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

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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 3-3X-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 Indo-Pacific 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, C18:3, C20:4x-6 and C22:5x-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 polysaturated fatty acids (PUFA) are rich in long-chains of x-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 hypotriacylglycerolemia, antiarrhythmic, antiatherogenic, 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 reduct lipid peroxidation (Pazos et al., 2005), with α-tocopherol being the most bioactive component. In nature, it exists at high concentrations when x-3 PUFA 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 (Oeiras-Oliveira, 2008), which should not affect intraluminal 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 commer-cially 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; Ozoek et al., 2008; Salman et al., 2007). Cholesterol concentration was previously described for L. gahi (Garcia-Garrido et al., 2011; Valver-de et al., 2012) and L. duvauceli (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 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). Further, 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 MQ cm−1) for quantitative analysis. Standard solutions of Ca (0.10–0.50 mg/L), Mg (0.05–0.35 mg/L), Zn (0.04–0.40 mg/L), Cu (2.50–22.5 mg/L), Fe (1.25–25 mg/L), Mn (2.50–30 mg/L), Cr (0.50–5.00 mg/L), Ni (2.00–20.0 mg/L), Cd (0.10–2.00 mg/L), Pb (1.00–2.00 mg/L) and As (10.0–60.0 mg/L) were prepared from their corresponding 1000 mg/L stock solutions (Pan-reac, 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%, Rie- del-de Hain, Seelze, Germany), sodium chloride (99.8%, Riedel-de Hain, Seelze, Germany) and potassium dihydrogen phosphate (99.5%, Riedel-de Hain, Seelze, Germany) dissolution in ultrapure water, respectively. Standards and samples were acidified with 1% (v/v) suprapur nitric acid (65%; Sigma–Aldrich, Steinheim, Ger- many). Cesium chloride (99.5%; Sigma–Aldrich, Steinheim, Germany) was used as ionization chemical suppressor for the quantification by high-resolution continu-
low for cephalopods safety monitoring purposes considering the maximum heavy metal levels established in cephalopods (without viscera) by the European Commis- sion 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.

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 propane-2-ol, dichloromethane and 0.9% (v/v) KCl, in the presence of the internal standards (n-undecane and triundecane), 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 M in methanol) at 100 °C (10 min) and further methylated with BF3, (30 min at 100 °C) to convert the acetyl- unsaponifiables 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 lipid chromatography consisted of a Jasco integrated system (Japan) equipped with an LC-Nellett/ADCO 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 detector (FP-2020 Plus; ksec = 290 nm and ksec = 330 - nm; gain, 10). The chromatographic separation was achieved on a Supelcosil™ LC- Si column (75 x 3.0 mm, 3 ᵃm; 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 eluant at a flow rate of 0.8 mL/min. Data were analyzed with the ChronNAV 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 stan- dard 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-9505 autosampler. The tempera- tures of the injector and detector were 250 and 270 °C, respectively. Separation was achieved on a 50 m x 0.25 mm i.d CP-Sil 88 column (0.19 ᵃm 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 C22:6a-3) was accomplished by comparing the relative retention times of FAMEs of commercially available fatty acids methyl esters (Supelco®-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 isolomers (9z and 9t), 18:1 (7z and 9z), 20:1z, 22:1z and 24:1z while PUFA include 18:2n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, 18:3n-3, 20:5n-3, 22:5n-3 and 22:6n-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 convert- 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-3A-6, DHA ± EPA sum, athereogenicity index (AI), thrombogenicity index (TI) and hypocholesterolemic/hypercholesterolemic ratio (HH) were determined (Guita irales et al., 2013). AI, TI and HH indexes were calculated using the Eqs. (1)-(3), respectively.

\[
AI = \frac{C_{18:2} + C_{18:3} + C_{20:3} + C_{22:5} + C_{22:6}}{C_{18:1} + C_{18:2} + C_{18:3} + C_{20:3} + C_{22:5} + C_{22:6}}
\]

\[
TI = \frac{C_{18:4} + C_{18:5} + C_{20:4}}{C_{18:3} + C_{20:3} + C_{22:3}}
\]

\[
HH = \frac{C_{18:1} + C_{18:2} + C_{18:3} + C_{20:3} + C_{22:3} + C_{22:4}}{C_{18:1} + C_{18:2} + C_{18:3} + C_{20:3} + C_{22:3} + C_{22:4}}
\]

