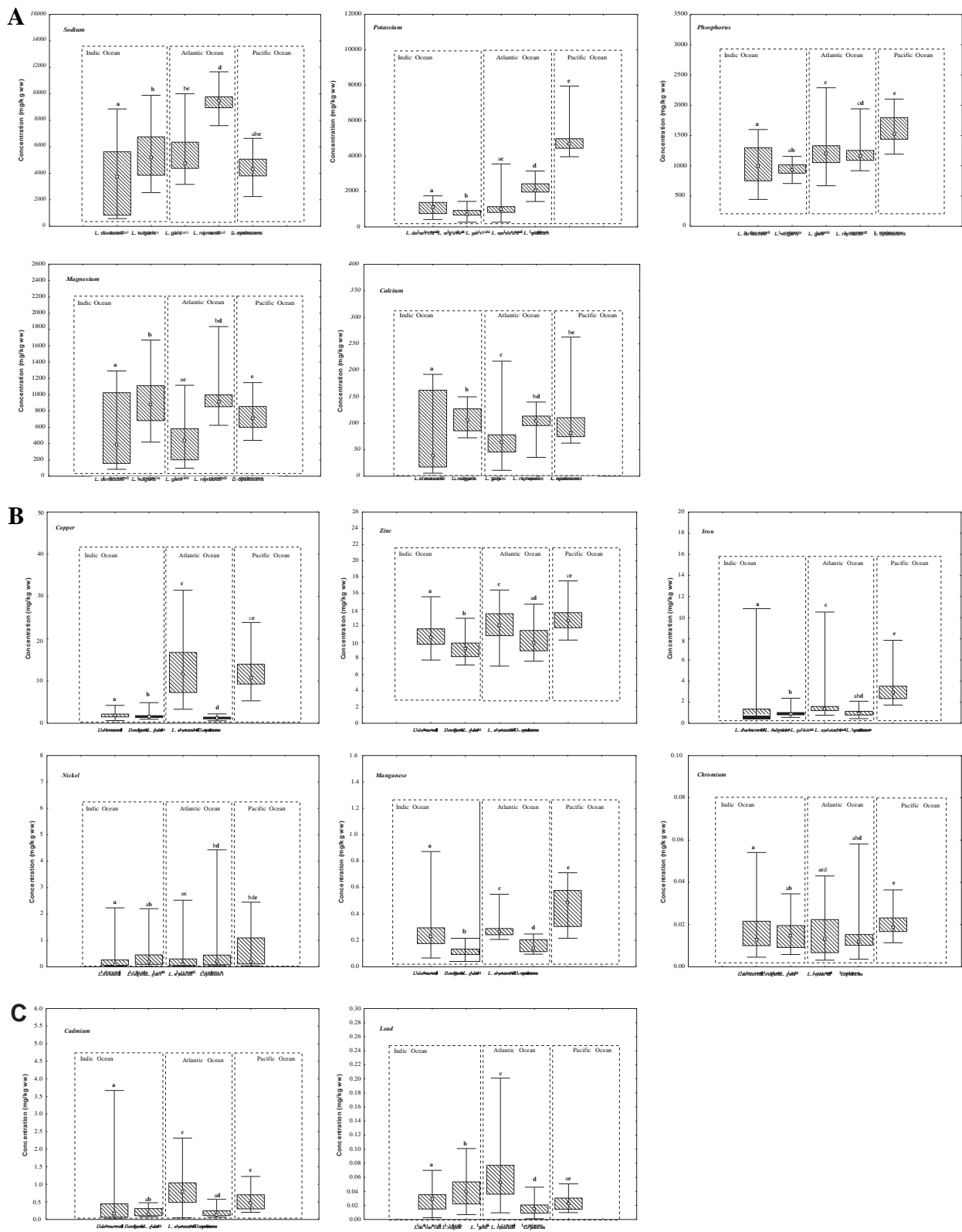


Fig. 1. Concentrations (h Median, 25–75% and range, mg/kg ww) of (A) macronutrients, (B) micronutrients and (C) toxic elements in the edible tissues of the characterized squid species. Distributions with different letters (a–e) are statistically different at  $p < 0.001$ .

(Fig. 1A), minor (Fig. 1B) and toxic elements (Fig. 1C). The concentrations of arsenic are not presented since all samples displayed values lower than the attained limit of detection (Table 2S) which

is in accordance with literature data that indicate very low As amounts for the only characterized species, *L. vulgaris* (Cirillo et al., 2010).

### 3.1.1. Macronutrients concentration

Na, K, P, Mg and Ca are macroelements that are critical for biological functions in the human body. The series that describe the interspecies comparison for each element median level are the followings: *L. reynaudii* > *L. vulgaris* > *L. gahi* > *L. opalescens* > *L. duvaucelii* for Na; *L. opalescens* > *L. reynaudii* > *L. gahi* > *L. duvaucelii* > *L. vulgaris* for K; *L. opalescens* > *L. reynaudii* > *L. gahi* > *L. duvaucelii* > *L. vulgaris* for P; *L. reynaudii* > *L. vulgaris* > *L. opalescens* > *L. duvaucelii* > *L. gahi* for Mg; *L. vulgaris* > *L. reynaudii* > *L. opalescens* > *L. gahi* > *L. duvaucelii* for Ca (Fig. 1A). Similar patterns among species were observed for K and P. *L. opalescens* presented the upper concentrations of these elements (4695 mg/kg ww for K and 1526 mg/kg ww for P) while the highest median levels of Na (9484 mg/kg ww) and Mg (914 mg/kg ww) were detected in

*L. reynaudii*. *L. vulgaris* and *L. reynaudii* showed the highest and very similar concentrations of Ca (105 mg/kg ww for *L. reynaudii* and 106 mg/kg ww for *L. vulgaris*).

*L. opalescens*, which was the only studied species from the Pacific Ocean, exhibited statistical significant differences ( $p < 0.001$ ) with all the other species for all the macro-elements except with

*L. vulgaris* for Na and Ca and *L. duvaucelii* for Na (Fig. 1A). Significant interspecific differences ( $p < 0.001$ ) were also observed between the two species from the Indic Ocean (*L. duvaucelii* and *L. vulgaris*) and also among those from the Atlantic Ocean (*L. gahi* and *L. reynaudii*) for all the elements except P.

The median content of each metal by species can be represented by Na > K > P > Mg > Ca for *L. duvaucelii* and *L. gahi*; Na > K > P > Mg > Ca for *L. reynaudii*; Na > P > Mg > K > Ca for *L. vulgaris*, and K > Na > P > Mg > Ca for *L. opalescens*. The most different profiles correspond to *L. opalescens*, and also to *L. vulgaris* but in a lesser extent. The concentrations of these minerals reflect the biological characteristics of each species but also the environmental growing conditions.

### 3.1.2. Micronutrients concentration

The concentrations of Zn, Cu, Fe, Mn, Cr and Ni are displayed in Fig. 1B. As in the case of the macroelements K and P, *L. opalescens* had the highest concentration values of the analysed elements (12.61 (10.25–17.53) mg/kg ww for Zn, 2.97 (1.73–7.86) mg/kg ww for Fe, 0.49 (0.22–0.71) mg/kg ww for Mn and 0.019 (0.011–0.036) mg/kg ww for Cr. *L. vulgaris* was the poorest species regarding median concentrations of Zn, Fe and Mn. All the characterized species had significant interspecific differences ( $p < 0.001$ ) concerning their Mn concentrations. Similarly with the statistical results of the macronutrients, the two species from the Indic Ocean (*L. duvaucelii* and *L. vulgaris*) and also those from the Atlantic Ocean (*L. gahi* and *L. reynaudii*) presented significant differences ( $p < 0.001$ ) among them for all the elements except for Ni and Cr. In accordance with Ca and Mg, no significant differences were observed between *L. vulgaris* and *L. reynaudii* ( $p = 0.269$ ) for iron levels.

The order that describe the micro-nutrient concentrations in the analyzed species are: Zn > Cu > Fe > Mn > Ni > Cr for *L. duvaucelii*, Zn > Cu > Fe > Ni > Mn > Cr for *L. vulgaris* and *L. reynaudii*; Zn > Cu > Fe > Mn > Ni > Cr for *L. gahi* and *L. opalescens*. Zn and Cu were the highest concentration elements ranging from 7.05 (*L. gahi*) to 17.53 mg/kg ww (*L. opalescens*), and 0.60 (*L. reynaudii*) to 31.50 (*L. gahi*) mg/kg ww, respectively. Cr was detected globally in similar mean levels but in a quite wide concentration range (from 0.003 for *L. gahi* to 0.058 mg/kg ww for *L. reynaudii*). Ni levels showed the higher variability ranging about 400 times between the minimum detected in *L. vulgaris* (0.011 mg/kg ww) and the maximum values (4.43 mg/kg ww) in *L. reynaudii*.

### 3.1.3. Concentrations of toxic elements

The concentrations of Cd and Pb are exhibited in Fig. 1C. The concentrations of Cd were approximately one order of magnitude

greater than those of Pb. The orders that represent the interspecies comparison are: *L. gahi* > *L. opalescens* > *L. duvaucelii* > *L. vulgaris* > *L. reynaudii* for Cd and *L. gahi* > *L. vulgaris* > *L. duvaucelii* > *L. opalescens* > *L. reynaudii* for Pb. *L. gahi* was the most contaminated species (0.79 (0.047–2.32) mg/kg ww for Cd and 0.053 (0.010–0.20) mg/kg ww for Pb) while *L. reynaudii* (0.14 (0.065–0.57) mg/kg ww for Cd and 0.015 (0.001–0.046) mg/kg ww for Pb) was the less contaminated one.

