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ARTICLE

Effect of Season on the Chemical Composition and Nutritional Quality of the Edible Crab *Cancer pagurus*

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Cancer pagurus is most appreciated in southern Europe for its muscle and brown meat content. In Portugal, consumption occurs mostly in summer and at Christmas. In this study the seasonal nutritional quality of edible tissues of female and male *C. pagurus* was determined. Tissue composition was affected by season and sex. All tissues had a well-balanced essential amino acid composition. Muscle and gonads of females had n-3/n-6 ratios in the range of the usual recommended values, and all tissues had PUFA/SFA above the recommended level. Autumn was the season with the highest brown meat yield, total essential amino acids (muscle), taurine (all tissues), EPA (male gonads), and n-3/n-6 ratio (gonads). However, it was also the season with the highest fat content and cholesterol concentration in ovaries. Therefore, people with restricted diets should moderate the consumption of ovaries in autumn. The remaining tissues pose no risks with respect to their proximate chemical composition.

KEYWORDS: Hepatopancreas; gonads; cholesterol; atherogenic index; thrombogenic index; seafood; crustaceans

INTRODUCTION

In recent years, there has been a notable promotion of seafood consumption, based on its importance as part of a healthy diet, mainly due to its content of high-quality protein, low saturated fat, and high omega-3 fatty acids (*1*). Among seafood, crustaceans are nutritionally valuable sources of protein and minerals, but they have often been rejected as a healthy food due to their presumably high levels of cholesterol (*2*). Nevertheless crustaceans are widely appreciated and, in particular, the European edible crab, *Cancer pagurus*, is one of the most important commercial crabs targeted by fishing fleets throughout Britain (31079 tones), Ireland (11525 tones), Norway (8510 tones), and France (5724 tones) (*3*, *4*). The major markets are located in France, Spain, Portugal, and Italy, where the muscle (white meat from claws, legs, and abdomen) and brown meat (hepatopancreas and reproductive organs) are much appreciated. In Portugal, where *C. pagurus* is the most marketed live crustacean, crabs are consumed all year round but with consumption peaks during the summer holidays and Christmas festivities (*5*). Also, larger males are traditionally more expensive than smaller males and females (*6*). In their habitats, crustaceans are exposed to a large number of environmental variables, which follow annual and daily cycles according to the geographical region and that affect their behavior, migration patterns, feeding, and metabolism. Therefore, biological responses to seasonal variations may have a significant effect on the crustaceans' biochemical composition, particularly in hepatopancreas and gonads (*7*). Because the nutritional data of this species is still incomplete, the purpose of this study was to determine the seasonal nutritional quality of *C. pagurus* by determining the proximate chemical composition, amino acid and fatty acid profiles, and cholesterol content of males and females. Moreover, considering the controversy of the benefits related to shellfish consumption and the potential impact in consumer's diet regarding the different edible tissues and consumption seasonality, samples were taken during the four seasons. Data was also analyzed to evaluate the nutritional quality in human consumption by calculating the atherogenic (AI) and thrombogenic (TI) indices, the essential amino acid scores (AS), and three ratios related with fatty acid content, namely, the DHA/EPA (docosahexaenoic acid/eicosapentaenoic acid), EPA/DHA, and PUFA/SFA (polyunsaturated fatty acids/saturated fatty acids).

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MATERIAL AND METHODS

Ethical Statement. All live animals utilized in the experiments have been treated with proper care, minimizing discomfort and distress, and were painlessly killed. Also, the number of animals was kept to the minimum necessary to obtain scientific results, considering that the gain in knowledge and long-term benefit to the subject species are high.

Biological Material. *C. pagurus* caught in the Scottish coast was sampled during spring (May, $n = 24$; 12 females, 12 males), summer

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(August, n = 20, 10 females, 10 males), autumn (October, n = 20, 10 females, 10 males), and winter (March, n = 20, 10 males, 10 females) of 2008. Every season, intermoult crabs were live-transported to the laboratory. 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 centers by means of a stainless steel rod. The rod was inserted through one of the eyes and through the vent as recommended in Codex Alimentarius, 1983 (*8*). For each crab several parameters were recorded: sex, total weight, gonad and hepatopancreas weight, carapace width and length, and maturity stage (only for females). The ovarian maturity scale was adapted from Edwards (*9*). Females were classified as follows: stage 1 (S1), immature, that is, no ovarian tissue was observed; stage 2 (S2), initial ovary development, that is, ovaries with a small spot of light orange found near the posterior part of the carapace; stage 3 (S3), developing orange ovaries extending into carapace; and stage 4 (S4), ripe ovaries, that is, carapace full of bright red ovary material. For both sexes, gonadosomatic (GSI) and hepatosomatic (HI) indices were calculated, as well as the claw muscle meat yield (MY) and the total meat yield (TMY). The GSI, HI, and MY were calculated as follows: tissue wet weight (g)/body wet weight (g) × 100. The TMY was calculated according to the formula (gonad wet weight+hepatopancreas wet weight+claw muscle wet weight)/(body wet weight) × 100. For the biochemical analyses pools of two to three individual crabs were performed (depending on the amount of sample) taking into account sex, season, and maturity stage. These pools were homogenized with a grinder (Retasch Grindomix GM200; 5000 rpm; material: PP cup and stainless steel knifes); a portion of this homogeneized sample was vacuum packed and stored at −20 °C, whereas the remaining was frozen in a Petri dish (−80 °C) and subsequently freeze-dried for 48 h at −50 °C and low pressure (approximately 10^{-1} atm). Samples were powdered and stored at −20 °C under controlled moisture conditions (vacuum packed) until further analyses.

Proximate Chemical Composition and Energy Content. Moisture, ash, protein, and lipid contents were determined in each tissue according to the AOAC methodologies (*10*). 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, TYP. MR170); crude protein content was determined according to the Kjeldahl method using an automatic distillation and titration unit (VELP Scientifica, UDK152, Milano, Italy) with a conversion factor of 6.25; and total lipid was determined with the Soxhlet extraction method using ethyl ether. The results were expressed in grams per 100 g of wet weight. The energy content was estimated as protein, 4.27 kcal g^{-1} of wet weight; lipids, 9.02 kcal g^{-1} of wet weight (1 kcal = 4.184 kJ).

Cholesterol. The quantification of cholesterol content was based on the modified procedure of Naeemi et al. (*11*). 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 (Varian Star 3400 Cx, Walnut Creek, CA) using helium as carrier gas at a flow rate of 1 mL min^{-1} in a flame ionization detector and a fused silica capillary CP-Sil 8 CB columm (30 m length × 0.25 mm internal diameter, 0.25 μm film thickness; J&W Scientific, Folsom, CA). The temperatures of the oven, injector, and detector were 280, 285, and 300 °C, respectively. Cholesterol was identified and quantified by comparison with the calibration curve of a pure cholesterol standard with 5α-cholestane (Sigma). Cholesterol/cholestane peak area ratios obtained with the Varian software were plotted with cholesterol concentrations, and a straight line was fitted to data points by linear regression.

Fatty Acid Analysis. The percent distribution of fatty acids methyl esters (FAME) of nonpolar and polar lipids was based on the experimental procedure of Cohen et al. (*12*). Each sample (300 mg of dry weight) was dissolved in 5 mL of acetyl chloride/methanol (1:19 v/v; Merck), shaken, and heated (80 °C; 1 h). After cooling, 1 mL of Milli-Q distilled water and 2 mL of *n*-heptane pro analysis (Merck) were added, and samples were shaken and centrifuged (2000*g*; 5 min, Sigma 2k15) until phase separation. The moisture content of the upper phase was removed with anhydrous sodium sulfate (Panreac). An aliquot (2 μL) of the upper phase was then injected onto a gas chromatograph (Varian Star 3800 Cp) equipped with an autosampler and fitted with a flame ionization detector at 250 °C for FAME analysis. The separation was carried out with helium as carrier gas at a flow rate of 1 mL min^{-1}, in a capillary column DB-WAX (30 m length × 0.32 mm internal diameter; 0.25 μm film thickness; Hewlett-Packard, Albertville, MN) programmed at 180 °C for 5 min, raised to 220 at 4 °C min^{-1}, and maintained at 220 °C for 25 min, with the injector at 250 °C. FAME were identified by comparing retention times with those of Sigma standards. Quantitative data was calculated using the peak area ratio (percent of total fatty acids) and the Varian software.

