

## Chemical composition of blue crabs from Adriatic sea

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## ORIGINAL ARTICLE

### Abstract

The population of the blue crab (*Callinectes sapidus* Rathbun 1896) is booming in the Mediterranean Sea, and it has been classified as an invasive alien species. In this study, blue crabs were caught in the Adriatic Sea and separated into meat and shell, and their composition was analyzed. The two fractions were low in total lipids despite their excellent fatty acid profile, characterized by a high content of unsaturated fatty acids. The shell contained high amounts of ash and chitin. In addition, meat and shell proved to be excellent sources of protein (7–10%) with high nutritional value, albeit some significant differences in the essential amino acid pattern.

**Keywords:** Amino acids, ash, *Callinectes sapidus*, chitin, lipids, protein

### Introduction

The blue crab (*Callinectes sapidus* Rathbun 1896) is an ecologically important component of various ecosystems. Its native distribution in the Atlantic Ocean extends from Nova Scotia to Argentina (Mancinelli *et al.*, 2017b). The main differences between this crustacean and most of its congeners are its broad geographic distribution, which includes temperate regions, and its ability to adapt to the full salinity gradient of estuaries (Williams, 1984). The crab is an important predator in coastal areas and supports a large commercial fishery (Epifanio, 2019). In their natural habitat, blue crabs are reported to not only feed mainly on molluscs, crustaceans, and fish but also on polychaetes, algae, detritus, and sediment (Marchessaux, Mangano, *et al.*, 2023). In addition, they are characterized by advantageous biological properties, such as early maturity, rapid growth, high reproduction rate, wide larval dispersal, and aggressive, opportunistic behavior (Marchessaux, Chevalier, *et al.*, 2023). All these characteristics facilitate

the invasion of the blue crab in different regions. Its recent spread has particularly affected the Mediterranean Sea, raising serious concern. *Callinectes sapidus* is indeed currently considered an invasive alien species with dramatic impacts on human activities (Zenetos *et al.*, 2005). Over the past decades, the Mediterranean Sea has experienced an extraordinary increase of nonindigenous species (NIS) in general. NIS are among the major biological problems (Giangrande *et al.*, 2020), and their spread is favored by several stressors, mainly climate change and anthropogenic interventions (Sarà *et al.*, 2018). The problematic impacts of the spread of NIS concern both ecology, through habitat modification and biodiversity loss (Gallardo *et al.*, 2016), and the economic effects of human activities, particularly small-scale fishing and shellfish farming (Mancinelli *et al.*, 2017a; Marchessaux, Mangano, *et al.*, 2023). In Tunisia, the invasion of another similar blue crab species (*Portunus segnis*) has led to a 37% decline in catches, while the average annual income per fisherman has fallen by more than 70% (Khamassi *et al.*, 2019).

In order to contain the spread of *Callinectes sapidus* and prevent the economic damage to the fisheries sector from worsening, the Italian government has approved €2.9 million for 2023 in favor of consortia and aquaculture and fishing companies that catch and dispose of the above-mentioned species (Decreto-Legge n. 104, 2023). However, it seems that the development of a specific blue crab fishery is the only possible effective management measure to control the populations (Marchessaux, Chevalier, *et al.*, 2023). Nevertheless, Fishermen's Cooperative of the Polesine has reported that in 2023 about 12 tons of crabs were caught per day, but with minimal impact on the *Callinectes sapidus* population. Although the blue crab has been the subject of scientific study for more than a century, information on its composition and quality as a seafood is minimal. A few studies have been conducted, but these are limited to the eastern Mediterranean coast of Turkey and to the basic analysis of breast meat, claw meat, and hepatopancreas (Çelik *et al.*, 2004; Gökoolu & Yerlikaya, 2003; Küçükgülmez *et al.*, 2006; Küçükgülmez & Çelik, 2008; Tufan, 2023). More recently, NMR profiling of aqueous and lipid extracts from raw claw muscle was also performed, which, as expected, revealed an interesting amino acid and lipid composition (Zotti *et al.*, 2016). It is also worth noting that the crab processing industry generates enormous quantities of by-products, accounting for up to 85% of the crab's total live weight (Tamburini, 2024). The primary use of these residues is as animal feed, carbonaceous materials (for applications such as bio-sorbents, biocatalysts, or bio-fillers), and the extraction of bioactive compounds (Tamburini, 2024). Among these, chitin is undoubtedly the most significant, as some authors pointed out a good similarity with commercial chitin (Jabeen *et al.*, 2023). Chitin and its derivatives have great potential and economic value for various applications, such as in the food, pharmaceutical, cosmetic, and textile industries (Caligiani *et al.*, 2018). In nature, chitin is closely associated with proteins, minerals, lipids, and pigments (Gortari & Hours, 2013), which is why a fractionation approach to extract proteins, chitin, and astaxanthin from the exoskeleton is also possible (Antunes-Valcareggi *et al.*, 2017).