In relation to Cd concentrations, statistically significant differences ( $p < 0.001$ ) were verified between all species except *L. duvaucelii* and *L. vulgaris* (from the same ocean), and *L. duvaucelii* and *L. reynaudii* (from different geographical origin). Regarding Pb, only *L. duvaucelii* and *L. opalescens* presented similarities ( $p = 0.089$ ).

### 3.2. Total fat and fatty acids in edible tissues of squids

The trend of total fatty acids median was *L. opalescens* > *L. gahi* > *L. duvaucelii* > *L. vulgaris* > *L. reynaudii*, with the highest concentration reaching 1.22 mg/100 g ww and the lowest 0.84 mg/100 g ww (Table 1). Significant differences were observed between

*L. gahi* and *L. duvaucelii* and *L. vulgaris*. No statistical differences were verified between *L. vulgaris*, *L. duvaucelii* and *L. reynaudii* as well as between *L. opalescens* and *L. gahi*.

The fatty acid concentrations (as relative percentage of total fatty acids) in the five characterized species were: SFA 34.9%, MUFA 9.9% and PUFA 55.2% for *L. duvaucelii*; SFA 34.3%, MUFA 9.3% and PUFA 56.4% for *L. vulgaris*; SFA 32.0%, MUFA 10.7% and PUFA 57.3% for *L. gahi*; SFA 33.5%, MUFA 10.1%, and PUFA 56.4% for *L. reynaudii*; SFA 30.9%, MUFA 9.6% and PUFA 59.5% for *L. opalescens*.

Regarding the fatty acids composition on an edible basis (mg/100 g ww) only the ones with more quantitatively/nutritional importance are detailed (Table 1).

PUFA ranged from 327 mg/100 g ww (52.2% of total fatty acids) for *L. gahi* to 903 mg/100 g (63.7% of total fatty acids) for *L. opalescens*. *L. duvaucelii* was not statistically different from *L. vulgaris* (which are both from the same ocean) and *L. reynaudii* (from another ocean), being all significantly different ( $p < 0.05$ ) from *L.*

*opalescens*. DHA (C22:6x-3) varied from 210 mg/100 g ww for *L. duvaucelii* and *L. gahi* to 612 mg/100 g ww for *L. opalescens* and presented the same statistical pattern as total PUFA. EPA (C20:5x-3) concentrations ranged from 68 mg/100 g ww for *L. vulgaris* to 244 mg/100 g ww for *L. opalescens*. Statistical similarities were detected between *L. duvaucelii* and *L. vulgaris*, as well as among *L. gahi* and *L. reynaudii* and *L. opalescens*.

SFA concentrations varied from 197 mg/100 g ww (29.5%) for *L. gahi* to 482 mg/100 g ww (35.4%) for *L. opalescens* (*L. opalescens* > *L. gahi* > *L. duvaucelii* > *L. vulgaris* > *L. reynaudii*). Statistical differences were verified among squid species except between *L. duvaucelii* and the other species ( $p = 0.174$ ), *L. opalescens* and *L. gahi* ( $p = 0.086$ ), as well as *L. vulgaris* and *L. reynaudii* and *L. gahi*. The major fatty acid was palmitic acid (C16:0) that ranged from 137 mg/100 g ww (20.7%) for *L. vulgaris* to 336 mg/100 g ww (22.8%) for *L. opalescens* and presented the following order of median concentrations: *L. gahi* > *L. opalescens* > *L. duvaucelii* > *L. reynaudii* > *L. vulgaris*. Statistical differences ( $p < 0.05$ ) were observed between *L. gahi* and the other species with the exception of *L. opalescens* and *L. reynaudii*, as well as among *L. opalescens* and the other squids.

MUFA global median values ranged from  $82 \pm 11$  mg/100 g ww (9.2  $\pm$  0.7%) for *L. vulgaris* to  $119 \pm 19$  mg/100 g ww (10.6  $\pm$  1.8%) for *L. gahi*. Statistical differences ( $p < 0.05$ ) were observed between *L. gahi* and the other species with the exception of *L. opalescens*, as well as among *L. opalescens* and *L. duvaucelii*, *L. vulgaris* and *L. reynaudii*. C18:1, which is the sum of C18:1x-7 and C18:1x-9, con-

Table 1

Fatty acids composition (as mg/100 g), total fatty acids, cholesterol and vitamin E concentrations (median  $\pm$  standard deviation and range; wet weight) of the edible tissues of the characterized squid species. Each letter (a–e) corresponds to a species (a – *L. duvaucelii*, b – *L. vulgaris*, c – *L. gahi*, d – *L. reynaudii* and e – *L. opalescens*) and the same letters in a row indicate that the given means are not statistically significant ( $p > 0.05$ ).

	Indic Ocean		Atlantic Ocean		Pacific Ocean
	<i>Loligo duvaucelii</i>	<i>Loligo vulgaris</i>	<i>Loligo gahi</i>	<i>Loligo reynaudii</i>	<i>Loligo opalescens</i>
Total fat g/100 g ww	0.93 $\pm$ 0.18a (0.63–1.18)	0.89 $\pm$ 0.13 ab (0.63–1.06)	1.12 $\pm$ 0.23 c (0.60–1.40)	0.84 $\pm$ 0.19 abcd (0.68–1.17)	1.22 $\pm$ 0.18 ce (0.96–1.53)
C14:0	22 $\pm$ 6a (15–34)	19 $\pm$ 5 ab (14–31)	28 $\pm$ 5 ac (19–36)	21 $\pm$ 7 abd (14–39)	24 $\pm$ 8 acde (19–48)
C16:0	213 $\pm$ 38a (141–252)	185 $\pm$ 30 ab (137–238)	262 $\pm$ 45 c (157–322)	198 $\pm$ 43abcd (159–273)	248 $\pm$ 42 cde (212–336)
C18:0	74 $\pm$ 14 a (50–93)	70 $\pm$ 11 ab (48–86)	52 $\pm$ 14 c (17–63)	55 $\pm$ 9cd (42–66)	67 $\pm$ 12 abe (50–91)
SFA <sup>a</sup>	330 $\pm$ 57 a (227–387)	297 $\pm$ 46 ab (216–373)	355 $\pm$ 68 ac (197–436)	282 $\pm$ 59 abd (230–393)	378 $\pm$ 54 ace (306–482)
C16:1	11 $\pm$ 2 a (8–14)	10 $\pm$ 2 ab (8–13)	11 $\pm$ 2 abc (8–13)	11 $\pm$ 3abcd (8–19)	11 $\pm$ 3abcde (7–16)
C18:1	40 $\pm$ 14 a (28–76)	34 $\pm$ 5 ab (29–48)	53 $\pm$ 11 ac (37–73)	39 $\pm$ 8 abd (25–52)	45 $\pm$ 11 acde (35–70)
C20:1	13 $\pm$ 4 a (9–24)	13 $\pm$ 3 ab (9–19)	38 $\pm$ 6 c (28–49)	24 $\pm$ 6 d (19–38)	40 $\pm$ 9 ce (34–60)
MUFA <sup>b</sup>	83 $\pm$ 21 a (61–130)	82 $\pm$ 11 ab (62–95)	119 $\pm$ 19 c (79–148)	87 $\pm$ 19 abd (65–125)	116 $\pm$ 17 ce (95–153)
C18:2x-6	3.7 $\pm$ 2.7 a (2.5–9.4)	3.2 $\pm$ 0.8 ab (2.5–5.1)	6.3 $\pm$ 1.4 ac (3.7–8.7)	6.8 $\pm$ 2.4 acd (3.0–12)	6.7 $\pm$ 1.9 acde (4.0–11)
C20:2x-6	2.2 $\pm$ 0.6 a (1.3–3.3)	2.0 $\pm$ 0.4 ab (1.0–2.5)	4.3 $\pm$ 1.0 c (3.2–6.8)	2.8 $\pm$ 1.2 ad (1.5–6.5)	4.1 $\pm$ 0.9 ce (2.9–5.5)
C20:4x-6	48 $\pm$ 13 a (25–69)	45 $\pm$ 8 ab (33–59)	18 $\pm$ 7 c (6–31)	12 $\pm$ 4 cd (8–22)	27 $\pm$ 7 e (19–41)
C22:5x-6	12.7 $\pm$ 2.4 a (8.3–16.7)	12.1 $\pm$ 1.9 ab (8.6–15)	3.6 $\pm$ 1.8 c (0.8–6.3)	3.8 $\pm$ 1.0 cd (2.6–5.9)	5.5 $\pm$ 1.9 cde (2.0–8.9)
x-6PUFA	72 $\pm$ 18 a (42–101)	67 $\pm$ 10 ab (50–84)	35 $\pm$ 10 c (16–53)	27 $\pm$ 7 cd (19–44)	47 $\pm$ 10 e (32–67)
C18:3x-3	1.7 $\pm$ 0.6 a (1.4–2.9)	2.4 $\pm$ 0.7 ab (1.6–3.9)	2.5 $\pm$ 1.3 abc (1.0–5.6)	1.7 $\pm$ 0.5 abcd (1.4–2.9)	4.5 $\pm$ 0.8 e (2.9–5.8)
C20:5x-3 (EPA)	102 $\pm$ 26 a (69–155)	98 $\pm$ 18 ab (68–120)	178 $\pm$ 36 c (87–212)	128 $\pm$ 32cd (101–187)	184 $\pm$ 30 ce (142–244)
C22:5x-3	13.4 $\pm$ 2.5 a (8.0–18.7)	10.9 $\pm$ 2.1 ab (7.7–15.9)	5.7 $\pm$ 1.4 c (3.0–8.3)	5.6 $\pm$ 1.8 cd (3.9–9.4)	5.1 $\pm$ 2.1 cde (3.0–12)
C22:6x-3 (DHA)	306 $\pm$ 59 a (210–393)	314 $\pm$ 51 ab (217–382)	430 $\pm$ 102 c (210–566)	300 $\pm$ 68 abcd (253–428)	491 $\pm$ 82 ce (332–612)
x-3PUFA	426 $\pm$ 85 a (292–564)	426 $\pm$ 70 ab (295–516)	612 $\pm$ 138 c (309–789)	438 $\pm$ 103 abcd (360–629)	694 $\pm$ 111 ce (481–844)
PUFA <sup>c</sup>	503 $\pm$ 102 a (336–668)	499 $\pm$ 78 ab (346–588)	652 $\pm$ 147 c (327–844)	464 $\pm$ 110 abcd (382–664)	751 $\pm$ 120 ce (517–903)
x-3/x-6	5.6 $\pm$ 0.7 a (4.9–7.1)	6.1 $\pm$ 0.6 ab (5.6–7.7)	17.6 $\pm$ 2.2 c (14.6–22.3)	17.7 $\pm$ 1.9 cd (13.8–20.3)	14.7 $\pm$ 1.1 e (12.3–16.3)
AI <sup>d</sup>	0.50 $\pm$ 0.02 a (0.48–0.54)	0.48 $\pm$ 0.03 b (0.44–0.53)	0.49 $\pm$ 0.05 abc (0.42–0.61)	0.50 $\pm$ 0.03 abcd (0.44–0.57)	0.44 $\pm$ 0.04 e (0.37–0.48)
TI <sup>e</sup>	0.23 $\pm$ 0.01 a (0.20–0.24)	0.22 $\pm$ 0.01 ab (0.19–0.23)	0.17 $\pm$ 0.01 c (0.15–0.20)	0.18 $\pm$ 0.01 cd (0.17–0.20)	0.16 $\pm$ 0.02 cde (0.14–0.21)
HH <sup>f</sup>	2.3 $\pm$ 0.4 a (0.8–2.5)	2.4 $\pm$ 0.2 ab (1.9–2.6)	2.4 $\pm$ 0.6 abc (0.8–2.7)	2.2 $\pm$ 0.7 acd (0.8–2.4)	2.5 $\pm$ 0.6 bce (0.9–3.0)
Cholesterol mg/100 g ww	191 $\pm$ 21 a (146–219)	209 $\pm$ 29 ab (140–241)	239 $\pm$ 45 bc (167–307)	170 $\pm$ 26 abd (148–230)	412 $\pm$ 77 e (290–549)
Vitamin E mg/100 g ww	0.76 $\pm$ 0.41 a (0.47–1.79)	0.81 $\pm$ 0.18 ab (0.35–0.97)	1.6 $\pm$ 0.3 c (1.2–2.4)	1.5 $\pm$ 0.2 cd (1.2–1.9)	3.0 $\pm$ 0.4 e (2.2–3.4)