Amino Acids. To extract total amino acids (protein bound + free), 40−90 mg of sample was placed in 10 mL ampules with 3 mL of 6 M HCl, according to the method described by AOAC methodologies (*10*). Ampules were vacuum sealed, and samples were hydrolyzed at 110 °C for 24 h; hydrolysates were frozen at −80 °C, freeze-dried, dissolved in 5 mL of 0.1 M HCl, 0.2 μm pore size filtered, and stored at −80 °C until amino acid separation. Separation was performed with high-performance liquid chromatography (Agilent 1100 HPLC, Palo Alto, CA) using precolumn *o*-phthalaldehyde and 3-mercaptopropionic acid in borate buffer (OPA, Agilent Technologies) and 9-fluorenylmethylchloroformate in acetonitrile (FMOC; Agilent Technologies) derivatization, a Phenomenex Gemini ODS C18 guard column (4 × 3 mm), and a Phenomenex Gemini ODS C18 110A column (4.6 × 150 mm, 5 μm). The solvents and gradient conditions were those described by Henderson et al. (*13*). Detection wavelengths were set at UV 338 and 262 nm and fluorescence 340/450 and 266/305 nm. The identity and quantity of the amino acids were assessed by comparison with the retention times and peak areas of standard amino acids (Sigma-Aldrich) using norvaline as internal standard.

Nutritional Quality. To measure the propensity of each edible tissue to influence the incidence of coronary heart disease, atherogenic (AI) and thrombogenic (TI) indices were calculated according to the following equations: AI = [12:0 + (4 × 14:0) + 16:0]/[MUFA + PUFA (n-6) + (n-3)]; TI = (14:0+16:0+18:0)/[(0.5 × MUFA)+(0.5 × PUFA (n-6))+(3 × PUFA (n-3)+(n-3)/n-6))] (MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids) by Ulbricht and Southgate (*14*). Three ratios related with fatty acid content were also calculated, DHA/EPA, EPA/DHA, and PUFA/SFA, to allow comparisons with the U.K. Department of Health recommendations (*15*). Essential amino acid scores (AS) were also calculated according to reference amino acid requirements of adults (*16*). This score compares the content of each essential amino acid in the protein/diet with its content in the requirement pattern: AS (%) = (mg of amino acid in 1 g of test protein/mg of amino acid requirement) × 100. The ratio of essential to nonessential amino acids was also calculated.

Statistical Analysis. All analyses were carried out in duplicate. Results were expressed as mean values ± standard deviation (SD). Data was analyzed by one-way ANOVA followed by multiple-comparison test (Tukey HSD). Whenever necessary, data was transformed to satisfy normal distribution and homoscedasticity requirements. If transformed data could not meet these assumptions, differences were analyzed with nonparametric analysis of variance (Kruskall−Wallis) followed by a nonparametric multiple-comparison test (Mann−Whitney). Principal component analysis (PCA) was also employed to reduce the multidimensional data sets of several elements to lower dimensions, thus simplifying the presentation and interpretation of data. All statistical analyses were tested at the 0.05 level of probability with the software STATISTICA 6.1. (Statsoft, Inc., Tulsa, OK).

RESULTS

Proximate Chemical Composition and Energy Content. The biometric data of animals sampled in this study is presented in **Table 1**. The meat yields are represented in **Table 2**. In general, males had more muscle meat yield (10.3−16.2%) than females (7.9−8.5%) but less gonad meat yield (males, 0.9−2.2%; females, 1.8−7.3%), whereas in hepatopancreas the only difference was observed in winter females (7.8%). The meat yield patterns in gonads during the four seasons were similar for males and

females, with increasing values from spring to autumn followed by a decrease in winter. The dominant maturity stage in autumn was S4 (100% females), in winter S2 and S3 were found in equal proportions (50%), and S3 was predominant in summer (40%; remaining females were in S2 and S4 with 30% each) and also in spring (58%; remaining females were S2). Significant differences were detected in the proximate chemical composition of edible tissues between tissues, seasons, and sexes (**Table 2**). In general, female gonads and muscle were rich in protein, whereas hepatopancreas had more fat. Gonads and hepatopancreas had higher energy and cholesterol contents compared to muscle. Female gonads showed significantly lower ash and moisture than male gonads, whereas no major differences were found in hepatopancreas and gonads. The protein content of muscle and hepatopancreas did not strongly vary through seasons (except in summer, muscle), whereas testes had a lower amount of protein than ovaries (except in winter). The fat content of muscle was significantly lower in winter males (0.1%), in the hepatopancreas it decreased in autumn (males) and winter (both sexes), and in ovaries it (3.1−5.6%) was always higher than in testis (0.9−1.3%), with maximum values obtained in winter in both sexes. The cholesterol content in hepatopancreas was constant throughout seasons, whereas muscle crabs sampled in spring showed the highest values. The cholesterol content increased in female gonads from spring (120.5 mg/100 g) to autumn (200.8 mg/100 g) followed by a strong decrease in winter (93.7 mg/100 g), whereas in males there was a decrease in winter (65.0 mg/100 g). Overall, females always had more cholesterol in gonads than males throughout the seasons. Statistical variations in the energy content were observed only in gonads, with ovaries showing higher energy content than testes regardless of season.

Table 1. Biometric Data [Weight, Carapace Width (CW), Carapace Length (CL), and Total Meat Yield (TMY)] of Female and Male *C. pagurus* Sampled in Each Season (Average ± Standard Deviation)

	weight (g)	CW (mm)	CL (mm)	TMY (%)
females				
spring	770.3 ± 2.8	163.9 ± 4.1	108.4 ± 3.0	21.6 ± 2.8
summer	748.0 ± 3.8	163.3 ± 2.3	107.4 ± 1.8	22.5 ± 3.8
autumn	770.0 ± 6.4	167.5 ± 18.2	107.7 ± 7.7	27.6 ± 6.4
winter	693.5 ± 2.1	159.8 ± 7.0	99.4 ± 3.7	17.5 ± 2.1
males				
spring	828.3 ± 2.4	160.9 ± 4.7	100.2 ± 2.1	25.6 ± 2.4
summer	751.0 ± 2.2	156.3 ± 3.0	98.4 ± 1.9	23.5 ± 2.2
autumn	869.0 ± 8.2	167.7 ± 18.0	103.6 ± 8.5	30.1 ± 8.2
winter	650.5 ± 3.5	153.7 ± 6.3	93.4 ± 3.4	21.1 ± 3.5

Fatty Acids. The fatty acid profiles were statistically different between tissues (**Tables 3** and **4**). Muscle and gonads had a similar pattern dominated by PUFA, followed by MUFA and SFA; in contrast, hepatopancreas had proportionally more MUFA, followed by PUFA and SFA (**Table 3**). Muscle had the most homogeneous fatty acid composition with fewer differences between sexes and seasons compared to gonads and hepatopancreas. In muscle the lowest and highest levels of SFA were obtained for females in winter and summer, respectively; MUFA values were generally lower in autumn and winter and higher in females during spring, and PUFA levels were constant throughout the year. Hepatopancreas had proportionally more SFA in autumn (both sexes) and less in winter (females); MUFA was lower in spring (females) and summer (males); and PUFA levels

Table 2. Tissue Indices [Muscle Meat Yield, Hepatosomatic, and Gonadosomatic Indices], Proximate Chemical Composition, Cholesterol Content, and Energy Content in Muscle, Hepatopancreas, and Gonads of Female and Male Crabs (Average ± Standard Deviation)[a]