The non-carcinogenic and carcinogenic risks through elemental intake were esti- mated 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 con-sumption 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 le-vel 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 carcinogenic effects. For carcinogenic, TR were estimated as the incremental probability of an individ- ual to develop cancer, over a lifetime, as a result of exposure to that potential car- cinogen (i.e., incremental or excess individual lifetime cancer risk; US EPA, 1989). Acceptable risk levels for carcinogens range from 10-⁶ (risk of developing cancer over a human lifetime is 1 in 10,000) to 10⁻¹² (risk of developing cancer over a hu-man lifetime is 1 in 1,000,000).

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

\[
THQ = \frac{E}{RfD}
\]

\[
TR = \frac{THQ}{RfD}
\]

where E is the exposure frequency (350 days per year), ED is the exposure dura- tion, IR is the food ingestion rate (1.37 x 10⁻³ g and 1.12 x 10⁻³ g kg per day per person for World and for Portuguese population, respectively; FAO, 2012); C is the metal concentration in cephalopods (g/g); RfD is the oral reference dose (0.001 g/kg/ day for Cd, 0.004 g/kg/day for Pb, 0.0030 g/kg/day for Cr (VI), 0.04 g/kg/day for Cu, 0.3 g/kg/day for Zn, 0.02 g/kg/day for Ni, 0.7 g/kg/day for Fe and 0.02 g/kg/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 x 365 days/year for non-carcinogenic ef-fects and 70 years x 365 days/year for carcinogenic effects); CSF is the oral carcin- ogenic slope factor from the Integrated Risk Information System database (8.5 x 10⁻³ (g/kg/day)⁰ 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 multi- ple 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 se-lected 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 (b 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. reynaudi > L. vulgaris > L. gahi > L. opalescens > L. duvauceli for Na; L. opalescens > L. reynaudi > L. gahi > L. duvauceli > L. vulgaris for K; L. opalescens > L. reynaudi > L. gahi > L. duvauceli > L. vulgaris for P; L. reynaudi > L. vulgaris > L. opalescens > L. duvauceli > L. gahi for Mg; L. vulgaris > L. reynaudi > L. opalescens > L. gahi > L. duvauceli 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. reynaudi. L. vulgaris and L. reynaudi showed the highest and very similar concentrations of Ca (105 mg/kg ww for L. reynaudi 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 macroelements except with L. vulgaris for Na and Ca and L. duvauceli for Na (Fig. 1A). Significant interspecific differences (p < 0.001) were also observed between the two species from the Indic Ocean (L. duvauceli and L. vulgaris) and also among those from the Atlantic Ocean (L. gahi and L. reynaudi) 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. duvauceli and L. gahi; Na > K > P > Mg > Ca for L. reynaudi; Na > K > Mg > K > Ca for L. vulgaris, and K > Na > P > Mg > Ca for L. opalescens. The most different profiles correspond to L. gahi and L. opalescens, and also to L. vulgaris but in a lesser extent. The concentrations of these minerals reflect the bio-logical 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, 297 (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. duvauceli and L. vulgaris) and also those from the Atlantic Ocean (L. gahi and L. reynaudi) 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. reynaudi (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. duvauceli, Zn > Cu > Fe > Ni > Mn > Cr for L. vulgaris and L. reynaudi; 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. reynaudi) 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. reynaudi). 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. reynaudi.