<sup>a</sup> SFA = saturated fatty acids.

<sup>b</sup> MUFA = monounsaturated fatty acids.

<sup>c</sup> PUFA = polyunsaturated fatty acids.

<sup>d</sup> AI = atherogenic index.

<sup>e</sup> TI = thrombogenic index.

<sup>f</sup> HH = hypocholesterolaemic/hypercholesterolaemic ratio.

tributed the most for the MUFA ranging from 25 mg/100 g ww (3.57%; *L. reynaudii*) to 76 mg/100 g ww (6.67%; *L. duvaucelii*).

### 3.3. Cholesterol concentrations

The order that describes the cholesterol median concentration by species is *L. opalescens* > *L. gahi* > *L. vulgaris* > *L. duvaucelii* > *L. reynaudii* (Table 1). Levels ranged from 140 (*L. vulgaris*) to 549 mg/100 g ww (*L. opalescens*). No statistical differences were

observed between *L. vulgaris*, *L. duvaucelii* and *L. reynaudii* while *L. opalescens* presented significant differences with the others.

### 3.4. Vitamin E concentrations

The order that describes the vitamin E concentration by species is *L. opalescens* > *L. gahi* > *L. reynaudii* > *L. vulgaris* > *L. duvaucelii*. Overall, vitamin E median values ranged from 0.76  $\pm$  0.41 mg/100 g ww for *L. duvaucelii* to 3.0  $\pm$  0.4 mg/100 g ww for *L. opales-*

cens (Table 1). A strong similarity was found between *L. vulgaris* and *L. duvaucelii* ( $p = 0.97$ ). Also, *L. gahi* showed no statistical differences with *L. reynaudii* ( $p = 0.58$ ), both from the Atlantic Ocean.

### 3.5. Estimation of potential public health benefits and risks

Recommended daily allowances (RDA) are regularly updated by health authorities for each element, on the basis on scientific evidences or, when inconsistent, adequate intakes (AI), being this the actual case for Na, K, P, Mg, Ca, Cu, Zn, Fe, Mn and Cr (European Commission, 2008; EMEA, 2008; FNIC, 2010). Ni, although recently included in the list of essential minerals, should be observed with caution due to its toxicity, with recommendation regarding restricted tolerable upper intake level (UL) (EMEA, 2008; FNIC, 2010). These guidelines represent intakes where there is essentially no risk, as far as it can be judged from the available scientific evidence.

The daily mineral intakes (DMI,%) were estimated based on the consumption of 100 g portion of fresh squids and on the RDA, AI and UL (European Commission, 2008; EMEA, 2008; FNIC, 2010) (Table 2). The attained ranges are, by descending order, 12.6–116.2% for Cu > 25.0–63.2% for Na > 10.2–24.4% for Mg > 13.4–

21.8% for P > 3.6–23.5% for K > 9.2–12.6% for Zn > 2.8–6.9% for Ni > 3.0–4.8% for Cr > 0.6–2.4% for Mn > 0.6–2.1% for Fe > 0.5–1.3%

for Ca. The richest species is *L. opalescens* (1.0% for Ca to 118.4% for Cu) and the poorest is *L. vulgaris* (0.6% for Mn to 34.9% for Na). Concerning Cd and Pb, the respective estimated mean monthly and daily intakes were calculated based on world (0.5 kg *per capita* per year) and Portuguese (4.1 kg *per capita* per year) cephalopod consumptions (FAO, 2012) since no specific data concerning squids were found. These values characterize well the potential public health risks for low (France, Russian Federation, U.S.A, Chile and Indonesia) and top (Mediterranean and Asian countries) consumers (FAO, 2012). Furthermore, since the analyzed squid species are those commercially existing in Portugal, risk assessment based on the Portuguese consumption is pertinent.

In view of the long half-life of Cd, the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2011) decided recently to express its tolerable intake as a monthly value in the form of a PTMI. The reached estimated intakes were clearly below the respective PTMI established (25 µg/kg body weight) (Table 3) (JECFA, 2011).

Regarding Pb, the previous PTWI (25 µg/kg body weight) was recently withdrawn (JECFA, 2011). The JECFA also concluded that it was not possible to establish a new PTWI that would be considered to be health protective. The attained dietary Pb exposure esti-

Table 2  
Recommended daily allowance (RDA; mg/day)/adequate intake (AI; mg/day) and estimated daily mineral intake (DMI, %) derived from the consumption of squid (100 g).

Element	RDA/ AI <sup>a</sup> (mg/ day)	DMI (%) <sup>a</sup>				
		(Indic Ocean)		(Atlantic Ocean)		(Pacific Ocean)
		<i>Loligo</i>	<i>Loligo</i>	<i>Loligo</i>	<i>Loligo</i>	<i>Loligo</i>
<i>duvaucelii</i>	<i>vulgaris</i>	<i>gahi</i>		<i>reynaudii</i>	<i>opalescens</i>	
Na	1500	25.0	34.9	31.7	63.2	28.9
K	2000	5.6	3.6	5.0	10.3	23.5
P	700	14.3	13.4	17.2	16.7	21.8
Mg	375	10.2	23.5	11.8	24.4	19.0
Ca	800	0.5	1.3	0.8	1.3	1.0
Cu	1	19.2	15.4	116.2	12.6	108.4
Zn	10	10.6	9.2	12.1	9.9	12.6
Fe	14	0.8	0.6	1.0	0.7	2.1
Ni	0.3	2.8	6.9	3.2	6.7	6.3
Mn	2	1.2	0.6	1.3	0.7	2.4
Cr	0.04	3.3	3.8	3.3	3.0	4.8

<sup>a</sup> According to European Commission (2008), EMEA (2008) and FNIC (2010).

mates ranged from  $0.001 \times 10^{-2}$  to  $3.23 \times 10^{-2}$  µg/kg body weight per day. They are considerably below the exposure level of 1.2 µg/kg body weight per day calculated by the Committee to be associated with a population increase in systolic blood pressure of 1 mmHg. Consequently, it may be considered that any health risk that would be expected to occur at the estimated exposure level is negligible (JECFA, 2011).