		tissue indices (%)	moisture (%)	ash (%)	proteins (%)	fat (%)	cholesterol (mg/100 g of wet wt)	energy (kcal/100 g of tissue wet wt)
muscle								
females	spring	8.1 ± 0.5 b	77.8 ± 0.8 a	2.0 ± 0.2 a	18.2 ± 1.4 ab	0.3 ± 0.0 a	46.0 ± 3.6 a	365.7 ± 15.3 a
	summer	8.5 ± 1.8 b	77.8 ± 2.1 a	2.1 ± 0.2 a	16.4 ± 4.2 b	0.3 ± 0.1 a	37.3 ± 4.7 b	398.9 ± 39.4 a
	autumn	8.1 ± 3.2 b	76.2 ± 1.6 a	2.1 ± 0.2 a	19.1 ± 1.1 ab	0.3 ± 0.1 a	41.3 ± 1.7 ab	359.5 ± 42.5 a
	winter	7.9 ± 1.1 b	77.8 ± 1.7 a	2.1 ± 0.1 a	18.6 ± 1.4 ab	0.3 ± 0.1 a	35.7 ± 5.5 b	399.0 ± 43.0 a
males	spring	12.0 ± 2.1 a	76.0 ± 2.0 a	1.9 ± 0.1 a	19.2 ± 1.8 ab	0.3 ± 0.1 a	47.3 ± 2.2 a	370.6 ± 73.8 a
	summer	11.9 ± 2.2 a	74.6 ± 2.2 a	1.9 ± 0.1 a	20.5 ± 1.7 a	0.2 ± 0.1 a	37.0 ± 9.1 b	365.9 ± 59.9 a
	autumn	16.2 ± 3.0 a	77.4 ± 2.6 a	1.9 ± 0.1 a	17.3 ± 2.9 ab	0.3 ± 0.2 a	40.0 ± 4.3 ab	364.4 ± 32.8 a
	winter	10.3 ± 1.7 b	76.6 ± 2.0 a	2.0 ± 0.2 a	18.7 ± 1.9 ab	0.1 ± 0.1 b	36.7 ± 9.3 ab	382.1 ± 36.5 a
p		<0.001	0.002	0.052	0.001	<0.001	<0.001	0.807
hepatopancreas								
females	spring	11.1 ± 2.4 a	64.1 ± 4.7 a	3.2 ± 1.1 bc	11.6 ± 1.2 b	16.6 ± 5.1 a	110.6 ± 33.8 a	936.7 ± 193.5 a
	summer	10.4 ± 1.8 a	67.9 ± 7.0 a	3.0 ± 0.4 c	12.2 ± 1.6 b	10.2 ± 3.6 ab	116.6 ± 27.3 a	926.4 ± 130.9 a
	autumn	12.3 ± 3.2 a	64.3 ± 3.7 a	3.2 ± 0.3 b	14.5 ± 1.8 ab	10.2 ± 2.0 a	93.4 ± 12.9 a	700.1 ± 220.5 a
	winter	7.8 ± 1.1 b	64.0 ± 6.2 a	3.5 ± 0.3 b	12.9 ± 1.4 ab	7.2 ± 3.2 b	104.3 ± 11.7 a	818.8 ± 196.0 a
males	spring	12.5 ± 2.1 a	59.3 ± 4.4 a	4.4 ± 0.7 b	14.5 ± 0.9 ab	14.3 ± 3.0 a	92.5 ± 16.6 a	790.90 ± 88.1 a
	summer	10.3 ± 2.2 a	61.8 ± 7.3 a	5.0 ± 1.2 a	13.3 ± 1.9 ab	12.2 ± 3.4 a	112.4 ± 10.1 a	690.6 ± 167.1 a
	autumn	11.7 ± 3.0 a	67.6 ± 2.6 a	3.8 ± 1.8 b	13.2 ± 3.0 ab	6.5 ± 0.2 b	104.0 ± 27.1 a	714.9 ± 167.2 a
	winter	10.0 ± 1.7 a	60.3 ± 3.8 a	6.4 ± 0.7 a	15.8 ± 2.1 a	2.9 ± 0.6 c	94.1 ± 12.5 a	642.0 ± 56.2 a
p		<0.001	0.055	<0.001	0.002	<0.001	0.100	0.073
gonads								
females	spring	2.4 ± 1.0 b	61.9 ± 6.0 c	1.5 ± 0.0 c	23.1 ± 3.7 a	3.8 ± 1.5 ab	120.5 ± 7.7 b	721.7 ± 161.3 a
	summer	3.6 ± 2.0 ab	56.8 ± 1.6 c	1.6 ± 0.0 c	25.5 ± 0.0 a	3.1 ± 0.5 b	170.5 ± 5.2 a	804.6 ± 196.3 a
	autumn	7.3 ± 2.1 a	56.5 ± 2.5 c	1.7 ± 0.1 c	24.8 ± 5.9 a	3.3 ± 0.1 b	200.8 ± 15.3 a	815.5 ± 57.1a
	winter	1.8 ± 0.5 b	68.2 ± 5.5 c	1.6 ± 0.1 c	19.2 ± 3.4 ab	5.6 ± 2.4 a	93.7 ± 26.7 c	640.4 + 128.6 a
males	spring	1.1 ± 0.5 b	79.4 ± 0.0 a	2.9 ± 0.0 a	14.7 ± 0.4 c	0.9 ± 0.1 d	89.4 ± 9.5 c	331.7 ± 42b
	summer	1.4 ± 0.7 b	79.9 ± 0.6 a	3.0 ± 0.0 a	13.1 ± 0.0 c	0.9 ± 0.0 d	84.6 ± 4.3 c	268.0 ± 22b
	autumn	2.2 ± 1.1 b	80.4 ± 0.1 a	2.7 ± 0.0 b	13.9 ± 0.1 c	0.9 ± 0.1 d	83.4 ± 0.9 c	319.5 ± 15b
	winter	0.9 ± 0.3 c	77.0 ± 0.5 b	2.7 ± 0.0 b	14.7 ± 1.9 bc	1.3 ± 0.0 c	65.0 ± 0.5 d	387.1 ± 59b
p		<0.001	0.001	<0.001	0.002	0.004	<0.001	0.003

[a] In each column different letters indicate significant differences per tissue.

Table 3. Fatty Acid Profile (Percent) and Nutrition Quality Parameters of Edible Tissues of Female and Male Crabs during the Four Seasons (Average ± Standard Deviation)[a]

	females				males				
	spring	summer	autumn	winter	spring	summer	autumn	winter	*p*
				Muscle					
14:00	1.2 a	1.2 a	1.2 a	0.7 b	1.2 a	1.1 a	0.8 b	0.6 b	<0.001
15:00	0.5 a	0.5 a	0.5 a	0.5 a	0.5 a	0.6 a	0.6 a	0.6 a	0.060
16:00	9.8 a	9.5 a	9.8 a	7.9 b	9.7 a	9.6 a	8.9 a	9.6 a	0.005
18:00	4.5 ab	5.1 a	4.8 ab	3.8 c	4.0 bc	4.4 ab	4.1 b	3.6 c	<0.001
Σ SFA	**16.6 ab**	**17.4 a**	**17.1 ab**	**13.9 c**	**16.2 b**	**16.7 ab**	**15.3 b**	**15.4 b**	**<0.001**
16:1n-7	3.6 c	4.2 c	6.2 b	3.7 c	4.1 c	4.6 bc	5.1 b	6.9 a	<0.001
16:1n-5	0.5 b	0.5 ab	0.8 ab	0.9 a	0.5 ab	0.6 ab	1.0 a	0.6 ab	0.003
18:1n-9	17.6 a	17.0 ab	15.1 bc	13.7 c	17.2 ab	16.8 b	14.7 c	14.7 c	<0.001
18:1n-7	5.1 ab	5.1 ab	4.9 ab	4.5 ab	5.0 ab	5.3 a	4.5 ab	4.4 b	0.004
20:1n-9	2.0 a	2.3 a	1.0 b	1.1 b	1.8 ab	2.0 a	1.1 b	0.8 b	<0.001
Σ MUFA	**30.7 a**	**31.9 ab**	**30.4 ab**	**26.6 b**	**30.8 ab**	**30.9 ab**	**27.6 b**	**28.4 b**	**<0.001**
16:3n-4	1.1 ab	1.1 ab	1.4 ab	1.1 ab	1.2 ab	1.4 a	1.0 b	1.0 ab	0.004
16:3n-3	2.7 a	2.6 a	0.8 b	0.9 b	3.0 a	2.8 a	1.1 b	0.8 b	<0.001
16:4n-3	0.4 ab	0.2 ab	0.5 a	0.9 a	0.5 ab	0.2 b	0.8 a	1.0 a	<0.001
18:2n-6	1.0 ab	1.0 ab	0.8 ab	0.8 b	1.0 ab	1.1 a	1.0 ab	0.8 b	<0.001
20:2n-6	1.0 ab	1.3 a	0.7 b	0.9 ab	0.8 b	1.1 ab	1.1 ab	0.5 b	<0.001
20:4n-6	6.7 ab	7.0 a	4.5 b	5.9 ab	7.2 ab	7.5 ab	6.4 ab	7.3 a	0.002
20:5n-3	21.0 a	21.3 a	20.6 a	22.4 a	19.1 a	19.5 a	20.6 a	19.4 a	0.062
22:5n-3	0.9 a	1.1 a	1.1 a	1.3 a	1.2 a	1.2 a	1.2 a	1.2 a	0.288
22:6n-3	11.2 b	10.9 b	12.2 ab	13.8 a	11.7 ab	11.5 ab	12.6 ab	12.9 ab	0.012
Σ PUFA	**47.8 a**	**47.0 a**	**43.5 a**	**49.2 a**	**47.9 a**	**48.5 a**	**47.8 a**	**47.2 a**	**0.054**
Σ n-3	36.4 b	36.6 ab	35.5 ab	39.1 a	35.6 b	36.1 b	36.1 b	34.9 b	0.007
Σ n-6	9.2 a	9.6 a	6.9 b	8.4 a	9.9 a	10.3 a	9.4 a	9.9 a	<0.001
Σ (n-3/n-6)	4.0 b	3.8 b	5.2 a	4.7 a	3.6 b	3.8 b	3.9 b	3.5 b	<0.001
PUFA/SFA	2.9 b	2.7 b	2.6 b	3.5 a	3.0 b	2.9 b	3.1 ab	3.1 ab	<0.001
AI	0.2 a	0.2 a	0.2 a	0.1 b	0.2 a	0.2 a	0.2 ab	0.2 ab	<0.001
TI	0.1 a	0.1 a	0.1 a	0.1 b	0.1 a	0.1 a	0.1 ab	0.1 ab	<0.001
				Hepatopancreas					
14:00	3.7 a	4.2 a	4.6 a	2.3 b	3.2 ab	3.3 ab	3.3 ab	1.7 b	<0.001
15:00	0.7 b	0.7 ab	0.8 ab	0.5 b	0.8 ab	0.8 ab	1.1 a	0.8 ab	0.004
16:00	11.5 bc	12.6 bc	17.4 a	9.9 c	13.0 b	12.8 b	16.5 a	11.3 bc	<0.001
18:00	2.2 b	3.2 ab	3.3 ab	2.6 ab	2.6 ab	2.7 ab	3.5 a	2.8 ab	0.003
Σ SFA	**18.8 b**	**21.8 ab**	**27.0 a**	**16.3 c**	**20.4 b**	**20.5 bc**	**25.5 a**	**17.5 be**	**0.000**
16:1n-7	7.0 b	9.0 ab	15.6 a	7.9 ab	8.5 ab	8.8 ab	13.3 a	12.5 a	0.001
16:1n-5	0.2 a	0.3 a	0.3 a	0.1 b	0.2 a	0.3 a	0.2 ab	0.5 a	0.006
18:1n-9	17.5 a	17.8 a	13.5 b	13.5 b	17.0 a	15.7 ab	14.1 b	14.0 b	<0.001
18:1n-7	4.5 b	5.7 b	8.3 a	5.6 b	5.1 b	5.2 b	7.2 a	6.3 ab	<0.001
20:1n-9	6.4 a	6.7 a	3.2 b	6.3 a	5.1 ab	4.6 b	4.7 ab	4.1 b	<0.001
Σ MUFA	**42.9 b**	**46.2 a**	**45.7 ab**	**38.2 ab**	**40.8 ab**	**38.8 b**	**44.5 ab**	**42.3 ab**	**0.006**
16:3n-4	0.9 a	1.0 a	0.8 a	0.8 a	0.9 a	1.0 a	1.1 a	1.0 a	0.060
16:3n-3	0.4 b	0.5 ab	0.6 ab	0.5 ab	0.5 ab	0.6 ab	0.8 a	0.4 b	0.007
16:4n-3	0.9 a	1.2 a	1.1 a	0.1 b	1.4 a	1.7 a	0.1 b	0.3 b	<0.001
18:2n-6	1.2 a	0.9 b	0.7 b	0.8 b	1.1 ab	0.9 b	0.7 b	0.5 c	<0.001
20:2n-6	1.2 ab	1.6 a	0.8 ab	1.2 ab	1.1 ab	1.6 ab	1.0 ab	0.7 b	<0.001
20:4n-6	2.7 b	2.6 b	1.5 b	3.0 a	3.3 a	3.7 a	1.7 b	3.8 a	<0.001
20:5n-3	7.0 a	5.5 b	7.7 a	8.5 a	7.9 a	7.4 a	4.6 b	9.0 a	<0.001
22:5n-3	1.3 a	0.8 b	0.4 b	1.6 a	1.3 a	1.3 a	0.6 b	1.4 a	<0.001
22:6n-3	12.0 ab	6.5 c	4.1 c	14.6 a	11.3 ab	10.4 b	5.5 c	12.6 ab	<0.001
Σ PUFA	**31.3 a**	**24.2 b**	**20.9 b**	**34.9 a**	**31.9 a**	**32.8 a**	**18.8 b**	**33.5 a**	**<0.001**
Σ n-3	23.3 b	15.4 c	14.3 c	27.2 a	22.6 b	21.5 b	12.5 c	24.7 ab	<0.001
Σ n-6	5.8 b	5.8 b	3.5 c	6.0 a	6.1 b	6.9 ab	4.0 bc	7.1 a	<0.001
Σ (n-3/n-6)	4.1 ab	2.6 b	4.2 ab	4.6 a	3.7 ab	3.5 ab	3.1 ab	3.5 ab	<0.001
PUFA/SFA	1.7 b	1.1 c	0.8 c	2.2 a	1.6 b	1.6 b	0.8 c	1.9 a	<0.001
AI	0.4 ab	0.4 ab	0.6 a	0.3 b	0.4 ab	0.4 ab	0.5 a	0.2 b	<0.001
TI	0.2 b	0.3 ab	0.4 a	0.1 c	0.2 b	0.2 b	0.4 a	0.2 b	<0.001
				Gonads					
14:00	1.8 a	1.9 a	2.1 a	2.2 a	1.1 b	0.6 c	0.6 c	0.8 c	0.001
15:00	0.8 ab	0.9 ab	0.9 ab	0.9 ab	0.5 ab	0.4 b	0.6 ab	1.1 a	0.002
16:00	11.9 b	11.7 ab	13.8 a	16.2 a	8.5c	8.7 c	10.5 b	12.7 ab	0.002

