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Original Article

Chemical composition, cholesterol, fatty acid and amino acid in two populations of brown crab *Cancer pagurus*: Ecological and human health implications

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ABSTRACT

The brown crab, *Cancer pagurus*, is a valued decapod species captured mainly in the UK and France. In Portugal, Scottish crabs and females of all species are less expensive than crabs caught in the English Channel and males. In this work the proximate chemical composition, cholesterol, fatty acid and amino acid content of female and male *C. pagurus* edible tissues from the Scottish coast and English Channel were compared. There was no evidence that the fishing ground influenced the chemical composition of tissues, but there were significant differences between tissues and sexes. Muscle was richer in protein, but poorer in fat and cholesterol, than gonads and hepatopancreas. Ovaries had more protein, fat, cholesterol and amino acids than testes. The fatty acid profiles in muscle and gonads were dominated by PUFA, while hepatopancreas was richer in MUFA and SFA. Lower *n*-3 fatty acid content and *n*-3/*n*-6 fatty acid ratio in hepatopancreas contributed to higher atherogenic and thrombogenic indices. Considering the chemical composition, there is no reason for price differentiation between crabs from different locations. As far as sex is concerned the principal difference that might increase male crabs' value is the meat yield content of claws, which was higher than females' claws.

1. Introduction

Worldwide, crustaceans are highly appreciated and are considered luxury seafood items. Although their frequent consumption is not advisable in general, either because of allergenic reactions or the supposedly high cholesterol content, there are a growing number of studies promoting crustacean consumption (Rosa and Nunes, 2003a; Gökoolu and Yerlikaya, 2003; Küçükgülmez et al., 2006; Chen et al., 2007; Teixeira et al., 2008; Barrento et al., 2008a, 2009a,b).

Despite this controversy, crustaceans are widely appreciated and consumed in Mediterranean countries. In particular, the crab *Cancer pagurus* is a valued decapod species in France, Spain, Portugal and Italy, where the muscle from claws and abdomen and the brown meat (gonads and hepatopancreas) are appreciated. In these countries the market trend is still the commercialization of live crabs harvested in the waters off Britain (31,079 tonnes), Ireland (11,525 tonnes) and France (5724 tonnes) (Tully et al., 2006; data from 2007, EUROSTAT, 2009). The major export countries to Portugal are UK and France. This species shows different market prices according to the fishing ground and sex. In fact, crabs caught off the English Channel are usually more expensive than those caught off Welsh, Scottish and Irish coasts, presumably due to the intrinsic quality of these populations (e.g. bigger animals that undergo fewer hours of transport and which are therefore more vigorous upon arrival) (Barrento et al., 2008b). In addition, males are more expensive than females (Brown and Bennett, 1980).

It is well known that the biochemical composition of edible tissues of marine invertebrates is influenced by their nutritional habits, age, sex, season, seawater temperature and salinity (Chapelle, 1977; Oliveira et al., 2007; Souchet and Laplante, 2007). Since crab populations from different fishing grounds might have distinct biochemical patterns in the edible tissues, the purpose of this study was to determine the nutritional quality of

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Table 1
Summary of reference material elemental concentration ($\mu g\,g^{-1}$ DW; $n=4$) and detection limits (DL) of certified reference material (±standard deviation) analyzed by the different techniques.

Elements	Technique	DL	Certified biological material	Certified	Present work
Moisture	Drying	nd	Canned matrix meat – SMRD 2000	68.8 ± 0.1	68.7 ± 0.0
Protein	Kjeldahl	nd	Canned matrix meat – SMRD 2000	1.63 ± 0.05	1.62 ± 0.06
Total lipids	Soxhlet	0.01	Canned matrix meat – SMRD 2000	14.3 ± 0.4	14.4 ± 0.1
Ash	Combustion	0.1	Canned matrix meat – SMRD 2000	2.65 ± 0.07	2.69 ± 0.03
Fatty acids					
14:0	GC	0.4-1	Beef pork fat blend – BCR-163	2.29 ± 0.04	2.23 ± 0.04
16:0	GC	0.4-1	Beef pork fat blend – BCR-163	25.96 ± 0.30	25.39 ± 0.017
16:1	GC	0.4-1	Beef pork fat blend – BCR-163	2.58 ± 0.16	2.22 ± 0.03[a]
18:0	GC	0.4-1	Beef pork fat blend – BCR-163	18.29 ± 0.16	17.65 ± 0.06
18:1	GC	0.4-1	Beef pork fat blend – BCR-163	38.34 ± 0.36	38.65 ± 0.16[b]
18:2	GC	0.4-1	Beef pork fat blend – BCR-163	7.05 ± 0.17	7.19 ± 0.03[c]
18:3	GC	0.4-1	Beef pork fat blend – BCR-163	0.86 ± 0.14	0.81 ± 0.01[d]
Amino acids	HPLC	nd	No reference material used	nd	nd
Cholesterol	GC	nd	No reference material used	nd	nd

Reference materials suppliers and location: SMRD-2000 (Swedish Meats R&D and Scan Foods/National Food Administration, Sweden), BCR-163 (Institute for Reference Materials and Measurement, Belgium). *Abbreviations*: gas chromatography (GC), high-performance liquid chromatography (HPLC), not determined (nd).
[a] 16:1*n*-7 + 16:1*n*-5.
[b] 18:1*n*-9 + 18:1n-7.
[c] 18:2*n*-6.
[d] 8:3*n*-3.