The results of the THQ and TR established by the U.S. Environmental Protection Agency (US EPA, 2010) are also displayed in Table 3. The total THQ values obtained were overall lower than the threshold of the unity indicating that consumption of the characterized squid species may be considered safe regarding potential non-carcinogenic risks. Still, THQ values reached ca. 0.62 and

0.52 through *L. duvaucelii* and *L. gahi*, respectively, based on Portuguese consumption mainly due to the contribution of Cd. This conclusion is also supported by the detection of some samples of these species that presented Cd concentrations higher than the maximum level recommended by the EC Regulation (1 mg/kg ww for Cd) (European Commission, 2006).

The target carcinogenic risks derived from the intake of Pb were calculated since this element may promote both non-carcinogenic and carcinogenic effects depending of the exposure dose (Table 3). Pb is classified as probable carcinogen based on animal studies (US EPA Group B2). Although Cd is also categorized in US EPA group B1 (probable carcinogen due to human studies), respectively, its TR was not estimated since the ingestion slope factor (CSF<sub>o</sub>; chemical-specific) is not established for this element (US EPA, 2010). For carcinogens the EPA believes that setting a  $10^{-6}$  risk level for individual chemicals and pathways will generally lead to insignificant cancer risks. However, caution is recommended to ensure that the cumulative cancer risk for all potential carcinogenic contaminants does not have a residual cancer risk exceeding  $10^{-4}$  (US EPA, 2010). Whatever the squid species taken into consideration in this study, the TR values were clearly lower than the upper ( $10^{-4}$ ) health-based guideline level indicating that there are no carcinogenic risks concerning Pb ingestion.

For the lipid components, several studies have revealed that increasing dietary x-3/x-6 ratio helps reducing some health risks.

The recommended ratio differs between authors but it is always superior to 1 (Chow, 2008). These squid species have x-3/x-6 ratios ranging from  $5.7 \pm 0.7$  (*L. duvaucelii*) to  $17.7 \pm 2.2$  for (*L. gahi*).

Furthermore, a portion of 37 g (*L. opalescens*) to 62 g (*L. vulgaris*) provides the recommended daily EPA + DHA intake of 250 mg (EFSA, 2010).

The attained mean AI ( $0.43 \pm 0.04$  to  $0.51 \pm 0.02$ ) and TI ( $0.17 \pm 0.01$  to  $0.23 \pm 0.01$ ) (Table 1) can be considered favorable for the consumption of all species due to the cardioprotective effect of their PUFA concentrations.

The ingestion of higher cholesterol containing squid species, particularly *L. opalescens*, should probably be moderated since a 100 g portion will surpass the recommended maximum daily intake by the American Heart Association Nutrition Committee (300 mg/day) (Lichtenstein et al., 2006). Still the PUFA content is also high, particularly of long-chain PUFA, which tends in opposite

ways regarding cholesterol effects (Chow, 2008). Overall, the mean HH values (Table 1) varied from  $1.9 \pm 0.7$  (*L. reynaudii*) to  $2.5 \pm 0.6$  (*L. opalescens*).

Being present in reduced amounts, vitamin E does not represent an important nutritional attribute. Its main function is to protect the squid lipids from oxidation, particularly the PUFA, being therefore associated with the total fat and PUFA amounts.

### 3.6. Discriminant analysis

Firstly, CDA was tested to distinguish the oceanic origin of the species via their standardized macro and micronutrients level

Table 3

Estimated monthly intake (EMI) ( $\mu\text{g}/\text{kg}$  body weight), estimated daily intake (EDI) ( $\mu\text{g}/\text{kg}$  body weight), target hazard quotients (THQ) and carcinogenic risks (TR) (mean and range) for some metals detected in the characterized squidspecies.

Per capita consumption <sup>a</sup>		(Indic Ocean)		(Atlantic Ocean)		(Pacific Ocean)	
		<i>Loligo duvaucelii</i>	<i>Loligo vulgaris</i>	<i>Loligo gahi</i>	<i>Loligo reynaudii</i>	<i>Loligo opalescens</i>	
EMI ( $\times 10^{-2}$ )	World	Cd	10.3 (1.2–215.6)	9.3 (4.4–27.9)	46.4 (2.8–135.9)	7.9 (3.9–33.6)	27.6 (12.0–72.0)
	Portuguese		84.7 (9.7–1766)	75.9 (36.3–229.3)	379 (22.8–1114)	65.0 (31.5–275.6)	226.7 (98.1–591.4)
EDI ( $\times 10^{-2}$ )	World	Pb	0.06 (0.006–0.14)	0.08 (0.01–0.20)	0.10 (0.02–0.39)	0.03 (0.001–0.09)	0.04 (0.02–0.10)
	Portuguese		0.46 (0.04–1.12)	0.62 (0.11–1.61)	0.85 (0.15–3.23)	0.24 (0.01–0.74)	0.36 (0.16–0.81)
THQ ( $\times 10^{-3}$ )	World	Zn	0.66 (0.49–0.97)	0.58 (0.45–0.81)	0.76 (0.44–1.03)	0.62 (0.48–0.92)	0.79 (0.64–1.10)
		Cu	0.90 (0.31–1.99)	0.72 (0.45–2.30)	5.45 (1.56–14.8)	0.59 (0.28–1.05)	5.09 (2.50–11.2)
		Fe	0.03 (0.02–0.29)	0.02 (0.02–0.06)	0.04 (0.02–0.28)	0.03 (0.01–0.06)	0.08 (0.05–0.21)
		Mn	0.22 (0.06–0.82)	0.10 (0.04–0.20)	0.25 (0.20–0.52)	0.13 (0.09–0.23)	0.46 (0.20–0.67)
		Cr	0.08 (0.03–0.34)	0.09 (0.04–0.22)	0.08 (0.02–0.27)	0.08 (0.02–0.36)	0.12 (0.07–0.23)
		Ni	0.08 (0.03–2.09)	0.19 (0.01–2.07)	0.09 (0.02–2.36)	0.19 (0.04–4.16)	0.18 (0.04–2.30)
		Cd	3.30 (0.38–68.9)	2.96 (1.41–8.93)	14.8 (0.89–43.5)	2.53 (1.23–10.7)	8.84 (3.83–23.0)
		Pb	0.14 (0.01–0.33)	0.18 (0.03–0.47)	0.25 (0.05–0.94)	0.07 (0.004–0.22)	0.10 (0.05–0.24)
	Portuguese	* THQ	5.41 (1.32–75.7)	4.86 (2.45–15.1)	21.7 (3.19–63.6)	4.24 (2.15–17.7)	15.7 (7.37–38.9)
		Zn	5.44 (3.99–7.99)	4.72 (3.68–6.63)	6.20 (3.61–8.41)	5.08 (3.93–7.52)	6.47 (5.26–8.99)
		Cu	7.38 (2.52–16.3)	5.91 (3.68–18.8)	44.7 (12.8–121)	4.86 (2.30–8.62)	41.7 (20.5–91.8)
		Fe	0.24 (0.13–2.39)	0.20 (0.13–0.52)	0.30 (0.17–2.32)	0.21 (0.10–0.46)	0.65 (0.38–1.73)
		Mn	1.77 (0.51–6.72)	0.85 (0.32–1.67)	2.06 (1.60–4.23)	1.10 (0.73–1.91)	3.74 (1.67–5.48)
		Cr	0.67 (0.24–2.78)	0.77 (0.30–1.77)	0.67 (0.16–2.21)	0.62 (0.19–2.99)	0.97 (0.59–1.87)
		Ni	0.64 (0.22–17.2)	1.58 (0.08–16.9)	0.73 (0.18–19.4)	1.55 (0.32–34.1)	1.46 (0.30–18.8)
		Cd	27.1 (3.09–565)	24.3 (11.6–73.3)	121 (7.28–356)	20.8 (10.1–88.1)	72.5 (31.4–188)
TR ( $\times 10^{-7}$ )	World	Pb	1.12 (0.10–2.69)	1.50 (0.26–3.88)	2.04 (0.37–7.73)	0.58 (0.04–1.77)	0.85 (0.37–1.95)
		* THQ	44.3 (10.8–621)	39.9 (20.1–124)	178 (26.2–522)	34.8 (17.8–146)	128 (60.4–319)
Portuguese	Pb	0.05 (0.004–0.1)	0.06 (0.01–0.16)	0.09 (0.02–0.3)	0.02 (0.001–0.07)	0.04 (0.002–0.08)	
	Pb	0.5 (0.04–0.9)	0.5 (0.09–1.3)	0.5 (0.12–2.6)	0.4 (0.01–0.6)	0.7 (0.13–0.66)	

<sup>a</sup> World (0.5 kg) and Portuguese (4.1 kg) *per capita* cephalopods consumption in 2009 according to FAO (2012).



(Fig. 2a), as well as their individual fatty acids content (Fig. 2b). Three clusters corresponding to each one of the ocean are clearly individualized reflecting the marked influence of the environmental conditions in the levels of essential mineral and fatty acids in the studied squid species. Concerning the CDA scatter plot based on mineral amounts (Fig. 2a), the first canonical function, CF1 (variance of 82.3%) is mainly correlated with K and to a lesser extent with Cu and P, while Na, Cu and P are the major contributors for CF2 (variance of 17.2%). Regarding Fig. 2b, the two functions issued from CDA on the data matrix of fatty acids explain up to 100% of the observed variance (92.8% for CF1 and 7.2% for CF2). CF1 is mostly influenced by C20:4x-6, C20:5x-3 and C18:1 whereas CF2 is mainly affected by C16:0, C18:1, C20:1x-9 and C20:5x-3.