Table 3. Continued

	females				males				
	spring	summer	autumn	winter	spring	summer	autumn	winter	*p*
18:00	2.2 c	2.5 c	3.1 b	2.4 c	4.3 b	5.5 a	6.3 a	5.7 a	<0.001
Σ SFA	**17.2 b**	**17.6 ab**	**20.1 a**	**21.9 a**	**15.3 b**	**16.6 b**	**18.9 ab**	**21.1 a**	**0.010**
16:1n-7	0.1 ab	0.2 ab	0.1 ab	0.1 ab	0.1 ab	0.0 b	0.5 a	0.3 ab	0.006
16:1n-5	0.5 ab	0.6 ab	0.7 ab	1.0 ab	0.6 ab	0.6 b	1.1 a	0.7 ab	0.001
18:1n-9	19.4 a	17.3 ab	15.0 ab	18.0 a	13.2 ab	13.0 ab	12.7 b	14.9 ab	0.001
18:1n-7	4.9 ab	4.8 b	5.4 ab	5.9 ab	4.9 ab	5.2 ab	6.0 ab	6.3 a	0.004
20:1n-9	2.3 ab	2.4 ab	1.5 ab	1.3 ab	3.7 a	2.4 ab	1.4 ab	1.1 b	0.001
Σ MUFA	**35.5 a**	**34.3 a**	**35.3 a**	**36.9 a**	**39.4 a**	**32.2 a**	**33.0 a**	**38.7 a**	**0.060**
16:3n-4	1.0 a	1.0 ab	0.5 ab	0.7 ab	0.6 ab	0.4 b	0.8 ab	0.8 ab	0.002
16:3n-3	0.5 b	0.5 b	0.5 b	0.7 ab	1.0 a	1.2 a	0.9 a	1.4 a	0.001
16:4n-3	1.6b	2.4a	1.1 b	1.5b	1.6b	1.5b	1.3 b	1.1 b	0.002
18:2n-6	1.0 a	0.9 a	0.7 b	0.8 b	0.7 b	0.6 b	0.6 b	0.5 c	0.001
20:2n-6	0.7 ab	0.7 ab	0.5 b	0.5 b	1.0 a	1.3 a	0.9 ab	0.5 b	0.001
20:4n-6	4.7 c	4.9 c	2.6 d	3.2 d	6.7 b	9.2 a	7.5 b	7.4 b	<0.001
20:5n-3	14.2 b	13.9 b	13.1 b	13.5 b	17.1 ab	19.7 a	20.9 a	16.8 ab	0.001
22:5n-3	1.7 a	1.5 a	1.3 a	0.9 b	1.1 b	1.1 b	0.6 c	0.5 c	0.002
22:6n-3	16.3 a	14.2 ab	13.6 ab	10.7 ab	13.4 ab	15.5 ab	10.8 ab	8.7 b	0.012
Σ PUFA	**44.3 a**	**43.2 ab**	**37.8 ab**	**35.4 b**	**40.3 ab**	**48.6 a**	**43.3 ab**	**36.6 b**	**0.009**
Σ n-3	33.9 ab	31.6 ab	30.4 ab	27.2 b	33.6 ab	38.3 a	34.0 ab	27.9 b	0.005
Σ n-6	7.4 c	7.6 c	5.2 d	5.4 d	8.7 b	11.9 a	9.7 b	9.0 b	<0.001
Σ (n-3/n-6)	4.6 b	4.2 c	5.8 a	5.0 a	3.9 c	3.2 d	3.5 d	3.1 d	<0.001
PUFA/SFA	2.6 b	2.5 ab	1.9 c	1.7 c	2.6 ab	2.9 a	2.3 b	1.7 c	<0.001
AI	0.2 ab	0.3 a	0.3 a	0.4 a	0.2 b	0.1 b	0.2 b	0.2 b	<0.001
TI	0.1 b	0.1 b	0.2 a	0.2 a	0.1 b	0.1 b	0.1 b	0.2 a	<0.001

[a] Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; AI, atherogenic index; TI, thrombogenic index. In each row different letters indicate significant differences between sexes and seasons ($p < 0.05$).