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. duvauceli > L. vulgaris > L. reynaudi for Cd and L. gahi > L. vulgaris > L. duvauceli > L. opalescens > L. reynaudi 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. reynaudi (0.14 (0.065–0.75) 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. duvauceli and L. vulgaris (from the same ocean), and L. duvauceli and L. reynaudi (from different geographical origin). Regarding Pb, only L. duvauceli 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. gu-hi > L. duvauceli > L. vulgaris > L. reynaudi, 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. duvauceli and L. vulgaris. No statistical differences were verified between L. vulgaris, L. duvauceli and L. reynaudi 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. duvauceli; 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. reynaudi; 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 quantitative/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. duvauceli was not statistically different from L. vulgaris (which are both from the same ocean) and L. reynaudi (from another ocean), being all significantly different (p < 0.05) from L. opalescens. DHA (C22:6x-3) ranged from 210 mg/100 g ww for L. duvauceli 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. duvauceli and L. vulgaris, as well as among L. gahi and L. reynaudi 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. duvauceli > L. vulgaris > L. reynaudi). Statistical differences were verified among squid species except between L. duvauceli and the other species (p = 0.174). L. opalescens and L. gahi (p = 0.886), as well as L. vulgaris and L. reynaudi 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 med- ian concentrations: L. gahi > L. opalescens > L. duvauceli > L. reynaudi > L. vulgaris. Statistical differences (p < 0.05) were observed between L. gahi and the other species with the exception of L. opal- escens and L. reynaudi, as well as among L. opalescens and the other squids.

MUFA global median values ranged from 82 ± 11 mg/100 g ww (9.2 ± 0.7%) for L. gahi to 119 ± 13 mg/100 g ww (10.6 ± 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. duvauceli, L. vulgaris and L. reynaudi. C18:1, which is the sum of C18:1x-7 and C18:1x-9, con-
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. duvaucelli).

3.3. Cholesterol concentrations

The order that describes the cholesterol median concentration by species is L. opalescens > L. gahi > L. vulgaris > L. duvaucelli > 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. duvaucelli 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. duvaucelli. Overall, vitamin E median values ranged from 0.76 ± 0.41 mg/100 g ww for L. duvaucelli to 3.0 ± 0.4 mg/100 g ww for L. opalescens.
A strong similarity was found between *L. vulgaris* and *L. duvaucelli* (*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 of scientific evidence. In dietetics or, when inadequate, 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 re-stricken 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, Al and UL (European Commission, 2008; EMEA, 2008; FNIC, 2010) (Table 2). The attained ranges are, by descending order, 12.6–116.2% for Ca > 25.0–63.2% for Na > 10.2–24.4% for Mg > 13.4–21.8% for P > 5.6–23.5% 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 Cu, classified 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 tolerable intakes were clearly below the respective PTMI established (25 l/kg body weight) (Table 3) (JECFA, 2011).

Regarding Pb, the previous PTWI (25 l/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 estimates ranged from 0.001 x 10⁻² to 3.23 x 10⁻² l/g/kg body weight per day. They are considerably below the exposure level of 1.2 l/g/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 charac- terized squid species may be considered safe regarding potential non-carcinogenic risks. Still, THQ values reached ca. 0.62 and 0.52 through *L. duvaucelli* 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 on 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 (CSFcin chemi- cal-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 consider- ation in this study, the TR values were clearly lower than the upper (10⁻⁶) 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/A-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/A-6 ratios ranging from 5.7 ± 0.7 (L. duvaucelli) to 17.7 ± 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 Al (0.43 ± 0.04 to 0.51 ± 0.02) and TI (0.17 ± 0.01 to 0.23 ± 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 ± 0.7 (L. reynaudii) to 2.5 ± 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 there- fore associated with the total fat and PUFA amounts.

3.6. Discriminant analysis

Firstly, CDA was tested to distinguish the oceanic origin of *L. duvaucelli*) from the Atlantic Ocean) (Table 3). Still the PUFA content varied (300 mg/day) from 5.7 ± 0.7 (L. duvaucelli) to 17.7 ± 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).

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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 there- fore 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...
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* 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 C20:4x6, C20:5x3 and C18:1 whereas CF2 is mainly affected by C16:0, C18:1, C20:1x9 and C20:5x3.

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 −10.8, −8.57 and 4.21 for L. duvaucelii, L. vulgaris and L. reynaudii, respectively).

Interpretation of the standardized coefficients of canonical variables showed that CF1 is more correlated with C18:0, C20:4x6, C18:1, C18:3x3, C14:0 and C22:5x6. 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, C20:5x3, C18:1, C22:6x3 and C20:1x9, 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, C18:3x3, C20:4x6 and C22:5x6), 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, C18:3x3 and C20:4x6. The compounds K, C22:5x6 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.