In this study, *Callinectes sapidus* individuals from the Veneto region (Adriatic Sea, Italy) were collected and separated into meat and shell. They were then analyzed in terms of proximate composition, chitin, amino acids, minerals, and fatty acid profile in order to propose alternative ways to valorize this huge biomass.

## Materials and Methods

### Samples

All crabs (*Callinectes sapidus* Rathbun, 1896) were caught from the banks of the estuary of the river Po in

Rovigo County in the Adriatic Sea, Veneto Region, Italy. Captured crabs were killed by freezing. The crabs were manually opened and divided into two different samples: "Meat", which comprised "soft" material, namely, breast flesh, legs flesh, claws flesh, organs, and gills, and "Shell", containing the "hard" parts, namely, shell, claws, and legs. "Meat" was subsequently weighed and ground using a knife mill GRINDOMIX GM 200 (Retsch, Haan, Germany), whereas "Shell" was weighed, dried in oven at 40°C for 24h, then ground in the same way.

### Determination of moisture and ash content

Meat and shell samples were analyzed by AOAC official methods for determining moisture and ash content (AOAC, 2002). Moisture was determined in an oven at 105°C for 24h, and ash was quantified at 550°C for 5h. Both the samples were analyzed in triplicate.

### Determination of protein content, chitin content, and amino acid distribution

Total protein and chitin content, as well as amino acid distribution, were determined following closely the method reported in a recent work about insects (Luparelli *et al.*, 2022), based on acid hydrolysis and UPLC/ESI-MS analysis. Protein content was determined as the sum of total anhydro amino acid using norleucine as internal standard, whereas chitin was calculated based on its non-acetylated monomer, namely, glucosamine, using galactosamine as the internal standard. The two samples were analyzed in triplicate.

### Determination of lipid content and fatty acid profile

For the determination of the total lipid content and fatty acids distribution, lipid fraction was first extracted by the Folch method (Folch *et al.*, 1957) with slight modifications, as reported elsewhere (Lolli *et al.*, 2018). Briefly, the extraction was performed mixing 10 g of sample with 75 mL of dichloromethane:methanol (2:1 V/V), then the mixture was centrifuged and filtered. The procedure was repeated three times, and the three filtrates were subsequently added to 0.88% KCl. The solution was shaken vigorously, and the lower organic phase was withdrawn and filtered through anhydrous sodium sulfate. Lipids in the extract were dried by rotary evaporator and weighed to determine the total lipid content. Then, lipids were redissolved with 5 mL of n-hexane, 1 mL of tetracosane (50 mg/mL in n-hexane, internal standard), and 0.2 mL of 10% KOH dissolved in methanol. After the separation of phases, the upper one was taken and analyzed by gaschromatography-mass spectrometry (GC-MS).

GC-MS was performed using a Thermo Scientific Trace 1300 gas-chromatograph (Thermo Scientific, Waltham, Massachusetts, USA), coupled to a Thermo Scientific Trace ISQ mass spectrometer (Thermo Scientific, Waltham, Massachusetts, USA). A SLB-5 ms column, 30 m × 0.25 mm, 0.25 µm of thickness (Supelco, Bellafonte, PA, USA), was employed for the separation of analytes.