were lower during summer (females) and autumn (both sexes). Concerning gonads, in both sexes SFA values were lower in spring and summer; no seasonal differences were detected in the proportion of MUFA levels, and PUFA content was lower in winter (both sexes). PCAs considering all fatty acids, seasons, and sexes were applied to all edible tissues, and factors 1, 2, and 3 yielded a total of 65% of explainable results (**Figure 1A**). Most fatty acids loaded heavily on factor 1 except 18:1n-9, 18:2n-6, 20:2n-6, and 22:5n-3, which loaded on factor 2, and 16:3n-4, 16:3n-3; 16:4n-3, and 22:6n-3, which loaded on factor 3. Clear cluster separation is evidenced between muscle and hepatopancreas, whereas gonads overlap both tissues, although predominantly overlapping the muscle. This cluster pattern is in accordance with the statistical results obtained with one-way ANOVA in which differences between tissues were tested for each fatty acid independently of sex and season (**Table 4**). The content of several fatty acids did not differ between gonads and hepatopancreas (15:0, 16:0, and 18:1n-7) and between gonads and muscle (16:1n-5, 20:4n-6, and 22:6n-3). In contrast, hepatopancreas had proportionally more 14:0, 20:1n-9, and 22:2n-6, whereas 18:0 and 20:5n-3 had higher proportion in the muscle. The main saturated fatty acid in all tissues was palmitic acid (16:0), which was fairly constant in the muscle during the four seasons, whereas in hepatopancreas and gonads it predominated during autumn and winter. Among MUFA, oleic acid (18:1n-9) was the prevailing fatty acid in all tissues, with particularly higher values found in spring (all tissues), summer (hepatopancreas), and winter (gonads). The main n-3 PUFA was EPA (20:5n-3; muscle and gonads) and DHA (22:6n-3; hepatopancreas) acids (**Table 4**). In muscle the proportion of EPA was fairly constant during the four seasons independent of sex, whereas in hepatopancreas lower values were detected in summer (females) and autumn (males) and in testes had higher proportion during summer and autumn than ovaries. DHA in the muscle of females had higher proportion during winter than in spring and summer, in hepatopancreas DHA was predominant in winter and spring in both sexes, whereas in gonads there was a tendency of decreasing DHA levels from spring to winter in both sexes. The n-3 PUFA were higher in muscle (34.9−36.6%) and gonads (27.2−38.3%) than in hepatopancreas (14.3−27.2%) (**Tables 3** and **4**). Higher levels of these fatty acids occurred during spring, summer, and autumn in gonads and during winter in hepatopancreas. The major n-6 PUFA was arachidonic acid (AA, 20:4n-6), mostly in muscle and gonads. The AA values were usually higher in the hepatopancreas of male crabs compared to females and always higher in testes than in ovaries. The n-6 PUFA were also dominant in muscle (6.9−10.3%) and gonads (5.2−11.9%) in opposition to hepatopancreas (3.5−7.1%) (**Tables 3** and**4**). Overall, major differences in fatty acid composition of gonads were observed between sexes and seasons, which are evidenced in the PCA representation considering all fatty acids, seasons, and sexes and where the three factors yielded a total of 78% of explainable results (**Figure 1B**). Most fatty acids loaded heavily on factor 1, except 13:1n-5, 18:1n7, 20:1n-9, 18:2n-6, 22:5n-3, and 22:6n-3, which loaded on factor 2, and 16:1n-7, 16:3n-4, and 16:4n-3, loaded on factor 3. In hepatopancreas, PCA indicates a separation between seasons with the three factors having a total of 68% of explainable results (**Figure 1C**). Most fatty acids loaded in factor 1, but 16:1n-5, 18:1n-9, 20:1n-9, and 18:2n-6 loaded on factor 2 and 16:3n-4, 16:3n-3, 20:2n-6, and 20:4n-6 loaded on factor 3. The ratio n-3/n-6 was lower in hepatopancreas and generally higher in females than in males (muscle and gonads). The PUFA/SFA ratio was higher in muscle followed by gonads and hepatopancreas. This ratio was higher in winter in the muscle of females and in the hepatopancreas of both sexes, whereas in gonads higher values were found in the spring and summer for both sexes. Hepatopancreas had generally higher values of atherogenic and thrombogenic indices followed by gonads and muscle.

Amino Acids. The amino acid composition of the edible tissues is given in **Table 5**. In general, the crab protein in all tissues

Table 4. Fatty Acids Statistical Differences between Tissues with the Respective *p* Values[a]

	tissue	*p*
Σ14:0	hepatopancreas > (muscle = gonads)	<0.01
Σ15:0	(hepatopancreas = gonads) > muscle	<0.01
Σ16:0	(hepatopancreas = gonads) > muscle	<0.01
18:0	muscle > gonads > hepatopancreas	<0.01
16:1n-7	hepatopancreas > muscle > gonads	<0.01
16:1n-5	(muscle = gonads) > hepatopancreas	<0.01
18:1n-9	ND	0.68
18:1n-7	(hepatopancreas = gonads) > muscle	<0.01
20:1n-9	hepatopancreas > (muscle = gonads)	<0.01
16:3n-4	muscle > hepatopancreas > gonads	<0.01
16:3n-3	hepatopancreas > gonads > muscle	<0.01
16:4n-3	gonads > hepatopancreas > muscle	<0.01
18:2n-6	(muscle = hepatopancreas) > gonads	<0.01
20:2n-6	hepatopancreas > (muscle = gonads)	<0.01
20:4n-6	(muscle = gonads) > hepatopancreas	<0.01
20:5n-3	muscle > gonads > hepatopancreas	<0.01
22:5n-3	ND	0.98
22:6n-3	(muscle = gonads) > hepatopancreas	<0.01
Σ SFA	(hepatopancreas = gonads) > muscle	<0.01
Σ MUFA	muscle > gonads > hepatopancreas	<0.01
Σ PUFA	muscle > gonads > hepatopancreas	<0.01
Σ n-3	muscle > gonads > hepatopancreas	<0.01
Σ n-6	muscle > gonads > hepatopancreas	<0.01
Σ (n-3/n-6)	(muscle = gonads) < hepatopancreas	<0.01
PUFA/SFA	muscle > gonads > hepatopancreas	<0.01
AI	hepatopancreas > gonads > muscle	<0.01
TI	hepatopancreas > gonads > muscle	<0.01

[a] Each fatty acid was tested independently of sex and season. Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; AI, atherogenic index; TI, thrombogenic index; ND, no differences.

contained high amounts of glutamic acid, arginine, aspartic acid, glycine, and leucine. Generally, hepatopancreas had the lowest amounts of amino acids compared to muscle and gonads (**Table 6**). The only exception was taurine (lower in muscle than in the remaining tissues). Major differences were observed regarding sex and season, which were more pronounced in gonads and hepatopancreas than in muscle. These results are corroborated by the PCA presented in **Figure 2**, where factors 1 and 2 gave a total of 91% of explainable results. There is a clear cluster separation of the three tissues and of both sexes in gonads. All amino acids loaded on factor 1 except taurine. Female gonads had higher amounts of most amino acids during all seasons, compared to males. Ovaries had the lowest amino acid values in winter (leucine, arginine, and alanine) compared to the remaining seasons, whereas testes had some of the highest values in this season (threonine, isoleucine, leucine, histidine, lysine, aspartic acid, glutamic acid, tyrosine, proline, and hydroxyproline). Hepatopancreas of male crabs had higher concentration of amino acids during winter, but particularly of threonine, valine, isoleucine, leucine, phenylalanine, histidine, arginine, aspartic acid, serine, glutamic acid, glycine, alanine, tyrosine, and hydroxyproline. On the other hand, during autumn female hepatopancreas had higher concentrations of lysine, serine, alanine, and hydroxyproline, whereas in spring the lowest amino acid content was detected (threonine, valine, isoleucine, leucine, phenylalanine, lysine, serine, glutamic acid, glycine, hydroxyproline, and taurine). With regard to the muscle, generally no significant differences were detected between seasons and sexes. The ratio of essential amino acids to nonessential amino acids (EAA/NEAA) did not vary in muscle; in hepatopancreas it was fairly constant throughout the year with a peak in winter male crabs, and ovaries had generally higher ratios than testes except in

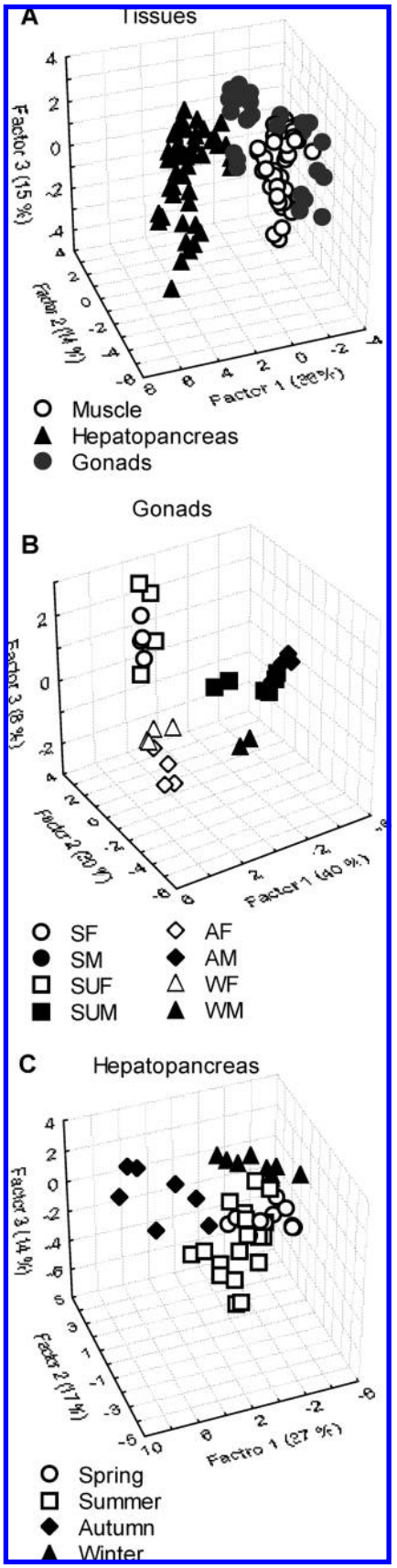

Figure 1. Results of PCAs considering the concentration of fatty acids: (**A**) in all tissues; (**B**) in gonads depending on sex and season; (**C**) in hepatopancreas depending on season. Abbreviations: SF, spring females; SM, spring males; SUF summer females; SUM, summer males; AF, autumn females; AM, autumn males; WF, winter females; WM, winter males.

