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ORIGINAL RESEARCH

Mechanical property and structural-elemental analysis of marine bivalve mollusc shells: *Cerastoderma edule, Chamelea gallina, Donax trunculus, Ruditapes decussatus*

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Abstract Mussels have great annual production in the world and their shells are discarded as a waste. Shells are calcium and carbon accumulators and, formed as a consequence of calcium carbonate biomineralisation. They can be used as bio-based composite materials in different fields. Hence, assessment of the chemical compound content and microstructure of shells is important for the prospective utility. For these reasons, structure of *Cerastoderma edule, Chamelea gallina, Donax trunculus, Ruditapes decussatus* shells were analyzed with X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FT-IR) and Scanning Electron Microscope-Energy Dispersive Spectroscopy (SEM-EDS). In addition, microhardness was measured in shells. Our results indicated that shells have a flake like structure with irregular grains appearance morphologically at micrometric scale. The fractions of aragonite in particular shells were assessed. EDS analysis showed that Ca, C and O were major elements of *C. edule, C. gallina, D. trunculus, R. decussatus* in accordance with XRD data. The lowest microhardness (2.68±0.16 GPa) was in *D. trunculus* while the highest microhardness (4.28±0.32 GPa) was in *C. edule.*

Keywords Cerastoderma edule . Chamelea gallina . Donax trunculus . Ruditapes decussatus . Microstructure . Microhardness

Introduction

The shells are main feature of Mollusca for protection the soft body against adverse environmental factor and predators and, formed as a consequence of calcium carbonate biomineralisation (Spann et al. 2010; Ituen 2015). Shells have three layers (periostracum, prismatic layer, nacreous) and are consisted of the calcium carbonate ($CaCO_3$) in different forms (crystal, calcite and aragonite) and organic components [chitin $(C_8H_{13}NO_5)n$, acidic polysaccharides and largely proteins] (Chakraborty et al. 2020; Yarra et al. 2021). Additionally, vaterite as a polymorph of $CaCO_3$ is present in gastropod mollusks shell (Medakovic et al. 2003; de Paula and Silveira 2005). Early studies have been mostly reported aragonite form of calcium carbonate ($CaCO_3$) in clam shells besides calcite form in a few species (Shimamoto 1986; Ituen 2015; Mu et al. 2018). The clam *Chamelea gallina* and the grooved carpet-shell *Ruditapes decussatus* are belonging Veneridae family, the wedge clam *Donax trunculus* is belonging Donacidae family and, they naturally distributed in the north-eastern Atlantic and the Mediterranean. The common cockle *Cerastoderma edule* is belonging

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Cardiidae family and inhabits the Mediterranean and Atlantic coasts of Western Europe. The global production of *C. gallina* was 55.546 tons in 2019 (FAO 2019). *R. decussatus* and *C. edule* have high market price and enhanced the global production from aquaculture and fisheries due to increased consumer demand (Saba 2011; Malham et al. 2012). As a popular food, *D. trunculus* is mostly consumed raw (Formiga-Cruz et al. 2003). Hydraulic dredges are used for mussel production in fisheries and caused shell breakage (3-30%) (Smolowitz and Nulk 1982; Lambert and Goudrean 1996; Bailey et al. 1998; Gaspar et al. 2001; Hauton 2003; Hauton et al. 2003; Addison et al. 2006; Goldberg et al. 2012; Mu et al. 2018). Shell breakage causes entering bacteria to the soft parts and enhancing mortality rates and, survival and commercial value are negatively affected due to be sold alive in market (Alexander 2001; Mondal et al. 2014: Mu et al. 2018). Therefore, mechanical properties in shells of these species were determined for protection of shells against to damage result from dredging and processing.

Soil acidity is neutralized by mussel shells (Akpabio 2006). Mussel shells have been reported as a calcium source for animal feeds (McNaughton et al. 1974). The synthesis of alternative materials has been increasingly interested in cement industry (Ravi et al. 2021). Therefore, clarification of structuring of biomaterials is important for new resource of construction materials and prospective use in industry (Parveen et al. 2020). Moreover, the stratigraphic age of geological formations and phylogenetic evolution are assessed by geologist in fossils (Chateigner et al. 2000). In addition, shell microstructures are realized for precise description using scanning electron microscopy in Palaeontology (Taylor and Layman 1972; Carter 1980; Carter and Clark 1985; Hedegaard 1990; Hedegaard 1997; Chateigner et al. 2000). In this context, this study is important and gives the first report about form, mechanical property, mineralogy and microstructure of *C. edule, C. gallina, D. trunculus, R. decussatus* shells.

