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Determination of toxic elements (Pb, Hg, Cd, As) and fatty acids in muscles and cephalothoraxes in a Mediterranean and a northern rose shrimp: a comparative study of *Parapenaeus longirostris* and *Pandalus borealis*.

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Introduction

Parapenaeus longirostris (Lucas 1846, commercial name: deep seawater rose shrimp) is one of the species of the Mediterranean Sea which is found at depths ranging from 20 to 750 m. *Pandalus borealis* (Kr¢yer 1838, commercial name: northern shrimp) is one of the species of the North Atlantic, found at depths ranging from 20 to 1380 m. Both have a characteristic rose colour, they are small in size and they are widely consumed, comprising an important source of nutrients such as ω-3 fatty acids and minerals to human. However, anthropogenic activities have increased the pollution to the seawater, increasing the levels of the toxic elements such as lead (Pb), mercury (Hg), cadmium (Cd) and arsenic (As) which their impact to human's health is worldwide-recognized.

Shrimps

Aim

The aim of the present work was to observe whether these species are suitable for human consumption in terms of toxicology and also whether the fatty acids of the total lipid (TL) are in desirable nutrition levels. Cephalothoraxes (head including hepatopancreas) were studied due to their economical value of the pharmaceutical industry for the production of medicines and food supplements.

Materials and Methods

Determination of Lead, Cadmium and Arsenic

Digestion in a microwave system by the use of acids (HNO_3 and H_2O_2)

Final determination by Inductively Coupled Plasma Mass Spectrometry (ICP-MS 7500ce, Agilent Technologies)

Instrumental conditions: RF power: 1500 W; Plasma gas flow: 15 L/min; Carrier gas flow: 0.9 L/min; Make-up gas flow: 0.16 L/min; Torch: 2.5 mm i.d.; Nebulizer: Babington; Integration time:1000 ms

Determination of Inorganic Arsenic

Extraction in waterbath with 0.1M HNO_3/ 3% H_2O_2.

Final determination by High Performance Liquid Chromatography coupled to ICP-MS (HPLC 1260, / ICP-MS 7500ce, both Agilent Technologies)

Instrumental conditions: Column: Anion Exchange (Trangenomic, 120*4.6mm); Method: Isocratic elution; Injection Vol.: 25 µl; Flow: 1 mL/min.; Column Temperature: 30°C; Mobile phase: 50 mM (NH4)2CO3 in 3% MeOH (10 min.)

Determination of Mercury

Digestion in a microwave system by the use of acids

Final determination by Cold Vapour Atomic Fluorescence Spectrometry (CVAFS) purge and trap dual amalgamation thermal extraction manual system coupled with Tekran detector, according to EPA method 1631

Determination of fatty acids of total lipids (TL)

Extraction of fat oil by the Bligh and Dyer method [1] and further transesterification to methyl esters (FAMEs) using a base-catalysed transesterification followed by a Borontrifluoride catalysed esterification according to AOCS [2]

Final determination by Gas Chromatography (GC) (Agilent 7890A) - equipped with a Flame Ionization Detector (FID)

Instrumental conditions: a DB-WAX fused silica capillary column (10 m* 0.1 mm, 0.1 lm; Agilent Technologies) using helium as carrier gas.

Oven temperature programme: initial 160 °C, 10.6 °C/min to 200 °C, hold 0.3 min and 10.6 °C/min to 220 °C, hold 1 min and 10.6 °C/ min to 240 °C, hold 3.8 min. A split ratio of 1:25 was used.

ICP-MS 7500ce, Agilent Technologies

Agilent 7890A GC-FID

Tekran CVAFS

Results & Conclusion

All toxic elements were found in higher levels in cephalothoraxes compared to muscles in both types of shrimp. Pb and Hg were found in higher levels in the muscles and cephalothoraxes of the Mediterranean shrimp in comparison with the northern. Nevertheless, both contained much lower concentrations than the maximum limits given by the European legislation (0.5 mg/kg for both elements) which is also the limit for Cd that was not exceeded in this study. In contrast, there is no regulatory limit yet for As, but the Environmental Protection Agency (US-EPA) and the World Health Organization (WHO) provide consumption advisories such as reference dose, Provisional Tolerable Weekly Intake (PTWI) [3,4] etc as calculated and shown in **Table 1**. Noticeably, the levels of total As were more than twice as high in the muscles of the northern shrimp, however it was not detected the inorganic form of As in the edible muscle tissue of the shrimps.