### Statistical analysis

Elaboration of statistical data was performed using SPSS software v. 29.0 (Chicago, IL, USA). Specifically, the Student's *t*-test was performed to compare proximate composition, fatty acids, and amino acids in blue crab meat and shell. The level of significance ( $\alpha$ ) was set at 0.05; therefore, *p*-values < 0.05 were considered as statistically significant.

## Results and Discussion

### Proximate composition of blue crab meat and shell

After opening the blue crabs, they were separated into meat and shell and both parts were weighed. The carapace accounted for about 50–55% of the total body weight of the crabs, while the meat made up 45–50%. Both parts were subjected to proximate composition analysis to determine the best way to utilize them. The results are reported in Table 1.

As expected, the meat and shell had quite different compositions; the moisture content was about 81% and 56%, respectively. Both parts of the crustacean contained a very small amount of lipids. The meat of *Callinectes sapidus* had a lipid content of about 0.6% (3% of dry matter), which is in line with what has been previously reported for the same species (Ayas, 2011; Tufan, 2023), although other authors found a content twice as high (Türeli *et al.*, 2000). The shell seemed to be a slightly more promising source of lipids, with a significantly higher quantity, equal to 1.5%, close to the results of Hamdi *et al.* (2018). On the other hand, total protein in blue crab meat and shell,

calculated as the sum of anhydroamino acids, proved to be much larger and not significantly different, with values around 7–10%. Authors in the literature have generally found larger amounts of protein in *Callinectes sapidus* flesh over time, averaging between 14% and 21% (Ayas, 2011; Tufan, 2023; Türeli *et al.*, 2000), and as high as 31% (Kuley *et al.*, 2008). Higher values have also been reported for other crab species such as the green crab and the sand crab (Skonberg & Perkins, 2002; Türeli *et al.*, 2000). However, it is known that the chemical composition of fish can vary greatly depending on the species, but also on the individual, age, food intake, spawning time, sex, season, environment, etc. (Kuley *et al.*, 2008). This could therefore indicate that the blue crabs recently distributed in Italy have a lower protein content than those found in other parts of the Mediterranean. The ash content was highly variable, with the proportion of shell being much higher at 28.4% (65% of dry matter). This result is in line with previous findings, reporting values around 58.6% of dry mass (Synowiecki & Al-Khateeb, 2003). The ash of the biomass needs to be analyzed to determine its chemical and physical properties and to fully exploit its potential benefits (Trivedi *et al.*, 2016). In addition to the ash, the carapace was quite rich in chitin (almost 25% of the dry mass), as expected. Limited data are available in the literature regarding the chitin content in the exoskeleton of the blue crab, with values ranging from 14% on a fresh weight basis (Tharanathan & Kittur, 2003) to 10–12% on a dry weight basis (Kaya *et al.*, 2016). However, it is important to note that the protocol used in this study for chitin quantification is a validated method, making it more accurate than others. This finding highlights the promising potential of using chitin from *Callinectes sapidus* in the future, as chitin from crustaceans is very extractable and the final product often achieves a good degree of purity, unlike other sources such as insects (Pedrazzani *et al.*, 2024).

### Fatty acid profile

Although the blue crab meat and carapace contained only small amounts of total lipids (as per the section on “Proximate Composition of Blue Crab Meat and Shell”),

Table 1. Proximate composition of blue crab meat and shell.

	% on fresh weight (g/100 g)				
	Moisture	Lipid	Ash	Protein	Chitin
Meat	80.82 ± 0.67 <sup>b</sup>	0.59 ± 0.04 <sup>a</sup>	3.14 ± 0.59 <sup>a</sup>	9.80 ± 0.97 <sup>a</sup>	n.d.
Shell	56.26 ± 0.15 <sup>a</sup>	1.50 ± 0.23 <sup>b</sup>	28.37 ± 0.53 <sup>b</sup>	7.33 ± 2.50 <sup>a</sup>	10.75 ± 0.62

Results are expressed as percentage on fresh weight (g/100 g) and reported as mean ± standard deviation of three independent analyses. Different letters within the same column indicate significant differences (*t*-test, *p*<0.05).

the lipid fraction extracted by the Folch method was analyzed by GC-MS analysis to obtain an overview of the fatty acid profile. The results are shown in Table 2.