In a second attempt, CDA was applied to group the different species (Fig. 3). The results based solely on individual fatty acids are depicted in Fig. 3a. They provide satisfactory differentiation of the characterized squid species. Only the two first CFs were plotted combining a total variance of 96.8%. CF1 of 89.6% variance separates all groups except *L. gahi* (mean canonical variance, MCV, of 6.40) and *L. opalescens* (MCV of 6.86) (MCV of -10.18, -8.57 and 4.21 for *L. duvaucelii*, *L. vulgaris* and *L. reynaudii*, respectively). Interpretation of the standardized coefficients of canonical vari-

ables showed that CF1 is more correlated with C18:0, C20:4x-6, C18:1, C18:3x-3, C14:0 and C22:5x-6. CF2 with a 7.2% variance distinguishes *L. opalescens* (MCV of 3.57) from the other squids while there is no significant separation between *L. gahi* (-1.21) and *L. duvaucelii* (-0.60). CF2 revealed to be more influenced by C16:0, C20:5x-3, C18:1, C22:6x-3 and C20:1x-9, of higher nutritional relevance. The best differentiation among *L. gahi*, *L. reynaudii* and *L. opalescens* was reached combining data from the major fatty acids (C14:0, C18:0, C18:1, C18:3x-3, C20:4x-6 and C22:5x-6), vitamin E, and the elements P, K and Cu (Fig. 3b). Two significant CF were obtained. CF1 (80.7% of variance) revealed to be strongly linked with C14:0, C18:0, C18:3x-3 and C20:4x-6. The compounds K, C22:5x-6 and Cu concentrations are the variables that contributed the most to CF2.

#### 4. Discussion

Concerning the macroelements, Na, K, Mg, Ca can be suitable chemical descriptors for *L. duvaucelii* and *L. vulgaris*, and for *L. gahi* and *L. reynaudii* from the same origin. P can allow differentiation of the oceanic provenance and discrimination of *L. opalescens* only

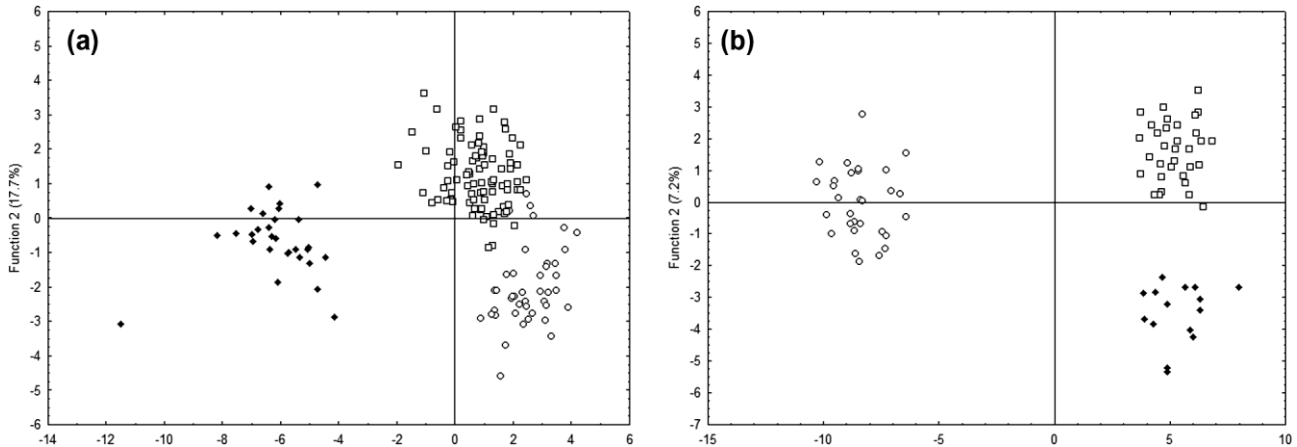


Fig. 2. Canonical discriminant analysis of the composition of squids (o—Indian Ocean, h—Atlantic Ocean, +—Pacific Ocean) based on (a) essential elements concentration, and (b) individual fatty acids concentration.

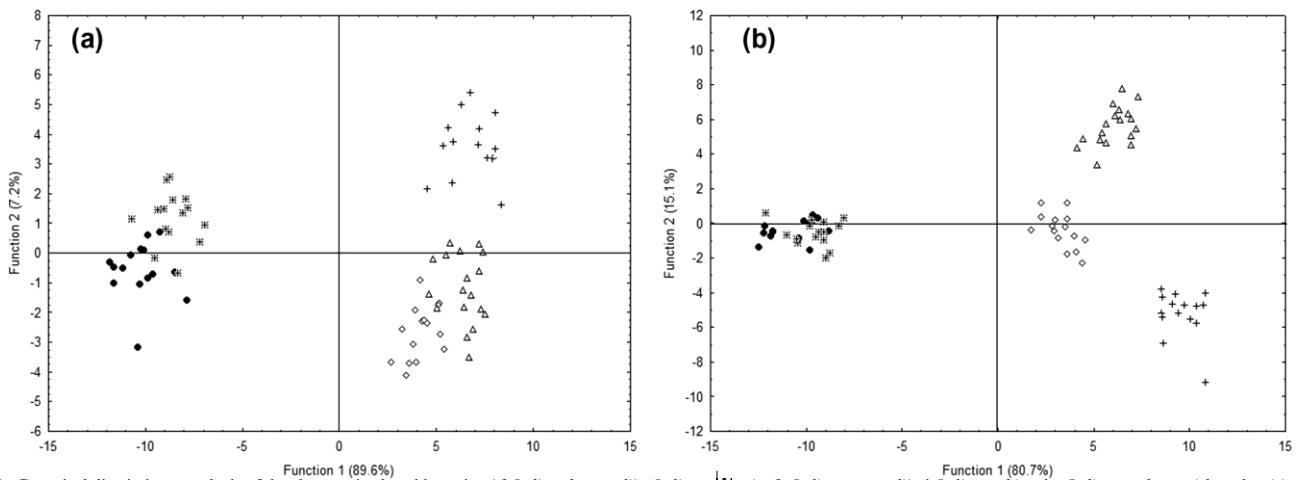


Fig. 3. Canonical discriminant analysis of the characterized squid species (◊ *Loligo duvaucelii*, ● *Loligo vulgaris*, ▲ *Loligo reynaudii*, ◻ *Loligo gahi* and + *Loligo opalescens*) based on (a) individual fatty acids content, and (b) C14:0, C18:0, C18:1, C18:3x-3, C20:4x-6, C22:5x-6, vitamin E, P, K and Cu concentration.

(this species can be differentiated by the K and Mg). *L. vulgaris* and *L. reynaudii*, which are from different origins, presented similarities in their Ca and Mg concentrations, probably due to their analogous biometric structure. *L. reynaudii* was previously considered as a subspecies of *L. vulgaris* (FAO, 2010). Literature data was only found for *L. vulgaris* from another origin, the Atlantic Ocean (Loureço et al., 2009). The results reached for this species are comparable with those published by Loureço et al. (2009) for Mg (283–610 mg/kg ww) and Ca (89–211 mg/kg ww) while being on the lower side of the reported values for K (1930–3430 mg/kg ww) and P (2300–2850 mg/kg ww), and in the higher range for Na (900–1940 mg/kg ww). Manso et al. (2007) reached values of 610 mg/kg ww for Ca and 12,750 mg/kg ww for K which are significantly different from ours.

Zn, Cu, Fe, Mn and Cr are also essential to human health but in lower quantities being therefore classified as microelements. Cr toxicity depends on its oxidation state. Cr(III) and Cr(VI) ions differ with regards to their biological, chemical, and toxicological properties. Cr(III) compounds are essential in the human body, playing a vital role in the metabolism of glucose, while Cr(VI) is toxic and carcinogenic (Vincent, 2010). Ni appears to be an essential micro-nutrient in animals, and so it is likely to be essential for humans, but its precise biochemical role and functions in humans are not defined (EMEA, 2008; FNIC, 2010). The attained composition micronutrient profiles are similar despite the different concentrations presented. Overall, the sequence is in accordance with previous studies regarding *L. vulgaris* (Manso et al., 2007; Loureço et al., 2009). However, *L. opalescens* and *L. gahi* can be distinguished due to their large (ca. 6 times for median values comparison) amounts of Cu. High levels of Cu in *L. gahi* were already reported by Seixas et al. (2005). Zn and Cu are cofactors in many enzyme systems and their involvement in many metabolic processes may explain the higher concentrations measured of these elements in comparison to the others. Cr and Ni correspond to the metals with less discriminating power between species. The results attained are consistent with those previously reported for *L. vulgaris* samples from the Atlantic Ocean and the NW Mediterranean sea (Loureço et al., 2009; Olmedo et al., 2013), and for *L. gahi* from Argentina and *L. opalescens* (Seixas et al., 2005). Prafulla et al. (2001) determined some micronutrients in several tissues and organs of *L. divaucelli* specimens from the Southwest coast of India. Their results are higher than our results. No information was found concerning microelements in *L. reynaudii*. The detected micronutrient concentrations in the studied species reflect the influence of each species (feeding preference, trophic level, behavior, size, and seasonal changes in physiology) (Gomes et al., 2013; Maioli et al., 2010), but also the environmental inputs showing the variability of metal concentrations originated from different sources present in Oceans. In terms of risk assessment, cephalopods can be useful since they accumulate high levels of essential and non-essential elements (Semedo et al., 2012). Due to their territorial nature and accordingly with a recent study of our research team (Semedo et al., 2012), cephalopods may provide useful information about the habitat and the quality of the environment where they live.