Materials and methods

Sample collection and preparation

Cerastoderma edule, Chamelea gallina, Donax trunculus, Ruditapes decussatus were collected from Black Sea (Giresun, Turkey) (41°01’39”N-38°54’02”E, 40°56’38”N-38°15’10”E, 40°55’46”N-38°33’35”E) by dredges in summer. Salinity (17.20±0.24 psu) is relatively stable in Black Sea and, pH (8.12±0.09) and temperature (23.72±0.08°C) were not largely different based on water samples and measurements taken at the time of sampling. The soft issue of the mussels was removed. Shells were washed and purified under running water. After washing, shells were dried at room temperature.

Microstructure observation

Shells of mussels were cut by a low-speed precision saw parallel to the axis of coiling to expose a longitudinal cross-section. For accessing the inner layers, shells were immersed in 0.1 M EDTA for 10 min as previously described by Mu et al. (2018). Samples were carefully rinsed with ultrapure water and dried in air. Samples were coated with Gold after chemical treatment. Some sections were observed without any further treatment. Hitachi SU3500 scanning electron microscope were used for SEM observations.

Elemental analysis

Energy-dispersive X-ray spectroscopy (EDS, Oxford INCA X-ray spectrometer) was used with AZtec and INCA software (EDS). The prepared samples were gold-coated for EDS measurements. An acceleration voltage for elemental distribution maps was 20 kV and count rates were between 1,000 and 2,000 s^{-1}.

Phase composition tests

For identification of phase composition, XRD analysis was realized in powdered shells by a Rigaku miniflex600 X-ray diffractometer (0.02º at scattering scope (2θ) ranging from 10 to 90º by using monochromatic Cu-Ka radiation [1.5406 (λ)] at 40 kV and 15 mA). To assess the aragonite transformation, $CaCO_3$ (ref JCPDS 9013800, 4001361, 4001361, 9013800, Rigaku) peaks were used as a reference.

Microhardness tests

For the assessment the microhardness, three samples were cut off for each species. In order to accomplish the high homogeneity of applied load direction and also for the convenience of microhardness test, samples were fixed with epoxy resin. After grinding off the epoxy resin on the surface of the sample, it was polished carefully to measure the microhardness of shells. Then the outer layer was rubbed off. The microhardness of shells was determined after the polishing process. The microhardness of the composite samples was measured using the Vicker hardness (Hv) (ASTM E92 standard) method under a load of 2 kg for a dwell time of 15 s with a Nemesis 9000 hardness testing machine. In the hardness tests, a diamond pyramid with a square base and an angle of 136°± 0.05 degrees between opposing surfaces was used. The Vickers hardness (Hv) was measured by inserting the diagonals (d) of the diamond-shaped indentations into the formula (1):

$$\frac{2Fsin\left(136^0/2\right)}{d^2}$$

In addition, the hardness tests, three measurements were taken in each sample to provide the repeatability of the test results. Data on microhardness were presented as means±standard deviation (S.D.) with $P < 0.05$ significance level. The data were analyzed using One-way ANOVA with Duncan test. The statistical package SPSS for Windows (Ver: 14.0) was used for computing all statistics.

Spectroscopic measurements

Infrared spectra (IR) were recorded by direct ATR transmission on powdered samples. The spectra were recorded by Bruker (Vector 22) in the range 4000–400 wave numbers (cm^{-1}), 4 cm^{-1} resolutions, 32 scans.

Results

External views of *C. edule, C. gallina, D. trunculus* and *R. decussatus* shells are given in Fig. 1. The mag-