Table 2 shows that the fatty acids of *Callinectes sapidus*, which were detected in both the meat and the carapace, were predominantly unsaturated. They showed an almost identical fatty acid profile. The ratio of SFA:MUFA:PUFA was 24:27:49 in both fractions, which is a typical fatty acid distribution for most crustaceans (Nanda *et al.*, 2021). The only significant difference was found for nervonic acid (C24:1), which is more abundant in meat than in shell. In general, arachidonic acid (C20:4) was the most abundant fatty acid (15–16% of total fatty acids), while other abundant compounds within the saponifiable fraction were, in the descending order, oleic acid (C18:1), eicosapentaenoic acid (C20:5), docosapentaenoic acid (C22:5), palmitic acid (C16:0), stearic acid (C18:0), and palmitoleic acid (C16:1). All these molecules together accounted for about 72% of total fatty acids. These results are partly consistent with the literature: On the one hand, previous studies report that palmitic acid, eicosapentaenoic acid, and oleic acid are the most abundant; on the other hand, they also emphasize the abundance of docosahexaenoic acid (C22:6) (Ayas, 2011; Çelik *et al.*, 2004; Krzynowek *et al.*, 1982), which in contrast was quite low in our samples. These results confirm that the dietary composition of blue crabs is strongly influenced by various factors, especially the environment in which they live. In both samples, iso-C15:0 and iso-C17 (saturated and monounsaturated), iso-C18:0, C18:1, iso-C19:0, C19:1 (only in meat), C22:2, and C24:1 were found in the form of two different isomers, while C22:1 was detected in the form of three different isomers.

### Amino acid profile

On the one hand, *Callinectes sapidus* is quantitatively not a major source of lipids; on the other hand, proteins constitute a significant proportion of both its flesh and its shell (Table 1). The amino acid composition of the protein fraction was therefore analyzed in more detail. The results are reported in Table 3.

As already indicated in Table 1, the total protein content of meat and shell of *Callinectes sapidus* was 9.8% and 7.3%, respectively. In contrast to the lipids, the proteins of meat and shell showed partially different profiles. Despite the same total amount of protein and essential amino acids (EAA), the amino acid pattern was different in some cases. Blue crab meat contained significantly higher amounts of lysine, alanine, and glutamic acid + glutamate. The carapace, on the other hand, was richer in serine, threonine, proline, and valine. Overall, the total content of EAA was more than satisfactory and perfectly