Cd and Pb have no beneficial effects in humans, and there is no known homeostasis mechanism for them (Castro-González and Méndez-Armenta, 2008; Vieira et al., 2011). They are among the most toxic elements to humans and animals. The adverse human health effects associated with exposure to them, even at low concentrations, are diverse and include, but are not limited to, neuro-toxic and carcinogenic actions (ATSDR, 2007, 2008). In general, for non-smokers and non-occupationally exposed workers, food products account for most of the human exposure burden to Cd and Pb (Castro-González and Méndez-Armenta, 2008). *L. gahi* and *L. reynaudii* corresponded to the most and less contaminated species, respectively. Geographical distribution, habitat, intrinsic factors

such as different rates of physiological processes of squids and feeding habits may influence metals bioaccumulation. Both species were originated from the Atlantic Ocean but their geographical distribution is quite different. *L. gahi* is almost exclusively captured in Argentina while *L. reynaudii* resides directly along the South African coast (FAO, 2010). Cd and Pb are ubiquitous in the environment and accumulate in various levels of the aquatic food chain by different mechanisms, directly from the dissolved phase, uptake of suspended particles, or by ingestion of lower trophic level organisms (fish, crabs and shrimp, mollusc, and other juvenile squids), detritus and sediments (Croxtton et al., 2012; FAO, 2010; Gomes et al., 2013). For both species the Cd and Pb median concentrations are lower than the maximum levels recommended by the EC Regulation (1 mg/kg ww for Cd and Pb) (European Commission, 2006). However, five samples of *L. divaucelli* (2.10–3.67 mg/kg ww), sixteen of *L. gahi* (1.03–2.32 mg/kg ww) and four of *L. opalescens* (1.03–1.22 mg/kg ww) had higher Cd concentrations than the established limit. Cephalopods are known for their ability to tolerate high concentrations of Cd (Semedo et al., 2012; Storelli et al., 2010) when compared to other seafood. The observed concentration ranges are similar to those indicated for *L. vulgaris* (Manso et al., 2007; Storelli, 2008), for *L. opalescens* (Galitsopoulou et al., 2009), and for *L. gahi* (Seixas et al., 2005) for Cd. The levels reported for *L. vulgaris* (Loureço et al., 2009) and for *L. gahi* (Falandyz, 1989) for Pb, as well as for *L. divaucelli* for Cd and Pb (Prafulla et al., 2001) were globally higher than the determined in this study. The results of *L. reynaudii* were not compared with others studies due to the lack of information concerning this species.

The fatty acid profiles (as relative percentage of total fatty acids) are in accordance to other previous studies concerning *L. divaucelli* from Thailand (SFA 41.3%, MUFA 14.1% and PUFA 44.7%; Chedoloh et al., 2011) and Egypt (SFA 44.6%, MUFA 6.7% and PUFA 48.7%; Gabr, 2010), *L. vulgaris* from the Mediterranean Sea (SFA 32.9%, MUFA 9.4% and PUFA 57.3% (Miliou et al., 2006); SFA 35.7%, MUFA 9.0% and PUFA 51.1% (Navarro and Villanueva, 2000); SFA 31.8–35.3%, MUFA 7.3–8.1% and PUFA 52.8–55.9% (Ozogul et al., 2008); SFA 36.8%, MUFA 7.4% and PUFA 51.1% (Salman et al., 2007)) and *L. gahi* caught in Falkland Islands (SFA 27.6%, MUFA 8.8% and PUFA 61.1% (Norambuena et al., 2012); SFA 30.9%, MUFA 13.6% and PUFA 55.2% (Phillips et al., 2003)). No complete profile description was found for *L. opalescens* (Tziouveli et al., 2011) and no data was found for *L. reynaudii*. The slight differences observed with our study may be due to variations in geographical provenance, water temperature and salinity, diet season of catch, gender, sexual maturity (Ozogul et al., 2008) and analytical methodology.

Regarding the fatty acids composition on an edible basis (mg/100 g ww), *L. gahi* and *L. opalescens* exhibited the major differences among the various profiles with particular relevance in the MUFA and PUFA concentrations. PUFA are the predominant fatty acids for all species and DHA (C22:6x-3) was the fatty acid that contributed the most for PUFA concentrations. These particular long-chain fatty acids are beneficial in preventing health problems but with their own specialized and distinctive functions. DHA is an important constituent of the building blocks of the biological membranes in the brain and retina affecting cognitive and visual functions (Chow, 2008; Connor, 2000). EPA is more efficient in reducing cellular inflammation preventing depression disorders (Martins, 2009) and in the reduction of serum triacylglycerols (formerly known as triglycerides). SFA was the most abundant class after PUFA. Some epidemiologic studies shown that SFA is positively associated with total cholesterol levels and coronary heart diseases (Caggiula and Mustad, 1997) although there is some controversy (Siri-Tarino et al., 2010). Compared to other food products, SFA content of squids are lower than meat products (about 40%) and higher than the majority of vegetables, fruits and cereals (<30%)

(Chow, 2008). Together with PUFA, MUFA are believed to reduce the incidence of cardiovascular diseases by decreasing low density lipoprotein (LDL) cholesterol in blood (Appel et al., 2005; Chow, 2008; Kris-Etherton, 1999). However, this fraction has a small representativeness in squids, being lower than meat products (about 40%), fruit (>15%) and cereals (>15%). Vegetables have, generally, the same composition or lower than squid (Chow, 2008).

Cholesterol concentrations were highly variable between species. Literature data was only found for *L. duvaucelii* and *L. gahi* (García-Garrido et al., 2011; Mathew et al., 1999; Uddin et al., 2001; Valverde et al., 2012). The obtained results are in agreement with previous reports for *L. duvaucelii* (188 and 198 mg/100 g ww (Mathew et al., 1999); 200 mg/100 g ww (Uddin et al., 2001)) and *L. gahi* (269.3 mg/100 g ww) (Valverde et al., 2012) but lower than the values (389.5 mg/100 g ww) mentioned by García-Garrido et al. (2011).

The higher levels of vitamin E were detected in the species with higher PUFA content which is in accordance with previous studies (Chow, 2008). Data regarding vitamin E levels in squids are quite limited; information was only found about *L. vulgaris* (Passi et al., 2002; Villanueva et al., 2009). The attained results for *L. vulgaris* are in agreement with those reported by Passi et al. (2002) (0.84 mg/100 g ww) while being clearly lower than those indicated by Villanueva et al. (2009). These authors referred 4.7 and 7.5 mg/100 g ww for hatchling and juvenile *L. vulgaris*, respectively. This discrepancy can be explained because hatchling and juvenile cephalopods have higher content of PUFAs compared to adult stage and since oxidation occurs more easily in long chain PUFAs, the content of vitamin E is also superior (Villanueva et al., 2009).

Squids can contribute significantly to the DMI of Cu, Na, Mg, K, P and Zn. Taking in consideration the results obtained, moderate consumption of *L. gahi* and *L. opalescens* is advised due to the potentially excessive Cu intake (8–16%). Moreover, high Na/K ratios are observed for all species except for *L. opalescens* which may suggest an increased risk of developing cardiovascular problems when consumed at high levels (Lourenço et al., 2009). Overall, the results found for *L. vulgaris* are in agreement with those published for this species from Atlantic waters (Lourenço et al., 2009). Still, Lourenço et al. (2009) described upper values for P while lower DMI for Na, Cu, Mg and Ni were presented. No other information was found.

The characterized squid species have all very interesting x-3/x-6 ratios, potentially with positive effects if included in a balanced diet.

The AI and TI are indicative of potential cardiovascular disease risk factors and therefore must be kept low. AI represents the relation between the pro-atherogenic (mainly saturated) and the anti-atherogenic (unsaturated) fatty acids. The first enhances the adhesion of lipids to cells of the immunological and circulatory system, and the latter inhibit the aggregation of plaque and decrease the levels of esterified fatty acids, cholesterol, and phospholipids, thereby preventing the appearance of micro- and macro-coronary diseases (Garaffo et al., 2011; Guimarães et al., 2013). TI indicates the tendency to form blood clots. The obtained indexes values are comparable with a large group of relevant marine organisms such as the silver scabbardfish (AI: 0.37 and TI 0.21), hake (AI: 0.30 and TI 0.51), ray (AI: 0.33 and TI 0.40) and many others (Afonso et al., 2013).