Table 5. Amino Acid Profile (Grams per 100 g of Wet Weight; Average ± Standard Deviation) of Edible Tissues of Female and Male Crabs in Spring, Summer, Autumn, and Winter[a]

	females				males				
	spring	summer	autumn	winter	spring	summer	autumn	winter	*p*
Muscle									
THR	0.89 b	0.99 ab	1.10 a	1.09 a	0.95 ab	1.06 a	1.02 ab	1.12 a	<0.001
VAL	0.67 c	0.69 b	0.79 a	0.73 ab	0.76 b	0.77 a	0.77 a	0.79 a	0.001
MET	0.27 b	0.18 c	0.23 b	0.16 c	0.32 a	0.26 b	0.31 a	0.20 b	<0.001
ILE	0.65 b	0.67 b	0.76 ab	0.71 ab	0.75 ab	0.75 ab	0.75 a	0.79 a	0.004
LEU	1.09 b	1.11 b	1.26 ab	1.17 ab	1.22 ab	1.25 a	1.24 a	1.27 ab	0.004
PHE	0.61 b	0.63 ab	0.70 ab	0.65 ab	0.70 a	0.70 a	0.68 a	0.71 a	0.008
HIS	0.33 b	0.36 b	0.37 ab	0.36 b	0.37 ab	0.40 a	0.33 b	0.42 a	<0.001
LYS	0.77 b	0.89 ab	0.81 ab	0.96 a	0.87 ab	1.00 a	1.06 a	1.01 a	0.001
ARG	1.68 b	1.64 b	1.93 a	1.77 a	1.50 c	1.79 a	1.92 a	1.85 a	<0.001
ASP	1.50 b	1.49 b	1.74 a	1.59 a	1.65 a	1.73 a	1.66 a	1.68 a	0.003
SER	0.65 b	0.67 ab	0.75 ab	0.71 a	0.69 ab	0.74 a	0.74 a	0.74 a	0.022
GLU	2.40 b	2.50 b	2.88 a	2.46 b	2.63 a	2.79 a	2.83 a	2.74 a	0.016
GLY	1.36 a	1.34 a	1.23 a	1.29 a	1.27 a	1.24 a	1.28 a	1.26 a	0.090
ALA	0.90 b	0.91 b	1.04 ab	0.93 b	0.94 ab	0.98 ab	1.03 a	0.99 ab	0.004
TYR	0.63 b	0.62 b	0.72 ab	0.69 a	0.70 ab	0.71 a	0.72 a	0.76 a	0.001
PRO	0.61 b	0.73 a	0.63 ab	0.60 b	0.69 a	0.75 a	0.71 a	0.69 a	0.016
HYP	0.44 b	0.39 b	0.53 a	0.54 a	0.55 a	0.45 ab	0.52 a	0.50 a	<0.001
TAU	0.35 b	0.33 b	0.49 ab	0.51 ab	0.41 ab	0.43 ab	0.61 a	0.57 a	<0.001
EAA/NEAA	0.79 a	0.80 a	0.79 a	0.82 a	0.78 a	0.81 a	0.80 a	0.82 a	0.058
total AA	15.79 b	16.16 b	17.96 ab	16.92 b	16.99 b	17.78 ab	18.16 a	18.11 ab	0.043
Hepatopancreas									
THR	0.59 d	0.75 c	0.96 ab	0.83 b	0.78 c	0.75 c	0.74 c	1.03 a	0.001
VAL	0.49 d	0.55 c	0.69 b	0.63 b	0.63 c	0.60 c	0.57 c	0.75 a	0.004
MET	0.11 a	0.03 c	0.01 c	0.05 b	0.13 a	0.12 a	0.06 b	0.04 b	0.016
ILE	0.42 c	0.47 b	0.59 ab	0.55 b	0.56 b	0.52 b	0.49 c	0.67 a	0.004
LEU	0.67 d	0.75 b	0.96 b	0.86 b	0.87 b	0.81 b	0.78 c	1.03 a	0.008
PHE	0.40 d	0.46 b	0.60 ab	0.52 b	0.53 b	0.50 b	0.48 c	0.64 a	0.011
HIS	0.21 c	0.29 bc	0.38 ab	0.34 b	0.33 b	0.35 b	0.29 c	0.48 a	0.003
LYS	0.34 d	0.54 bc	0.72 a	0.61 b	0.60 b	0.60 b	0.47 c	0.70 ab	<0.001
ARG	0.78 c	0.92 bc	1.25 b	1.06 b	1.06 b	1.08 b	0.95 b	1.49 a	0.002
ASP	0.82 c	0.98 bc	1.26 ab	1.09 b	1.13 b	1.08 b	1.02 b	1.38 a	0.005
SER	0.40 d	0.47 bc	0.60 a	0.52 b	0.50 b	0.48 bc	0.45 c	0.60 a	0.003
GLU	1.10 d	1.30 bcd	1.63 ab	1.43 b	1.46 b	1.39 b	1.31 c	1.75 a	0.009
GLY	0.49 d	0.58 bc	0.67 b	0.64 c	0.61 c	0.61 c	0.54 c	0.84 a	0.001
ALA	0.52 c	0.57 bc	0.70 a	0.56 c	0.61 b	0.60 b	0.55 c	0.70 a	0.001
TYR	0.49 c	0.52 bc	0.71 b	0.66 b	0.63 b	0.58 c	0.55 c	0.81 a	<0.001
PRO	0.30 c	0.35 ab	0.45 a	0.31 c	0.37 b	0.39 ab	0.28 c	0.44 a	0.002
HYP	0.23 c	0.33 b	0.41 a	0.35 b	0.34 bc	0.28 c	0.34 bc	0.41 a	0.006
TAU	0.43 c	0.54 b	0.71 a	0.64 a	0.46 c	0.58 ab	0.58 ab	0.57 ab	<0.001
EAA/NEAA	0.84 b	0.84 b	0.86 b	0.88 b	0.89 b	0.89 b	0.86 b	0.92 a	0.002
total AA	8.79 d	10.39 c	13.30 b	11.67 bc	11.53 c	11.32 bc	10.46 c	14.42 a	0.007
Gonads									
THR	1.69 ab	2.03 a	1.87 a	1.49 b	0.89 d	0.81 d	0.86 d	1.07 c	<0.001
VAL	1.34 a	1.55 a	1.55 a	1.17 ab	0.76 b	0.68 b	0.70 b	0.88 b	<0.001
MET	0.32 b	0.43 b	0.53 a	0.28 b	0.13 c	0.09 c	0.08 c	0.12 c	<0.001
ILE	1.07 a	1.32 a	1.35 a	0.92 a	0.53 c	0.49 c	0.49 c	0.66 b	<0.001
LEU	1.67 a	2.04 a	2.04 a	1.46 b	0.74 d	0.75 d	0.73 d	0.95 c	<0.001
PHE	1.03 a	1.23 a	1.19 a	0.92 a	0.56 b	0.51 c	0.49 c	0.64 b	<0.001
HIS	0.59 a	0.72 a	0.71 a	0.62 a	0.25 c	0.29 c	0.26 c	0.39 b	<0.001
LYS	0.95 a	1.13 a	1.15 a	1.01 a	0.52 c	0.81 b	0.76 b	1.01 a	<0.001
ARG	1.73 a	2.13 a	2.12 a	1.56 b	0.92 c	0.96 c	1.00 c	1.30 c	<0.001
ASP	1.94 ab	2.37 a	2.23 a	1.77 b	1.25 d	1.13 e	1.16 e	1.50 c	<0.001
SER	1.31 ab	1.64 a	1.66 a	1.12 ab	0.78 b	0.66 c	0.68 c	0.81 b	<0.001
GLU	2.95 ab	3.60 a	3.57 a	2.66 b	1.71 c	1.64 c	1.66 c	2.09 b	<0.001
GLY	1.25 a	1.30 a	1.20 a	1.21 a	0.77 c	0.87 b	0.84 b	0.92 b	<0.001
ALA	1.14 a	1.28 a	1.26 a	1.04 b	0.56 d	0.67 c	0.64 c	0.69 c	<0.001
TYR	1.16 b	1.41 a	1.39 a	1.11 b	0.62 d	0.55 d	0.54 d	0.76 c	<0.001
PRO	1.10 ab	1.30 a	1.16 ab	0.85 b	0.70 c	0.70 c	0.69 c	0.83 b	<0.001
HYP	0.55 b	0.69 b	0.84 a	0.56 b	0.33 c	0.34 c	0.34 c	0.43 b	<0.001
TAU	0.73 b	0.73 b	0.80 a	0.77 a	0.24 d	0.49 c	0.50 c	0.46 c	<0.001
EAA/NEAA	0.85 a	0.88 a	0.90 a	0.85 a	0.76 b	0.77 b	0.76 b	0.83 ab	0.008
total AA	22.52 ab	26.90 a	26.76 a	20.51 b	12.26 d	12.44 d	12.42 d	15.50 c	0.003