**Table 2.** Fatty acid profile of blue crab meat and shell.

	Fatty acid distribution (g/100 g total lipid)	
	Meat	Shell
C12:0	0.63±0.53 <sup>a</sup>	0.25±0.14 <sup>a</sup>
C14:0 iso	0.04±0.01 <sup>a</sup>	0.05±0.04 <sup>a</sup>
C14:4	0.011±0.001 <sup>a</sup>	0.03±0.02 <sup>a</sup>
C14:3	0.063±0.001 <sup>a</sup>	0.10±0.08 <sup>a</sup>
C14:2	0.014±0.005 <sup>a</sup>	0.01±0.01 <sup>a</sup>
C14:1	0.0121±0.0004 <sup>a</sup>	0.05±0.05 <sup>a</sup>
C14:0	1.18±0.14 <sup>a</sup>	1.89±1.54 <sup>a</sup>
C15:0 iso	0.15±0.02 <sup>a</sup>	0.23±0.13 <sup>a</sup>
C15:0	0.661±0.002 <sup>a</sup>	0.81±0.33 <sup>a</sup>
C16:0 iso	0.32±0.03 <sup>a</sup>	0.42±0.27 <sup>a</sup>
C16:2	0.010±0.002 <sup>a</sup>	0.43±0.55 <sup>a</sup>
C16:1	5.76±0.15 <sup>a</sup>	6.58±1.93 <sup>a</sup>
C16:0	7.51±0.85 <sup>a</sup>	7.70±1.71 <sup>a</sup>
C17:1 iso	0.35±0.03 <sup>a</sup>	0.37±0.05 <sup>a</sup>
C17:0 iso	2.73±0.05 <sup>a</sup>	2.61±0.58 <sup>a</sup>
C17:1	1.39±0.11 <sup>a</sup>	1.23±0.20 <sup>a</sup>
C17:0	1.54±0.11 <sup>a</sup>	1.50±0.02 <sup>a</sup>
C18:0 iso	0.98±0.19 <sup>a</sup>	0.64±0.44 <sup>a</sup>
C18:2	1.42±0.16 <sup>a</sup>	1.31±0.14 <sup>a</sup>
C18:1	14.38±0.48 <sup>a</sup>	13.99±2.14 <sup>a</sup>
C18:0	6.52±0.62 <sup>a</sup>	6.57±0.26 <sup>a</sup>
C19:0 iso	0.51±0.05 <sup>a</sup>	0.54±0.12 <sup>a</sup>
C19:1	0.17±0.08 <sup>a</sup>	0.12±0.01 <sup>a</sup>
C19:0	0.19±0.03 <sup>a</sup>	0.17±0.04 <sup>a</sup>
C20:5	9.31±0.31 <sup>a</sup>	9.02±2.27 <sup>a</sup>
C20:4	16.27±0.08 <sup>a</sup>	15.06±3.97 <sup>a</sup>
C20:3	0.35±0.11 <sup>a</sup>	0.36±0.13 <sup>a</sup>
C20:2 iso	0.42±0.03 <sup>a</sup>	0.59±0.14 <sup>a</sup>
C20:2	1.8±0.09 <sup>a</sup>	1.88±0.62 <sup>a</sup>
C20:1 iso	2.09±0.04 <sup>a</sup>	2.40±0.77 <sup>a</sup>
C20:1	1.08±0.12 <sup>a</sup>	0.94±0.50 <sup>a</sup>
C20:0	0.35±0.03 <sup>a</sup>	0.28±0.08 <sup>a</sup>
C22:6	0.88±0.16 <sup>a</sup>	1.01±0.18 <sup>a</sup>
C22:5	12.36±0.24 <sup>a</sup>	12.83±1.41 <sup>a</sup>
C22:4	1.09±0.16 <sup>a</sup>	0.95±0.12 <sup>a</sup>
C22:3	2.31±0.33 <sup>a</sup>	2.57±0.05 <sup>a</sup>
C22:2	1.43±0.38 <sup>a</sup>	1.30±0.99 <sup>a</sup>
C22:1	0.39±0.05 <sup>a</sup>	0.33±0.10 <sup>a</sup>
C22:0	0.18±0.03 <sup>a</sup>	0.16±0.06 <sup>a</sup>
C24:1	0.84±0.07 <sup>b</sup>	0.09±0.08 <sup>a</sup>
C24:0	0.08±0.03 <sup>a</sup>	0.11±0.04 <sup>a</sup>

Compounds are reported in the order of chromatographic elution as mean ± standard deviation of three independent analyses. Noncharacterized isomers have been summed up. Different letters within the same row correspond to significant differences (*t*-test, *p*<0.05).

Table 3. Amino acid profile of blue crab meat and shell.