Cholesterol content in squids, and cephalopods in general, is very high compared with the majority of marine organisms and also higher than meat products, possibly affecting blood cholesterol on susceptible individuals. The HH index estimates specific effects of fatty acids on cholesterol metabolism, and high values are desired from a nutritional standpoint (Guimarães et al., 2013). The deter-

mined HH values are quite acceptable and similar to those reported for other highly consumed fish species (Afonso et al., 2013). Overall, moderate consumption of these squid species is advised, particularly by the most vulnerable population groups, such as children, pregnant and breast-feeding mothers to ensure that the recommended reference daily intake are not surpassed, and that cumulative non-carcinogenic and carcinogenic risks for all potential contaminants does not exceed the stipulated thresholds. *L. vulgaris* is mainly found throughout the Mediterranean Sea and in the eastern Atlantic Ocean from the North Sea to the Gulf of Guinea (FAO, 2010). According to data on Portuguese landings, *L. vulgaris* seems to be the more appreciated squid species in Portugal (INE, 2012) which does not pose any risks. Nevertheless, *L. duvaucelii*, *L. gahi*, *L. reynaudii* and *L. opalescens* are important squid species worldwide, with special significance for the populations at the sampling sites. *L. duvaucelii* is distributed throughout the Mediterranean Sea and Indian Ocean periphery, including the Red Sea and the Arabian Sea, extending eastwards from Mozambique to the South China Sea and the Philippines Sea, northward to Taiwan (Province of China) (FAO, 2010). *L. gahi* is widely dispersed along the Pacific coast of South America and is the second most important loliginid squid in commercial fisheries worldwide (FAO, 2010). A small part of the total catch is consumed in Europe, pre-dominantly in Spain. *L. reynaudii* is geographically distributed in the Eastern central Atlantic: from South Africa to southern Namibia. *L. opalescens* has become the most important fishery in California, USA, in both landings and commercial value (FAO, 2010). The combination of CDA based on different compounds permit cross-validation for authenticity control of these squid species.

## 5. Conclusions

This study fills the gap concerning the characterization and estimation of potential health benefits/risks for human consumption of five commercially valuable squid species from different geographical origins (Atlantic, Indian and Pacific Oceans). Attending to the daily intake of the majority of macro and micronutrients, and

to the nutritional lipid quality indexes such as total x-3/x-6, DHA + EPA sum, AI, TI and HH, the consumption of the selected squid species are advisable in a balanced diet. Still, the ingestion of higher cholesterol containing squid species, particularly *L. opalescens*, should be moderated. Concerning the daily minerals intake, moderate consumption of *L. gahi* and *L. opalescens* is advised due to the potentially excessive Cu intake. Also, assessment of the non-carcinogenic risks suggested that *L. duvaucelii* and *L. gahi*, may pose additional risks for consumers, if not eaten in moderation, due to the contribution of Cd. Overall, the assessment of health benefits/risks suggest that the characterized species should be consumed in moderation. These recommendations are particularly relevant for the most vulnerable population groups. Clear discriminations among oceanic origins and squid species were reached based on their minerals, individual fatty acids and vitamin E concentrations. These compounds exhibited the potential to proof authenticity of commonly consumed and commercially relevant squid species.

## Conflict of Interest

The authors declare that there are no conflicts of interest.

## Transparency Document

The Transparency document associated with this article can be found in the online version.

## Acknowledgements

The authors would like to thank to Fundação para Ciência e Tecnologia for the financial support of this work through the Project PTDC/AGR-AAM/102316/2008 (COMPETE and co-financed by FEDER). This work received financial support from the European Union (FEDER funds through COMPETE) and National Funds (FCT, Fundação para a Ciência e Tecnologia) through project Pest-C/ EQB/LA0006/2013. The work also received financial support from the European Union (FEDER funds) under the framework of QREN through Project NORTE-07-0124-FEDER-000069. To all financing sources the authors are greatly indebted. M. Oliveira is also grateful to Fundação para Ciência e Tecnologia for her fellowship SFRH/BD/80113/2011.

## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.fct.2014.02.014>.

## References

- Afonso, C., Lourenço, H.M., Cardoso, C., Bandarra, N.M., Carvalho, M.L., Castro, M., Nunes, M.L., 2013. From fish chemical characterization to the benefit-risk assessment—Part A. *Food Chem.* 137, 99–107.
- Appel, L.J., Sacks, F.M., Carey, V.J., Obarzanek, E., Swain, J.F., Miller III, E.R., Conlin, P.R., Erlinger, T.P., Rosner, B.A., Laranjo, N.M., Charleston, J., McCarron, P., Bishop, L.M., 2005. Effects of protein, monounsaturated fat, and carbohydrate intake on blood pressure and serum lipids: results of the omniscience randomized trial. *JAMA* 294, 2455–2464.
- Agency for Toxic Substance and Disease Registry (ATSDR), 2007. Toxicological Profile for Lead. US Department of Health and Human Services, Public Health Service, Centers for Disease Control, Atlanta.
- Agency for Toxic Substance and Disease Registry (ATSDR), 2008. Draft Toxicological Profile for Cadmium. US Department of Health and Human Services, Public Health Service, Centers for Disease Control, Atlanta.
- Caggiula, A.W., Mustad, V.A., 1997. Effects of dietary fat and fatty acids on coronary artery disease risk and total and lipoprotein cholesterol concentrations: epidemiologic studies. *Am. J. Clin. Nutr.* 65, 1597S–1610S.
- Cardoso, C., Lourenço, H., Afonso, C., Nunes, M.L., 2012. Risk assessment of methyl-mercury intake through cephalopods consumption in Portugal. *Food Addit. Contamin.* 29, 94–103.
- Castro-González, M.I., Méndez-Armenta, M., 2008. Heavy metals: implications associated to fish consumption. *Environ. Toxicol. Pharmacol.* 26, 263–271.
- Chedoloh, R., Kariila, T.T., Pakdeechanuan, P., 2011. Fatty acid composition of important aquatic animals in Southern Thailand. *Int. Food Res. J.* 18, 783–790.
- Chow, C.K., 2008. *Fatty Acids in Foods and their Health Implications*, third ed. CRC Press.
- Cirillo, T., Fasano, E., Viscardi, V., Arnese, A., Amodio-Cocchieri, R., 2010. Survey of lead, cadmium, mercury and arsenic in seafood purchased in Campania, Italy. *Food Addit. Contamin.* Part B 3, 30–38.
- Connor, W.E., 2000. Importance of n–3 fatty acids in health and disease. *Am. J. Clin. Nutr.* 71, 171S–175S.
- Croxton, A.N., Wikfors, G.H., Schulerbrandt-Gragg, R.D., 2012. Immunomodulation in eastern oysters, *Crassostrea virginica*, exposed to a PAH-contaminated, microphytobenthic diatom. *Aquat. Toxicol.* 118–119, 27–36.
- Cruz, R., Casal, S., Mendes, E., Costa, A., Santos, C., Morais, S., 2013. Validation of a single-extraction procedure for sequential analysis of vitamin E, cholesterol, fatty acids, and total fat in seafood. *Food Anal. Meth.* 6, 1196–1204.
- EFSA, 2010. EFSA panel on dietetic products, nutrition, and allergies (NDA): Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA J.* 8, 1461.
- EMA, 2008. Guideline on the specification limits for residue of metal catalysts or metal reagents. Doc. Ref. EMA/CHMP/SWP/4446/2000, p. 22, EMA – European Medicines Agency, London, UK.
- European Commission, 2006. Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Off. J. Eur. Union*, L 364/5.
- European Commission, 2007. Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs. *Off. J. Eur. Union*, L 88/29.
- European Commission, 2008. Commission directive 2008/100/EC of 28 October 2008 amending Council Directive 90/496/EEC on nutrition labelling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions. *Off. J. Eur. Union*, L 285/9.
- Falandysz, J., 1989. Trace metal levels in the raw and tinned squid *Loligo patagonica*. *Food Addit. Contamin.* 6, 483–488.
- FAO, 2010. Cephalopods of the world. An annotated and illustrated catalogue of cephalopod species known to date. In: Jereb, P., Roper, C.F.E. (Eds.), *Myopsid and Oegopsid Squids*. FAO Species Catalogue for Fishery Purposes. No. 4, vol. 2, Rome, pp. 34–81.
- FAO, 2011. Review of the state of world marine fishery resources. Food and Agriculture Organization of the United Nations. FAO fisheries and aquaculture technical paper 569, Rome.
- FAO, 2012. 1961–2009 Fish and fishery products: world apparent consumption statistics based on food balance sheets. FAO Yearbook, Fishery and Aquaculture Statistics. Food and Agriculture Organization of the United Nations, Rome.
- FNIC 2010. Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Elements; Food and Nutrition Board, Institute of Medicine, National Academy of Sciences.
- Gabr, H.R., 2010. Biochemical and bacteriological properties of fresh and frozen sold cephalopods in the Egyptian market. *Egypt. J. Aquat. Biol. Fisheries* 14, 27–42.
- Galitsopoulou, A., Georgantelis, D., Kontominas, M.G., 2009. Cadmium content in fresh and canned squid (*Loligo opalescens*) from the Pacific coastal waters of California (USA). *Food Addit. Contamin.* Part B 2, 38–43.
- Garaffo, M.A., Vassallo-Agius, R., Nengas, Y., Lembo, E., Rando, R., Maisano, R., Dugo, G., Giuffrida, D., 2011. Fatty acids profile, atherogenic (IA) and thrombogenic (IT) health lipid indices, of raw roe of blue fin tuna (*Thunnus thynnus* L.) and their salted product “Bottarga”. *Food Nutr. Sci.* 2, 736–743.
- García-Garrido, S., Hachero-Cruzado, I., Dominguez, P., López, N., Rosas, C., 2011. Effects of fish hydrolysate (CPSP®) on growth and digestive gland lipid composition of *Octopus vulgaris* (Cuvier, 1797) juveniles. *Aquacult. Nutr.* 17, e825–e839.
- Gomes, F., Oliveira, M., Ramalhosa, M.J., Delerue-Matos, C., Morais, S., 2013. Polycyclic aromatic hydrocarbons in commercial squids from different geographical origins: levels and risks for human consumption. *Food Chem. Toxicol.* 59, 46–54.
- Greenberg, A.E., Clesceri, L.S., Eaton, A.D. 1992. Standard methods for the examination of water and wastewater (4500-P), USA.
- Guimarães, R.C.A., Macedo, M.L.R., Munhoz, C.L., Filiiu, W., Viana, L.H., Nozaki, V.T., Hiane, P.A., 2013. Sesame and flaxseed oil: nutritional quality and effects on serum lipids and glucose in rats. *Food Sci. Technol. (Campinas)* 33, 209–217.
- INE, 2012. Estatística de Pesca 2011. Instituto Nacional de Estatística, IP, Lisbon, Portugal.
- Joint FAO/WHO Expert Committee on Food Additives (JECFA), 2011. Evaluation of certain food additives and contaminants. WHO Technical Report Series No. 960. World Health Organization, Geneva.
- Kris-Etherton, P.M., 1999. Monounsaturated fatty acids and risk of cardiovascular disease. *Circulation* 100, 1253–1258.
- Lichtenstein, A.H., Appel, L.J., Brands, M., Carnethon, M., Daniels, S., Franch, H.A., Franklin, B., Kris-Etherton, P.M., Harris, W.S., Howard, B., Karanja, N., Lefevre, M., Rudel, L., Sacks, F., Horn, L.V., Winston, M., Wylie-Rosett, J., 2006. Diet and lifestyle recommendations revision 2006. A scientific statement from the American Heart Association nutrition committee. *Circulation* 114, 82–96.
- Lourenço, H.M., Anacleto, P., Afonso, C., Ferraria, V., Martins, M.F., Carvalho, M.L., Limo, A.R., Nunes, M.L., 2009. Elemental composition of cephalopods from Portuguese continental waters. *Food Chem.* 113, 1146–1153.
- Maioli, O.L.G., Rodrigues, K.C., Knoppers, B.A., Azevedo, D.A., 2010. Polycyclic aromatic and aliphatic hydrocarbons in *Mytella charruana*, a bivalve mollusk from Mundaú Lagoon, Brazil. *Microchem. J.* 96, 172–179.
- Manso, M., Carvalho, M.L., Nunes, M.L., 2007. Characterization of essential and toxic elements in cephalopod tissues by EDXRF and AAS. *X-Ray Spectrometry* 36, 413–418.
- Martins, J.G., 2009. EPA but not DHA appears to be responsible for the efficacy of omega-3 long chain polyunsaturated fatty acid supplementation in depression: Evidence from a meta-analysis of randomized controlled trials. *J. Am. College Nutr.* 28, 525–542.
- Mathew, S., Ammu, K., Viswanathan Nair, P.G., Devadasan, K., 1999. Cholesterol content of Indian fish and shellfish. *Food Chem.* 66, 455–461.
- Miliou, H., Fintikaki, M., Tzitzinakis, M., Kountouris, T., Verriopoulos, G., 2006. Fatty acid composition of the common octopus, *Octopus vulgaris*, in relation to rearing temperature and body weight. *Aquaculture* 256, 311–322.
- Miller, J.N., Miller, J.C., 2000. *Statistics and Chemometrics for Analytical Chemistry*. Pearson Education, London.
- Navarro, J.C., Villanueva, R., 2000. Lipid and fatty acid composition of early stages of cephalopods: an approach to their lipid requirements. *Aquaculture* 183, 161–177.
- Norambuena, F., Estevez, A., Bell, G., Carazo, I., Duncan, N., 2012. Proximate and fatty acid compositions in muscle, liver and gonads of wild versus cultured broodstock of Senegalese sole (*Solea senegalensis*). *Aquaculture* 356–357, 176–185.
- Oehlenschläger, J., 2006. Cholesterol content in food, data from the last decade: a review. In: Luten, J.B., Jacobsen, C., Bekaert, K., Saebø, A., Oehlenschläger, J. (Eds.), *Seafood research from fish to dish: quality, safety and processing of wild and farmed fish*. Wageningen Academic Publishers, Netherlands.
- Olmedo, P., Hernández, A.F., Pla, A., Femia, P., Navas-Acien, A., Gil, F., 2013. Determination of essential elements (copper, manganese, selenium and zinc) in fish and shellfish samples. Risk and nutritional assessment and mercury–selenium balance. *Food Chem. Toxicol.* 62, 299–307.
- Ozogul, Y., Duygusak, O., Ozogul, F., Özkütük, A.S., Türeli, C., 2008. Seasonal effects in the nutritional quality of the body structural tissue of cephalopods. *Food Chem.* 108, 847–852.