C. pagurus harvested in the English Channel and Scottish coast by determining the proximate chemical composition, amino acid and fatty acid profiles, and cholesterol content. Data were also analyzed to evaluate the nutritional quality of *C. pagurus* edible tissues to human consumption by calculating the atherogenic and thrombogenic indices, the essential amino acid scores and three ratios related with fatty acid content: DHA/EPA (docosahexaenoic acid/eicosapentaenoic acid), EPA/DHA and PUFA/SFA (polyunsaturated fatty acids/saturated fatty acids).

2. Materials and methods

2.1. Biological material

Twenty *C. pagurus* specimens caught off the Scottish coast in summer (SC; 10 females and 10 males) and 20 specimens from the English Channel (EC; 10 females and 10 males) were purchased live and transported to the laboratory. The biological material was the same used in previous studies published by Barrento et al. (2009a,c). Animals were kept under refrigerated conditions (5 °C) during 1 h to decrease their metabolism, and stunned before being euthanized by piercing the two nerve centres by means of a stainless steel rod. The rod was inserted through one of the eyes and through the vent as recommended by the Codex Alimentarius Commission (FAO/WHO, 1983).

Several parameters were recorded for each crab: sex, total weight (Omega PP 50\1; d = 1 g), claw muscle, gonad and hepatopancreas weight (Mettler PM 400; d = 0.001 g), carapace width and length (digital caliper Comecta; 0.01 mm), and ovarian maturity stage. The ovarian maturity scale was adapted from Edwards (1979). Females were classified as immature/no ovary development (Stage 1: S1); initial ovary development (Stage 2: S2); developing orange ovaries extending into carapace (Stage 3: S3); and ripe, carapace full of bright red ovary material (Stage 4: S4). Gonadosomatic (GSI) and hepatosomatic (HI) indices were calculated as well as claws muscle meat yield (MY) and the total meat (TMY) yield. The GSI, HI and MY, were calculated as follows: (tissue wet weight/body wet weight) × 100; the TMY was calculated according to the formula: (gonad wet weight + hepatopancreas wet weight + claw muscle wet weight)/(body wet weight) × 100. For each tissue the edible content was also calculated as follows: (tissue wet weight/sum of all edible tissues wet weight) × 100. For the biochemical analyses, the edible tissues of at least two crabs were pooled taking into account sex, ovarian maturity stage and location, and were homogenized with a grinder (Retsch Grindomix GM200, Düsseldorf, Germany; 5000 rpm; material: PP cup and stainless steel knifes), vacuum packed and stored at −20 °C. A portion of each frozen sample was freeze-dried for 48 h at −50 °C and low pressure (approximately 10^{-1} atm; Heto power dry LL 3000), and stored at −20 °C under controlled moisture conditions (vacuum packed) until further analyses.

2.2. Proximate chemical composition and energy content

Moisture, ash, protein and lipid contents were determined in duplicate for each sample, according to the AOAC (2005) methods that were validated with reference material (Table 1). Briefly, the moisture content was obtained by drying the sample overnight at 105 °C (laboratory heater, P-Selecta 207); ash was quantified after combustion for 16 h at 550 °C (muffle furnace, Heraeus Hanau, TYPMR170); crude protein content was determined by the Kjeldahl method using an automatic distillation and titration unit (VELP Scientifica, UDK152) with a conversion factor of 6.25; and total lipid content was determined with the Soxhlet extraction method using ethyl ether (40–60 °C; 7 h; heater plate SBS Instruments PC6L). The results were expressed in g per 100 g wet weight. The energy content was estimated as: proteins, 4.27 kcal g^{-1} wet weight; lipids, 9.02 kcal g^{-1} wet weight; 1 kcal = 4.184 kJ (FAO, 1987).

2.3. Cholesterol

Cholesterol content was determined in duplicate for each sample, and was based on the modified procedure of Naeemi et al. (1995). Briefly, each sample (300 mg of dry weight) was combined with 260 μL of the internal standard solution (5α-cholestane Sigma; 5 mg mL^{-1} cyclohexane, Merck), 3 mL of saturated methanolic potassium hydroxide solution (2 M, Merck), and 3 mL of methanol pro analysis (Merck). Following heating (80 °C; 30 min water bath), samples were cooled and supplied with 250 μL of magnesium chloride solution (1 M, Merck) and 1.5 mL of cyclohexane pro analysis (Merck). Samples were shaken and centrifuged (1500 × *g*; 4 min, centrifuge Sigma 2K15) until phase separation. The moisture content of the upper phase was removed with anhydrous sodium sulfate (Panreac). The cholesterol in the upper phase (2 μL) was separated by gas chromatography