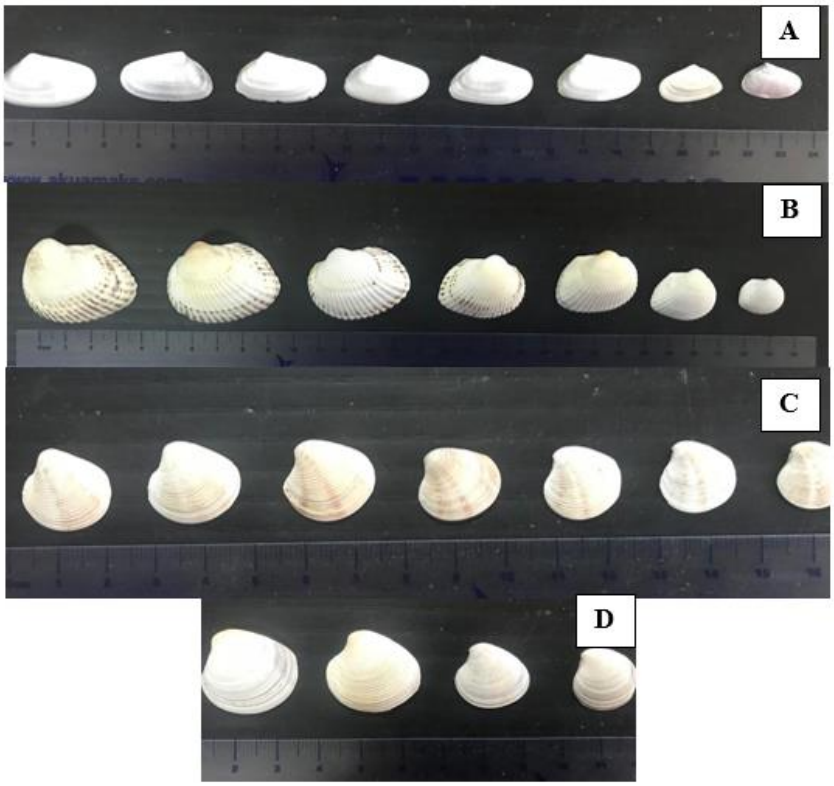

Fig. 1 External views of shells, A) *Donax trunculus*. B) *Cerastoderma edule*, C) *Chamelea gallina* and, D) *Ruditapes decussatus*

nified SEM micrographs of cross-sectional view of shells and structural aspect of the organic matrix of *C. edule, C. gallina, D. trunculus* and *R. decussatus* shells (SEM) after surface treatment by EDTA (surface decalcification) are given Figs. 2, 3, 4 and 5. Fig. 2 showed larger particles at irregular size and shape with heterogeneous distribution. In SEM micrographs of cross-sectional view of *C. edule*, aragonite sheets horizontally overlapped with one another to make continuous superimposed sheets in the area indicated in Fig. 3. Fig. 4 demonstrated homogeneous structure with equidimensional irregular-shaped crystallites. Fig. 5A-C indicates the external-most periostracum layer, intermediate cross-lamellar arranged layer and inner nacreous layer in vertical section. Different strata formed on the biological matrix with various arrangement patterns of $CaCO_3$ crystals. The areas indicated in Fig. 5D-F correspond to a homogeneous structure. Fig. 5G showed irregular grains at micrometric scale.

Energy Dispersive X-ray Spectroscopy (EDS) images indicating the distribution of some elements on part of a shell cross-section are presented in Fig. 6. O, C, Ca, and Na were obtained from analysis. The elemental composition of mineralized layer was confirmed by the EDS spectra. The polymorphs of calcium carbonate in *C. edule, C. gallina* and *D. trunculus* demonstrated strong O peaks as well as Ca peaks with the incidence of C and Na peaks while the polymorphs of calcium carbonate in *R. decussatus* exhibit strong Ca peaks as well as O peaks with the incidence of C and Na peaks.

XRD patterns of shells of mussels (*C. edule, C. gallina, D. trunculus* and *R. decussatus*) demonstrated similarities in crystalline peaks with the existence aragonite forms of calcium carbonate. The existence of orthorhombic aragonite phase in the XRD pattern was revealed with the intense peaks at (111) and (012) planes (Fig. 7).

Fig. 8 indicates the FTIR spectra of shells *C. edule, C. gallina, D. trunculus, R. decussatus.* FTIR spectra analysis showed similar features in the shell of *C. edule, C. gallina, D. trunculus, R. decussatus*. All four aragonite samples exhibited *v1* and *v2* bands at 1083 and 854 cm^{-1} respectively in their FTIR. The peak around 1786 cm^{-1} appeared due to alcohol C═H stretching. The spectral peak at 1082 cm^{-1} was showed the C–O stretching. The band at 1445 and 1458 cm^{-1} corresponded to the Alkyl Halide C═C stretch. The absorption peak at 854 cm^{-1}, 712 cm^{-1} and 700 cm^{-1} indicate occurrence of alkyl Halide C–Cl stretch. The