[a] Abbreviations: THR, threonine; VAL, valine; MET, methionine; ILE, isoleucine; LEU, leucine; PHE, phenylalanine; HIS, histidine; LYS, lysine; ARG, arginine; ASP, aspartic acid; SER, serine; GLU, glutamine; GLY, glycine; ALA, alanine; TYR, tyrosine; PRO, proline; HYP, hydroxyproline; TAU, taurine; EAA/NEAA, essential amino acids/nonessential amino acids; total AA, total amino acids). In each row different letters indicate significant differences per tissue ($p < 0.05$).

Table 6. Statistical Differences between Tissues with the Respective *p* Values[a]

	tissue	*p*
THR	(muscle = gonads) > hepatopancreas	<0.01
VAL	(muscle = gonads) > hepatopancreas	<0.01
MET	(muscle = gonads) > hepatopancreas	<0.01
ILE	(muscle = gonads) > hepatopancreas	<0.01
LEU	(muscle = gonads) > hepatopancreas	<0.01
PHE	(muscle = gonads) > hepatopancreas	<0.01
HIS	muscle = gonads > hepatopancreas	0.01
LYS	(muscle = gonads) > hepatopancreas	<0.01
ARG	gonads > muscle > hepatopancreas	<0.01
ASP	(muscle = gonads) > hepatopancreas	<0.01
SER	(muscle = gonads) > hepatopancreas	<0.01
GLU	(muscle = gonads) > hepatopancreas	<0.01
GLY	muscle > gonads > hepatopancreas	<0.01
ALA	(muscle = gonads) > hepatopancreas	<0.01
CYS	(muscle = gonads) > hepatopancreas	<0.01
TYR	(muscle = gonads) > hepatopancreas	<0.01
PRO	gonads > muscle > hepatopancreas	<0.01
HYP	muscle = gonads > hepatopancreas	<0.01
TAU	(gonads = hepatopancreas) > muscle	<0.01
EAA/NEAA	(gonads = hepatopancreas) > muscle	<0.01
total AA	(muscle = gonads) > hepatopancreas	<0.01

[a] Each amino acid was tested independently of season and sex. Abbreviations: THR, threonine; VAL, valine; MET, methionine; ILE, isoleucine; LEU, leucine; PHE, phenylalanine; HIS, histidine; LYS, lysine; ARG, arginine; ASP, aspartic acid; SER, serine; GLU, glutamine; GLY, glycine; ALA, alanine; TYR, tyrosine; PRO, proline; HYP, hydroxyproline; TAU, taurine; EAA/NEAA, essential amino acids/nonessential amino acids; total AA, total amino acids.

winter. The amino acid scores are presented in **Figure 3**; the highest scores were obtained for threonine, phenylalanine, and tyrosine in all tissues but with the highest values in gonads. Values below 100% were obtained for methionine (muscle, all seasons in females, summer and winter in males; hepatopancreas, all seasons in both sexes; gonads, spring and winter in females, all seasons in males), lysine (muscle, spring and autumn in females; hepatopancreas, spring in females, spring and autumn in males; gonads, spring in females and males), valine (muscle, spring in females and summer in males) and leucine (gonads, spring in males).

DISCUSSION

Meat Yield and Chemical Composition. The results indicate that the meat yield of edible crab varied throughout the seasons in relation to molting and breeding cycle as previously described (*9*). The variation is most significant in female crabs due to changes in the brown meat content following the development of gonads. The gonad development generally occurs in autumn, after molting and mating took place during summer; spawning occurs in late autumn and winter (*9*, *17*). After spawning, there is a period of egg incubation that can last up to 8 months before the broods hatch throughout spring, summer, and early autumn, depending on latitude and temperature (*9*, *17*). The GSI obtained in the present study is in agreement with the reproductive cycle of edible crab, as the highest values in autumn corresponded to the greater proportion of S4 (ripe ovaries). The gonadosomatic index as a function of gonadal development of marine invertebrates was described by several authors, and in general an increase in GSI of female ovaries as maturation approaches was observed (*18*). The proximate chemical composition and fatty acid and amino acid contents varied between tissues according to season and sex. The white meat from claws of males and females showed fewer seasonal variations than other tissues. Muscle is a structural tissue, the major site for protein storage, and its fat content is mainly composed by structural lipids with up to 72−74% of phospholipids (*19*), which are important components of cellular

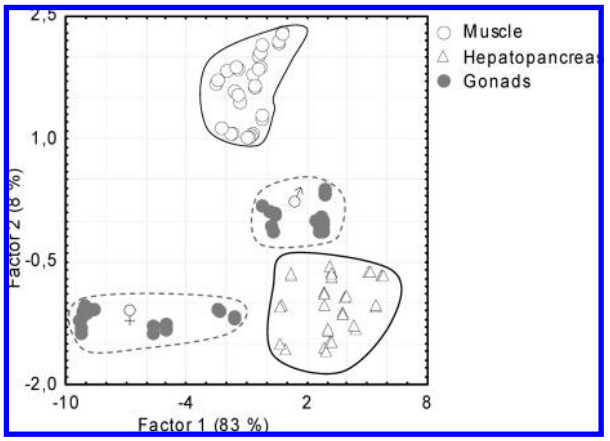

Figure 2. PCA considering the content of all amino acids in edible tissues. Solid lines represent clusters related to tissue, whereas dashed lines represent clusters related to sex. Symbols: ♂ (males); ♀ (females).

membranes. Therefore, this tissue has a more stable composition less exposed to seasonal variations (*20*). Levels of muscle lipids were comparatively low and remained nearly constant throughout the seasons, demonstrating the absence of lipid mobilization to other tissues. Sex differences were more pronounced in the muscle protein and amino acid contents; higher values were quantified in males than in females, particularly in summer. This might be due to an increase in the biosynthesis of hormones and enzymes involved in mating that occurs during this season. Major differences between sexes were also observed in the muscle meat yield, as the claws of mature males are larger and with higher meat content than those of mature females of similar size (*9*). Marked sex and seasonal variations in the proximate chemical composition and fatty acid, cholesterol, and amino acid contents were observed in gonads, which can be related to food availability, temperature, migration pattern, sexual maturity, and gametogenesis, which are variables that are influenced by seasons (*21*). This was evidenced by the higher amounts of protein, amino acids, fat, and cholesterol found in ovaries compared to testes. Also, the higher cholesterol and amino acid contents in summer and autumn and fat in winter (females) correspond to mating seasons and subsequent gonad development. Although in decapod crustaceans muscle is the major site of protein storage and hepatopancreas that of fat and cholesterol, these results indicate that ovaries become an additional center for protein, fat, and cholesterol metabolism during maturation (*21*). Protein and amino acid variations may indicate an increase in the biosynthesis of various proteins, including hormones, enzymes, and lipoproteins involved in mating, fertilization, gonad maturation, spawning, and normal development of embryo in decapods (*22*). An optimum protein to energy ratio is essential for efficient nutrient deposition, where available protein is used primarily for gonad development, with energy requirements being satisfied by lipids and, to a lesser extent, by carbohydrates (*23*). Cholesterol is an essential nutrient for crustaceans because they are not capable of de novo synthesis of steroid rings, and therefore cholesterol stores are derived from the diet (*18*). Hepatopancreas is the major site of cholesterol metabolism, which is consistent with the constant cholesterol values obtained throughout the seasons. Souchet and Laplante (*24*) have reported no seasonal variations in cholesterol content of hepatopancreas, but argued that this could be due to the proximity of their sampling periods (spring and summer). On the other hand, female gonads showed the highest cholesterol values in summer and autumn, whereas in muscle the lowest values were attained in summer. These seasonal differences might