	Meat		Shell		FAO/WHO Reference protein
	% on fresh weight (g/100 g)	mg/g protein	% on fresh weight (g/100 g)	mg/g protein	mg/g protein
<b>His</b>	<b>0.16±0.02</b>	<b>15±1<sup>a</sup></b>	<b>0.16±0.07</b>	<b>19±3<sup>a</sup></b>	<b>15</b>
Arg	0.81±0.12	74±11 <sup>a</sup>	0.76±0.31	91±7 <sup>a</sup>	
Ser	0.47±0.06	40±2 <sup>a</sup>	0.47±0.18	53±2 <sup>b</sup>	
Gly	1.24±0.15	96±3 <sup>a</sup>	0.87±0.24	91±6 <sup>a</sup>	
Asp+Asn	1.17±0.19	103±9 <sup>a</sup>	0.84±0.34	98±7 <sup>a</sup>	
Glu+Gln	1.99±0.28	178±11 <sup>b</sup>	1.26±0.42	151±6 <sup>a</sup>	
<b>Thr</b>	<b>0.54±0.06</b>	<b>47±1<sup>a</sup></b>	<b>0.44±0.17</b>	<b>51±2<sup>b</sup></b>	<b>23</b>
Ala	0.92±0.12	75±3 <sup>b</sup>	0.59±0.18	65±4 <sup>a</sup>	
Pro	0.52±0.06	45±2 <sup>a</sup>	0.53±0.16	61±2 <sup>b</sup>	
<b>Lys</b>	<b>0.6±0.03</b>	<b>54±3<sup>b</sup></b>	<b>0.35±0.15</b>	<b>41±4<sup>a</sup></b>	<b>45</b>
Tyr	0.25±0.04	23±3 <sup>a</sup>	0.21±0.13	24±7 <sup>a</sup>	
<b>Val</b>	<b>0.47±0.07</b>	<b>41±2<sup>a</sup></b>	<b>0.47±0.15</b>	<b>54±1<sup>b</sup></b>	<b>39</b>
<b>Ile</b>	<b>0.38±0.06</b>	<b>33±2<sup>a</sup></b>	<b>0.26±0.12</b>	<b>30±4<sup>a</sup></b>	<b>30</b>
<b>Leu</b>	<b>0.89±0.11</b>	<b>78±2<sup>a</sup></b>	<b>0.53±0.22</b>	<b>62±5<sup>a</sup></b>	<b>59</b>
<b>Phe</b>	<b>0.39±0.04</b>	<b>36±3<sup>a</sup></b>	<b>0.36±0.15</b>	<b>43±4<sup>a</sup></b>	<b>38</b>
<b>Met</b>	<b>0.37±0.1</b>	<b>33±11<sup>a</sup></b>	<b>0.23±0.06</b>	<b>31±14<sup>a</sup></b>	<b>22</b>
Cys	0.27±0.07	25±6 <sup>a</sup>	0.2±0.06	27±12 <sup>a</sup>	
<b>Trp</b>	<b>0.07±0.01</b>	<b>6±1<sup>a</sup></b>	<b>0.07±0.01</b>	<b>9±2<sup>a</sup></b>	<b>6</b>
<b>Sum EAA</b>	<b>-</b>	<b>343</b>	<b>-</b>	<b>340</b>	<b>277</b>

Compounds are reported in order of chromatographic elution as mean ± standard deviation of three independent analyses. Data are expressed in absolute (gAA/100 g fresh sample) and relative terms (mgAA/g protein). Essential amino acids ("EAA") are marked in bold. Different letters within the same row correspond to significant differences (*t*-test, *p*<0.05).

met the requirements proposed by the FAO for human nutrition (FAO, 2011). Furthermore, no amino acid could be defined as limiting, indicating that the blue crab is a very valuable source of high-quality protein. The ratio between essential and non-EAA was 0.51 in both samples, which is lower than other reports ranging from 0.79 to 1.03 (Küçükgülmez & Çelik, 2008).

## Conclusions

The blue crab is spreading with extreme and impressive speed along the Italian coasts, seriously endangering the ecosystem and the economy. As there is an urgent need for remedial action, it is essential to investigate whether and how this invasive alien species can be utilized. In this work, the nutritional composition of two parts of blue crabs collected from the Adriatic coast, namely, meat and shell, was investigated. The results showed an excellent fatty acid profile with a high proportion of polyunsaturated fatty acids (including omega 3), although the total amount of lipids was low. The proteins, which accounted

for about 10% of the meat and carapace weight, showed exceptional nutritional quality, with no limiting amount of amino acids and the amount of EAA well above the minimum requirement. As expected, the carapace was a potentially valuable source of chitin. In general, the data collected indicate that the surplus of blue crabs could be utilized as biomass for biorefinery processes, in addition to direct use as food, with the aim of fractionating their main nutrients. The fractions obtained (proteins, lipids, chitin, and minerals) could help meet the market demand for proteins, nutraceuticals, and bio-based materials.

## Authors Contribution

All authors contributed equally to this article.

## Conflicts of Interest

None.



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