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Amino acids, fatty acids and sterols profile of some marine organisms from Portuguese waters

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ABSTRACT

Marine organisms have been increasingly regarded as good sources of new drugs for human therapeutics and also as nutrients for human diet. The amino acids, fatty acids and sterols profiles of the widely consumed echinoderms *Paracentrotus lividus* Lamarck (sea urchin), *Holothuria forskali* Chiaje (sea cucumber), the gastropod molluscs *Aplysia fasciata* Poiret and *Aplysia punctata* Cuvier (sea hares), from Portuguese waters, were established by GC–MS analysis. Overall, 10 amino acids, 14 fatty acids and 4 sterols were determined. In general, all species presented the 10 amino acids identified, with the exceptions of *H. forskali*, in which no glycine, proline, *trans*-4-hydroxy-proline or phenylalanine were found, and of *A. fasciata* which did not contain proline. Unsaturated fatty acids were predominant compounds, with those from the ω-6 series, being in higher amounts than their ω-3 homologues, and cholesterol being the main sterol. The amino acids, fatty acids and sterols qualitative and quantitative composition of *A. fasciata, A. punctata* and *H. forskali* is reported here for the first time.

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1. Introduction

Marine organisms have been increasingly regarded as a promising source of new bioactive molecules for the pharmaceutical industry. In addition, many echinoderms and molluscs are regarded as excellent sources of nutritionally important compounds, such as fatty acids (Latyshev, Kasyanov, Kharlamenko, & Svetashev, 2009; Ozogul, Duysak, Ozogul, Özkütük, & Türeli, 2008; Usydus, Szlinder-Richert, Adamczyk, & Szatkowska, 2011), amino acids (Hamdi, 2011; Xu, Yan, & Xu, 2012; Zarai et al., 2011) and sterols (Kanazawa, 2001; Kandyuk, 2006; Zhukova, 2007).

Fatty acids are essential for life, due to their pivotal role as a source of energy, membrane constituents, as well as metabolic and signalling mediators. Compounds with two or more double bonds are named polyunsaturated fatty acids (PUFA) and the position of the first double bound is given by the (*n*–*x*) notation, counting the number of carbon atoms from the methyl group (the ω end). A review on the chemistry and distribution of fatty acids, in marine animals, was recently published (Pereira et al., 2011). Due to their positive impact on human health, alternative sources of PUFA are being pursued, with marine sources being increasingly regarded as good alternatives. Within PUFA, ω-3 compounds are particularly relevant, as they have already proved to have a positive impact on human health (Miles, Banerjee, & Calder, 2004; Miles & Calder, 2012; Rees et al., 2006). For example, ω-3 fatty acids have been shown to ameliorate some inflammatory conditions (Calder, 2008) and to improve cardiovascular status (Cawood et al., 2010; Studer, Briel, Leimenstoll, Glass, & Bucher, 2005).

Amino acids are determinant metabolites in the homeostasis of an organism, mainly due to their role as protein building blocks, in the regulation of several cellular processes and also as precursors of other molecules, such as hormones and nitrogenous bases (Wu, 2009). Given the fact that some of these compounds (essential amino acids) cannot be synthesised by humans, a diet rich in this class of metabolites is mandatory for a healthy organism.

The term sterol refers to a compound with a fused cyclopentanophenanthrene ring with a 3-hydroxyl moiety. These compounds have been regarded to have a positive impact on human health, mainly due to their hypocholesterolemic activity, that arises from their marked similarity with cholesterol, thus competing for absorption (Plat et al., 2012; Vanstone, Raeini-Sarjaz, Parsons, & Jones, 2002;Ostlund, 2002).

In a previous work, we have developed a method for the simultaneous determination of several classes of metabolites in marine organisms, including amino acids, fatty acids and sterols (Pereira et al., 2012). In the present work, four macro invertebrates were analysed. The metabolic profile of two molluscs (*Aplysia fasciata* Poiret and *Aplysia punctata* Cuvier) is reported here for the first time. Two echinoderms, *Holothuria forskali* Chiaje and

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Paracentrotus lividus Lamarck, were also studied and the metabolic profile of the former is described here for the first time; some studies are available regarding the latter (Arafa, Chouaibi, Sadok, & El Abed, 2012; Martínez-Pita, García, & Pita, 2010; Mol, Baygar, Varlik, & Tosun, 2008), but, to our knowledge, no absolute quantification has been reported. These echinoderms are consumed worldwide (Andrew et al., 2002; FAO, 2008) and a detailed knowledge of their chemical composition is needed.

2. Material and methods

2.1. Standards and reagents

Nonanoic acid (⩾99%), decanoic acid (⩾99%), lauric acid (⩾95%), myristic acid (⩾99%), pentadecanoic acid (⩾99%), palmitic acid (⩾99%), margaric acid (⩾98%), stearic acid (⩾99%), arachidonic acid (⩾99%), linolenic acid (⩾99%), methyl linolelaidate (⩾99%), oleic acid (⩾99%), 5,8,11,14,17-eicosapentaenoic acid (EPA) (⩾99%), *cis*-11,14-eicosadienoic acid (⩾98%), *cis*-11-eicosaenoic acid (⩾99%), docosahexaenoic acid (DHA) (⩾98%), arachidic acid (⩾97%), arginine (⩾98%), asparagine (⩾98%), aspartic acid (⩾98%), cysteine (⩾98%), glutamic acid (⩾98%), glutamine (⩾98%), histidine (⩾98%), lysine (⩾98%), methionine (⩾98%), tryptophan (⩾98%), tyrosine (⩾98%), alanine (⩾98%), glycine (⩾99%), valine (⩾98%), leucine (⩾98%), isoleucine (⩾98%), proline (⩾99%), serine (⩾99%), threonine (⩾98%), *trans*-4-hydroxy-proline (⩾98%), phenylalanine (⩾98%), norvaline (⩾99%), cholestan-3,5-diene (⩾95%), 5-α-cholestan-3-ol (⩾95%), cholestanol (⩾99%), ergosterol (⩾95%), β-sitosterol (⩾97%), desmosterol (⩾85%) and *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) were from Sigma and ethanol was from Panreac.