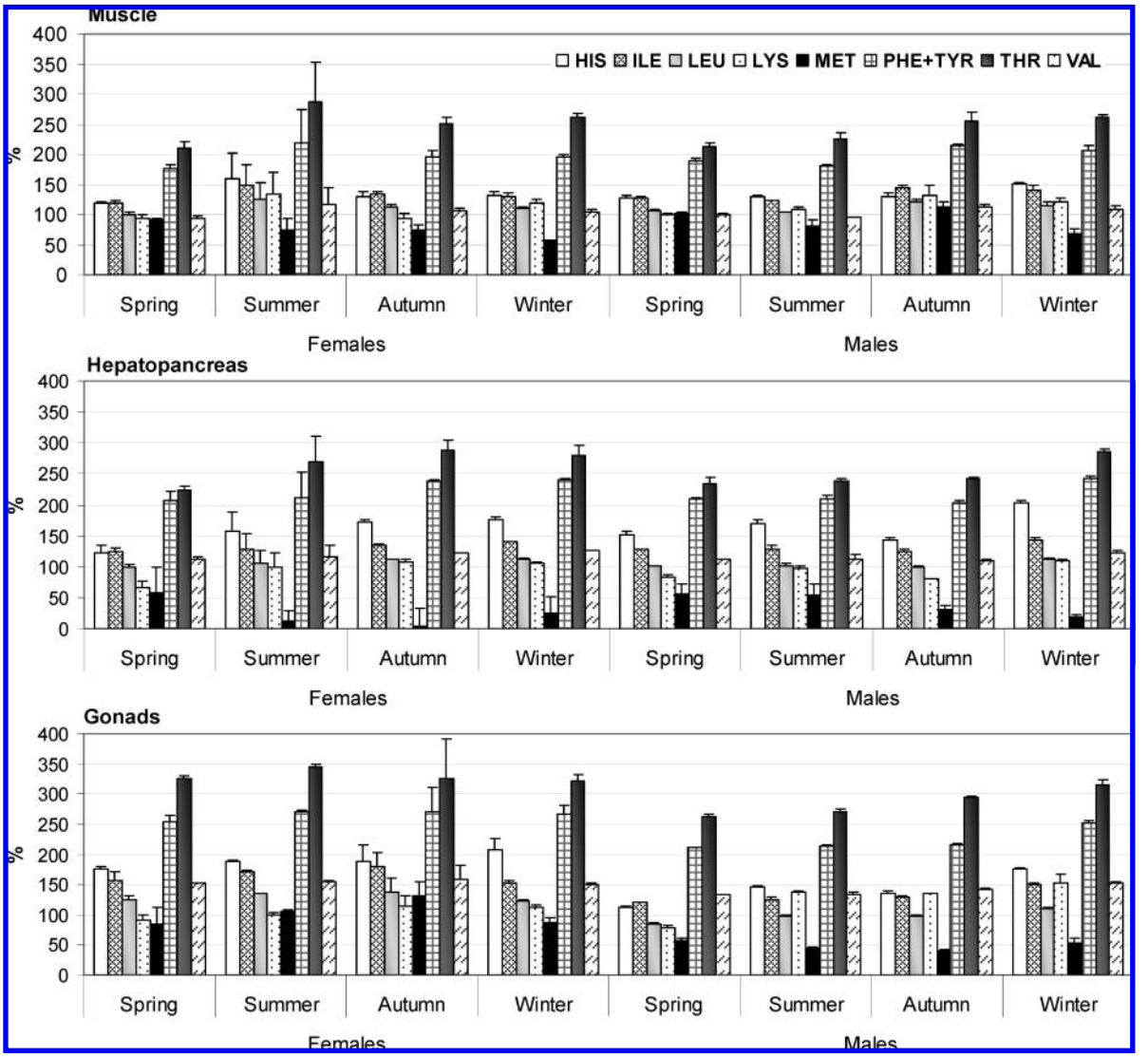

Figure 3. Essential amino acid score (percent) of edible tissues of female and male *C. pagurus* during spring, summer, autumn, and winter. Abbreviations: HIS, histidine; ILE, isoleucine; LEU, leucine; LYS, lysine; MET, methionine; PHE+TYR, phenylalanine + tyrosine; THR, threonine; VAL, valine.

be due to the mobilization of cholesterol from muscle to gonads, as previously reported by Teshima et al. (*25*). In the current study significant increases in the content of SFA in hepatopancreas and maturing ovaries suggest that these fatty acids might be important at this stage. Previous studies reported that SFA and MUFA are the major sources of energy during embryonic early larval development of the shrimp *Macrobrachium rosenbergii* (*26*). Also, PUFA from the n-3 series, especially EPA and DHA, have been identified in recent decades as essential nutrients for marine invertebrates in general (*27*). In crabs, these fatty acids are essential for maturation and reproduction and might be implicated in the molting process (*28*). EPA is an important structural component of cell membranes (*29*), which corroborates the present study considering that EPA levels were kept constant in muscle. EPA is also a precursor of prostaglandins (*29*), which might explain the differences observed between sexes in gonads, with males having higher EPA proportion during summer and autumn (main mating and gonad development period) than females.

Nutritional Quality. The present research evidenced the wide variation in the nutritional quality of edible crab tissues. Moreover, in each tissue the nutritional quality was affected by season and sex at different levels. Muscle composition was less variable than the remaining edible tissues, in hepatopancreas the chemical composition was mostly affected by season, and in gonads both season and sex contributed to the differences observed. The Western diet is based on the consumption of domesticated animals and cultured vegetables and fruits. Most food items are standardized and cultured under restricted conditions, those that are best for rapid growth. Wild seafood, on the other hand, faces the effect of different variables throughout the year, which are interlinked combinations of factors. Season is crucial and affects the life cycle, behavior, migration pattern, and type of food available. Season also affects water temperature, salinity, and light exposure, to name only a few. Wild crabs, such as *C. pagurus*, are affected by these factors, and their nutritional compositions reflect these influences. Muscle, being a structural tissue, did not suffer as many changes as hepatopancreas and gonads. *C. pagurus* is mainly appreciated in southern Europe because of its gonads and hepatopancreas. From a consumer's perspective, autumn is the best season to eat crab, especially due to the characteristic high brown meat yield. Nevertheless, sex is certainly also important in autumn, as females have higher brown meat yields and muscle is the predominant tissue in males. From a nutritional point of view it is also important to stress that the cholesterol and fat contents were higher in female gonads than in males, particularly in

autumn. Considering that the current dietary recommendations suggest a cholesterol intake of $< 200-300$ mg per day, people under a cholesterol-restricted diet should moderate the consumption of female gonads during autumn. Nevertheless, the remaining tissues of *C. pagurus* pose no risks during the four seasons even to a restricted cholesterol diet. The fat content was also higher in autumn and spring in female hepatopancreas, mainly composed of SFA and MUFA. On the other hand, taurine concentration in autumn and winter was higher in muscle (males), hepatopancreas (females), and gonads (females) than in remaining seasons. Beneficial effects of dietary taurine have been observed in animal and human studies, and there are indications of reduced cardiovascular disease risk and cholesterol absorption (*30*). The capacity for taurine biosynthesis varies remarkably among species. Humans have only limited ability for its biosynthesis; consequently, taurine needs to be acquired with the diet, but still there is no recommended dietary intake value. In general, muscle and female gonads were better sources of protein and amino acids than hepatopancreas. Particularly in autumn, muscle had the highest amino acid concentration. According to the amino acid score, protein in the edible tissues of *C. pagurus* was well balanced in the essential amino acid composition. Threonine and phenylalanine + tyrosine had the highest scores in all tissues but especially in female gonads. The limiting amino acids were methionine and lysine in the three tissues, but particularly in hepatopancreas and male gonads. Similar results were reported in the muscle of shrimps and Norway lobster (*31*). Tryptophan and the sulfur-containing amino acid cysteine are lost during the acid hydrolysis of meat products and therefore were not quantified, but they are typically available in low concentration in seafood products. The tissues are also well balanced with respect to EAA and show a favorable ratio and may be considered as a food source of high-quality protein. The ratio of n-3/n-6 fatty acids has been cited as an excellent index to compare the relative nutritional value of lipids, where high values correspond to better quality foods. In this case, the highest values were obtained in autumn, particularly in female gonads (5.8). Compared to other crustacean species, like the crabs *Callinectes sapidus* (muscle, 2.32; hepatopancreas, 1.57) and *Carcinus mediterraneus* (muscle, 1.4), all tissues of *C. pagurus* during all seasons had higher nutritional quality with respect to this ratio (*32*, *33*). The U.K. Department of Health stipulated an ideal n-3/n-6 ratio of 4:1 in the human diet and a minimum value of PUFA/SFA ratio recommended of 0.45 (*15*). In this regard, muscle and gonads of females had n-3/n-6 ratios in the range of the recommended values, and all tissues of both sexes in the four seasons had PUFA/SFA ratios above 0.45. In conclusion, the edible tissues of *C. pagurus* have distinct nutritional composition that varies between sexes and seasons, but overall it was a well-balanced nutritious food.

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