- Passi, S., Cataudella, S., Di Marco, P., Simone, F., Rastrelli, L., 2002. Fatty acid composition and antioxidant levels in muscle tissue of different Mediterranean marine species of fish and shellfish. *J. Agric. Food Chem.* 50, 7314–7322.
- Pazos, M., Sánchez, L., Medina, L., 2005.  $\alpha$ -Tocopherol oxidation in fish muscle during chilling and frozen storage. *J. Agric. Food Chem.* 53, 4000–4005.
- Phillips, K., Nichols, P., Jackson, G., 2003. Size-related dietary changes observed in the squid *Moroteuthis ingens* at the Falkland Islands: stomach contents and fatty-acid analyses. *Polar Biol.* 26, 474–485.
- Prafulla, V., Francis, L., Lakshmanan, P.T., 2001. Concentrations of trace metals in the squids, *Loligo duvauceli* and *Doryteuthis sibogae* caught from the Southwest Coast of India. *Asian Fisheries Sci.* 14, 399–410.
- Russo, G.L., 2009. Dietary  $n-6$  and  $n-3$  polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. *Biochem. Pharmacol.* 77, 937–946.
- Salman, Y., Salman, A., Ozkizilcik, S., 2007. The fatty acid profile of the marine cephalopod *Loligo vulgaris*. *Israeli J. Aquacult. – Bamidgeh* 59, 133–136.
- Seixas, S., Bustamante, P., Pierce, G.J., 2005. Interannual patterns of variation in concentrations of trace elements in arms of *Octopus vulgaris*. *Chemosphere* 59, 1113–1124.
- Semedo, M., Reis-Henriques, M.A., Rey-Salgueiro, L., Oliveira, M., Delerue-Matos, C., Morais, S., Ferreira, M., 2012. Metal accumulation and oxidative stress biomarkers in octopus (*Octopus vulgaris*) from Northwest Atlantic. *Sci. Total Environ.* 433, 230–237.
- Siri-Tarino, P.W., Sun, Q., Hu, F.B., Krauss, R.M., 2010. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. *Am. J. Clin. Nutr.* 91, 535–546.
- Storelli, M.M., 2008. Potential human health risks from metals (Hg, Cd, and Pb) and polychlorinated biphenyls (PCBs) via seafood consumption: Estimation of target hazard quotients (THQs) and toxic equivalents (TEQs). *Food Chem. Toxicol.* 46, 2782–2788.
- Storelli, M.M., Garofalo, R., Giungato, D., Giacomini-Stuffler, R., 2010. Intake of essential and non-essential elements from consumption of octopus, cuttlefish and squid. *Food Addit. Contamin.: Part B* 3, 14–18.
- Tziouveli, V., Hall, M., Smith, G., 2011. The effect of maturation diets on the reproductive output of the white-striped cleaner shrimp, *Lysmata amboinensis*. *J. World Aquacult. Soc.* 42, 56–65.
- Uddin, M., Jahan, P., Ahmad, M.U., 2001. Fat and cholesterol content in some fish and shellfish of Bay of Bengal. *Asian J. Chem.* 13, 1227–1230.
- US EPA, 1989. Risk assessment guidance for superfund, vol. I: Human Health Evaluation Manual. EPA/540/1–89/002. Office of Emergency and Remedial Response, Washington, DC.
- US EPA, 2010. Risk-Based Concentration Table. (Available from: <<http://www.epa.gov/reg3hwmd/risk/human/index.htm>>).
- Valverde, J.C., Hernández, M.D., García-Garrido, S., Rodríguez, C., Estefanell, J., Gairín, J.I., Rodríguez, C.J., Tomás, A., García, B.G., 2012. Lipid classes from marine species and meals intended for cephalopod feeding. *Aquacult. Int.* 20, 71–89.
- Vieira, C., Morais, S., Ramos, S., Delerue-Matos, C., Oliveira, M.B.P.P., 2011. Mercury, cadmium, lead and arsenic levels in three pelagic fish species from the Atlantic Ocean: intra- and inter-specific variability and human health risks for consumption. *Food Chem. Toxicol.* 49, 923–932.
- Villanueva, R., Escudero, J.M., Deulofeu, R., Bozzano, A., Casoliva, C., 2009. Vitamin A and E content in early stages of cephalopods and their dietary effects in *Octopus vulgaris* paralarvae. *Aquaculture* 286, 277–282.
- Vincent, J.B., 2010. Chromium: celebrating 50 years as an essential element? *Dalton Trans.* 39, 3787–3794.
- Wijendran, V., Hayes, K.C., 2004. Dietary  $n-6$  and  $n-3$  fatty acid balance and cardiovascular health. *Annu. Rev. Nutr.* 24, 597–615.