2.2. Organisms

Samples were from Portuguese waters: *H. forskali* and *P. lividus* were collected in the area of Peniche (west Portugal coast), in September 2009; *A. fasciata* was collected in October, 2009 and *A. punctata* in July, 2010, both in the Óbidos lagoon (Foz do Arelho). Specimens were placed on ice and transported to the laboratory. The organisms were then cleaned and washed with sea water. In the case of *P. lividus*, individuals were cracked and the coelomic fluid and gonads collected. Samples were kept at −20 °C, prior to freeze-drying in a Labconco 4.5 Freezone apparatus (Kansas City, MO, USA). The dried material was powdered and sifted (<910 μm) before extraction. Each sample corresponds to a mixture of 2–4 individuals.

2.3. Metabolites extraction

Each sample (100 ± 1.00 mg) was transferred to a glass vial and the internal standards for amino acids (norvaline), fatty acids (methyl linolelaidate) and sterols (desmosterol) were added. The volume was then made up to 2 ml with ethanol. The mixture was stirred at 200 rpm, for 20 min, at 40 °C. The extract was filtered and 50 μl was transferred to a glass vial. The solvent was evaporated under a nitrogen stream and 50 μl of the derivatization reagent, MSTFA, was added to the residue. The vial was capped, vortexed and heated for 20 min in a dry block heater, maintained at 40 °C. All extractions and analysis were performed in triplicate.

2.4. GC–MS analysis

The GC–MS conditions used herein were the ones used by Pereira et al. (2012). A Varian CP-3800 gas chromatograph coupled to a Varian Saturn 4000 mass selective ion trap detector (USA) and a Saturn GC/MS workstation software version 6.8 was used, with a VF-5 ms (30 m × 0.25 mm × 0.25 μm) column (VARIAN). A CombiPAL automatic autosampler (Varian, Palo Alto, CA) was used for all experiments. The injector port was heated to 250 °C and the injections were performed in split mode, with a ratio of 1/40. The carrier gas was helium C-60 (Gasin, Portugal), at a constant flow of 1 ml/min. The Ion Trap detector was set as follows: transfer line, manifold and trap temperatures were 280, 50, and 180 °C, respectively. The mass ranged from 50 to 600 *m*/*z*, with a scan rate of 6 scan/s. The emission current was 50 μA and the electron multiplier was set in relative mode to an auto tune procedure. The maximum ionisation time was 25.000 μs, with an ionisation storage level of 35 *m*/*z*. The injection volume was 2 μl and the analysis was performed in Full Scan mode. The oven temperature was set at 100 °C for 1 min, then increasing 20 °C/min to 250 °C, held for 2 min, 10 °C/min to 300 °C and held for 10 min. All mass spectra were acquired in the electron impact (EI) mode. Identification of compounds was achieved by comparison of their mass spectra, with those from pure standards analysed under the same conditions, and from the NIST05 MS Library Database.

For quantification purposes, each sample was injected in triplicate and the amount of metabolites was determined from the calibration curves of the respective trimethylsilyl (TMS) standards. All compounds were quantified in Full Scan mode, with the exception of linoleic (*m*/*z* 262, 337 and 352), linolenic (*m*/*z* 191, 335 and 350) and oleic (*m*/*z* 264, 339 and 354) acids that were quantified, by the area obtained from the re-processed chromatogram, using the characteristic *m*/*z* fragments.

3. Results and discussion

The technique used in this study was first validated with the echinoderm *Marthasterias glacialis* L. (Pereira et al., 2012). For this reason, in our work, recovery experiments for the analysed species were conducted (Table 1). Representative compounds of the three classes studied were used: isoleucine (amino acids), hexadecanoic acid (fatty acids) and 5-α-cholestan-3-ol (sterols). Two different concentrations were tested, one lower and the other higher, and the values of recovery were in the ca. 89–117% range, with the exception of the lower concentration of hexadecanoic acid in *H. forskali*, which corresponded to ca. 65%. However, this low value of recovery was found solely for *H. forskali* and for this particular compound, which could be a consequence of a matrix effect and not be related to the technique itself. Recovery for isoleucine was in the range ca. 93–112% and 5-α-cholestan-3-ol was ca. 92–117%.

3.1. Amino acids

The majority of the 10 amino acids detected (Fig. 1), could be found in all samples, with the exception of glycine, *trans*-4-hydroxy-proline and phenylalanine, which were not detected in *H. forskali*, and of proline which was not found in neither this species or in *A. fasciata* (Table 2). *A. punctata* contained the highest amount of amino acids, followed by *P. lividus* (Table 2).

In both *Aplysia* species and in *H. forskali* the major amino acid was alanine, which accounted for over 49% of the determined amino acids. In contrast to this, in the echinoderm *P. lividus* glycine was the major amino acid (Table 2), a trend that has already been reported in the echinoderm *M. glacialis*, collected in different seasons and in locations similar to those of the sample used herein (Pereira et al., 2012).

Threonine was the amino acid present in lower amounts in *Aplysia* sp., whilst in the echinoderms *P. lividus* and *H. forskali*, phenylalanine and valine were the minor amino acids, respectively (Table 2).

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