The cephalothoraxes contained higher fat content (~5g/100g w. w.) than the muscles which contained only ~1% of fat in both species. Polyunsaturated fatty acids (PUFA) were the major fatty acids of the total lipids (TL) at a range of 34-42% in the tissues of the Mediterranean shrimp. In the northern shrimp, PUFA were the major fatty acids only in the muscles, while in the cephalothoraxes monounsaturated fatty acids (MUFA) were the predominant (52%).The saturated fatty acids (SFA) varied between 15 and 25% of TL in both species. Moreover, the muscles of both species were observed to be rich in Σω-3 and the ratio of ω-3/ω-6 was more than 4:1, in agreement with the recommendations of the U.K. Department of Health. (**Figure 1**). According to the **Table 2**, C20:5 ω-3 (EPA /eicosapentaenoic), C22:6 ω-3 (DHA /docosahexaenoic), C18:1 ω-9 (oleic acid) and C16:0 (palmitic acid) were the most dominant fatty acids in all tissues. The atherogenic (AI) and thrombogenic (TI) indices were very low as expected in marine food [6].

In conclusion, both species were considered as suitable of consuming because they had low fat content, they were rich in fatty acids, especially in ω-3 and the levels of the toxic elements were lower than the European legislation. The significant differences of the fatty acids and the toxic elements were probably due to the native habitats and the trophic characteristics of the seas.

Table 1: Estimated Weekly Intake per meal size (EWIm) in adult compared with the PTWI (µg/kg bw/week) [3-5] BDL=Below Detection Limit.

PTWI	Species	Muscles *P. longirostris*	Muscles *P. borealis*
	Metals	EWIm	EWIm
21	iAs	BDL	BDL
withdrawn	Pb	0.0312	0.0151
4	Hg	0.2642	0.0690
6	Cd	0.0323	0.4070

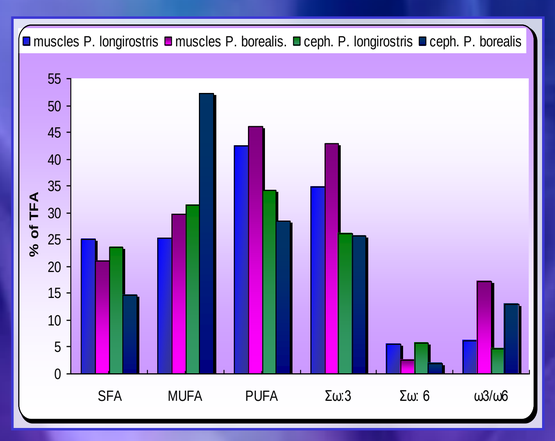

Figure 1: Fatty acid sum composition (% w/w) in total lipid of muscles and cephalothoraxes of both types of shrimp

Table 2: Fatty acid composition (%w.w.) and quality parameters of total lipids (TL) (muscles and cephalothoraxes of Mediterranean and northern shrimp)

	P. longirostris (Mediterranean shrimp)	*P. borealis* (northern shrimp)	*P. longirostis* (Mediterranean shrimp)	*P. borealis* (northern shrimp)
	Muscles	Muscles	Cephalothoraxes	Cephalothoraxes
C14:0	0.91±0.00a	2.24± 0.01b	1.78± 0.02c	2.97± 0.02d
C16:0	17.02± 0.03a	16.41± 0.01b	15.33± 0.01c	9.38± 0.04d
C18:0	5.21± 0.01a	1.58 ±0.00b	4.40± 0.01c	1.59± 0.01d
C 16 :1 (ω-7)	5.58± 0.00a	6.04± 0.00b	6.19± 0.06c	10.24± 0.00d
C18:1 (ω-9 cis)	14.23± 0.01a	11.44± 0.01b	14.59± 0.04c	11.68± 0.09d
C18:1 (ω-7 cis)	3.47± 0.01a	6.25 ±0.00b	4.53± 0.14c	4.65± 0.08d
C 20 :1 (ω-11)	0.89± 0.00a	3.44± 0.00b	3.41± 0.47c	9.92± 0.13d
C 20 :1 (ω-7)	0.45± 0.01a	0.53± 0.00b	1.47± 0.02c	2.02± 0.00d
C22:1 (ω-11)	0.09± 0.04a	1.55 ±0.01b	0.33± 0.04c	10.84± 0.09d
C18:2 (ω-6)	1.17± 0.01a	1.15 ±0.04b	1.15± 0.01c	1.00± 0.04d
C20:4 (ω-6 cis)	4.33± 0.01a	1.30± 0.01b	4.13± 0.04c	0.90± 0.01d
C 20 :5 (ω-3)	12.47± 0.02a	22.30± 0.00b	8.72± 0.12c	12.26± 0.03d
C22:6 (ω-3 cis)	19.93± 0.02a	18.30±0.01b	14.70± 0.23c	10.22±0.01d
AI	0.31	0.34	0.35	0.27
TI	0.18	0.12	0.21	0.12

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