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**Fig. 2.** Canonical discriminant analysis of the characterized squid species (a) L. duvaucelii, L. vulgaris, L. reynaudii, L. gahi, L. opalescens) based on (a) individual fatty acids content, and (b) C14:0, C18:0, C18:1, C18:3x3, C20:4x6, C22:5x6, vitamin E, P, K and Cu concentration.

**Fig. 3.** Canonical discriminant analysis of the characterized squid species (a) L. duvaucelii, L. vulgaris, L. reynaudii, L. gahi, L. opalescens) based on (a) individual fatty acids content, and (b) C14:0, C18:0, C18:1, C18:3x3, C20:4x6, C22:5x6, vitamin E, P, K and Cu concentration.
(this species can be differentiate 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 (Lour- enço et al., 2009). The results reached for this species are comparable with those published by Lourenço 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 proper-ties. 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 (Manso et al., 2007; Falandysz, 1989). 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; Lourenço 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 sam-plies from the Atlantic Ocean and the NW Mediterranean sea (Lour- enço 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. duvaucellii 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 con- centrations, 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 prod- ucts 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 dis- tribution is quite different. L. gahi is almost exclusively captured in Argentina while L. reynaudii resides directly along the South Afri- can coast (FAO, 2010). Cd and Pb are ubiquitous in the environ- ment 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 organ- isms (fish, crabs and shrimp, mollusc, and other juvenile squids), detritus and sediments (Croxton 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 Reg- ulation (1 mg/kg ww for Cd and Pb) (European Commission, 2006). However, five samples of L. duvaucellii (2.10–3.67 mg/kg ww), six- teen 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 concentra- tion ranges are similar to those indicated for L. vulgaris (Manso et al., 2007; Storelli, 2008), for L. opalescens (Galiotopoulou et al., 2009), and for L. gahi (Seixas et al., 2005) for Cd. The levels reported for L. vulgaris (Lourenço et al., 2009) and for L. gahi (Falundys, 1989) for Pb, as well as for L. duvaucellii 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. duvaucel- li from Thailand (SFA 41.3%, MUFA 14.1% and PUFA 44.7%; Chedolah 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 ob-served 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 acid compositions on an edible basis (mg/100 g w) 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 impor-tant 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 cel- lular 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 Mustard, 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%)
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 rep- resentativeness 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 spe- cies. Literature data was only found for L. duvauceli 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. duvauceli (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 accor- dance 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 hatching and juvenile L. vulgaris, respectively. This discrepancy can be explained because hatching and juvenile cephalo- pods have higher content of PUFA as compared to adult stage and since oxidation occurs more easily in long chain PUFA, 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 ra- tios are observed for all species except for L. opalescens which may suggest an increased risk of developing cardiovascular prob- lems when consumed at high levels (Lourenço et al., 2009). Overall, the results found for L. vulgaris are in agreement with those pub- lished 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 rela- tion between the pro-atherogenic (mainly saturated) and the anti-atherogenic (unsaturated) fatty acids. The first enhances the adhe- sion 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 seabass (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 re-port for other highly consumed fish species (Afonso et al., 2013). Overall, moderate consumption of these squid species is ad- vised, 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 po- tential 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. vul- garis seems to be the more appreciated squid species in Portugal (INE, 2012) which does not pose any risks. Nevertheless, L. duvauceli, L. gahi, L. reynaudii and L. opalescens are important squid spe- cies worldwide, with special significance for the populations at the sampling sites. L. duvauceli is distributed throughout the Medi- terranean 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 Nami- bia. L. opalescens has become the most important fishery in Califor- nia, 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 esti- mation of potential health benefits/risks for human consumption of five commercially valuable squid species from different geo-graphical origins (Atlantic, Indic and Pacific Oceans). Attending to the daily intake of the majority of macro and micronutrients, and to the nutritional lipiddescriptors 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. opal- escens, 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- carcinogetic risks suggested that L. duvauceli 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 Pro-ject PTDC/AGR-AMA/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 grate-ful 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