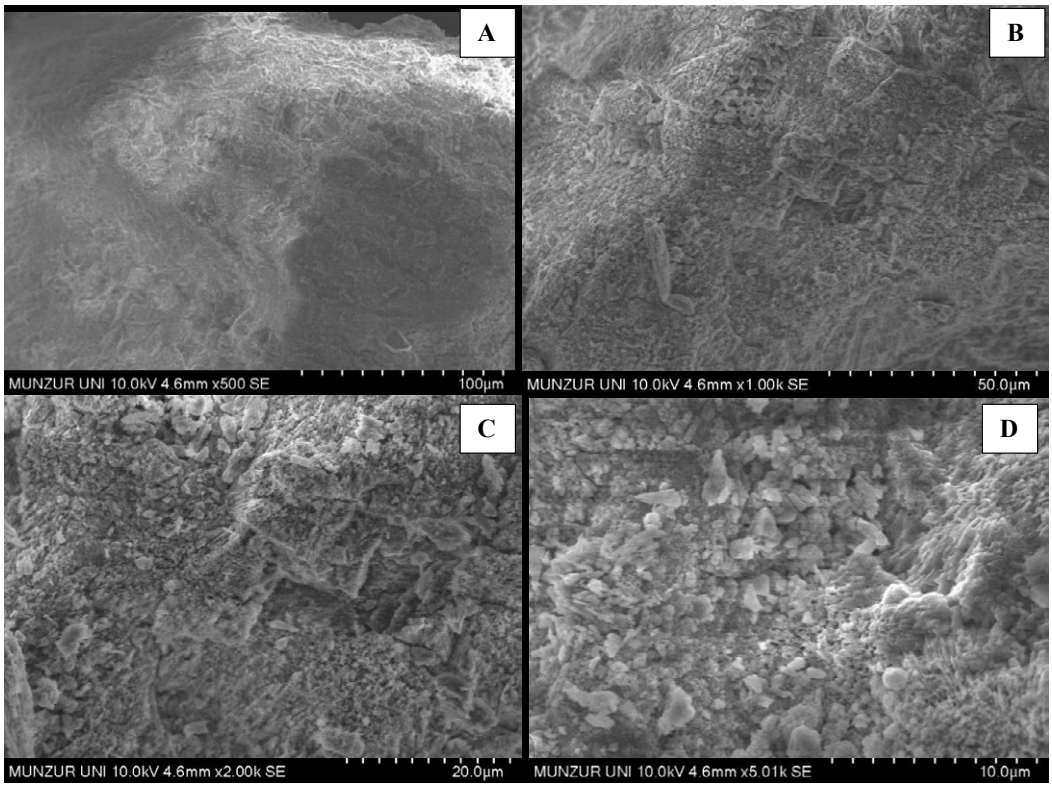

Fig. 2 SEM micrographs of cross-sectional view of shells of *Donax trunculus* at different magnifications, A) scale bar = 100 µm, B) scale bar = 50 µm, C) scale bar = 20 µm, D) scale bar = 10 µm.

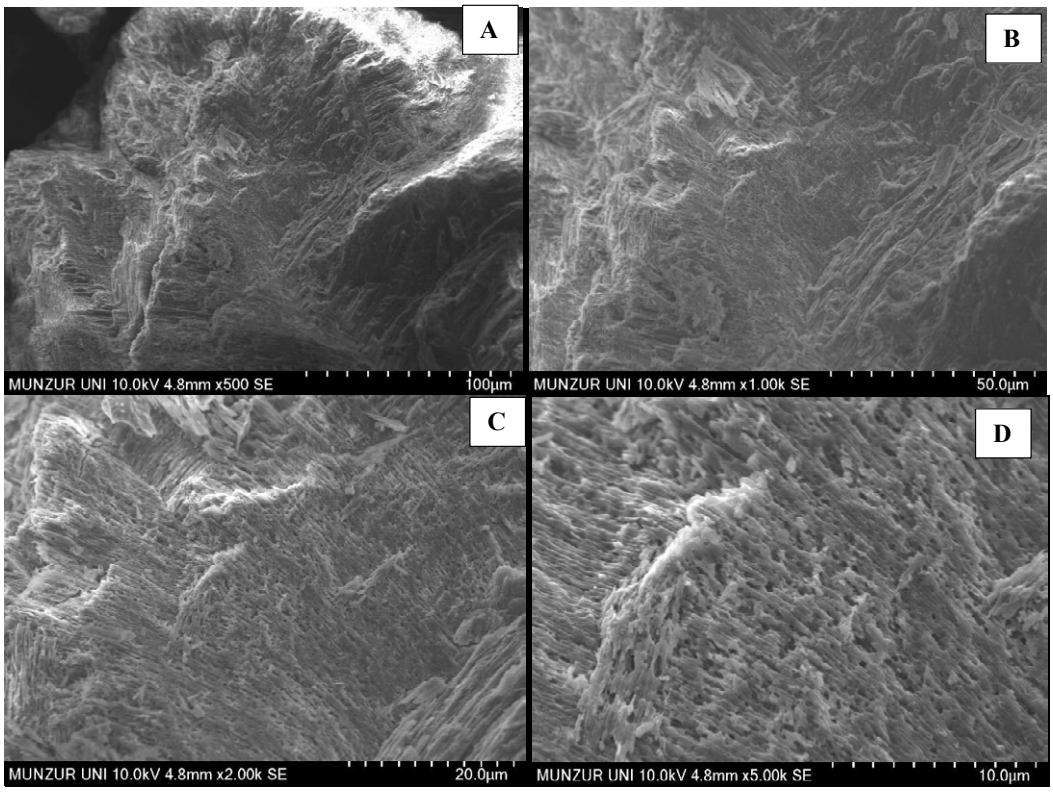

Fig. 3 SEM micrographs of cross-sectional view of shells of *Cerastoderma edule* at different magnifications, A) scale bar = 100 µm, B) scale bar = 50 µm, C) scale bar = 20 µm, D) scale bar = 10 µm.

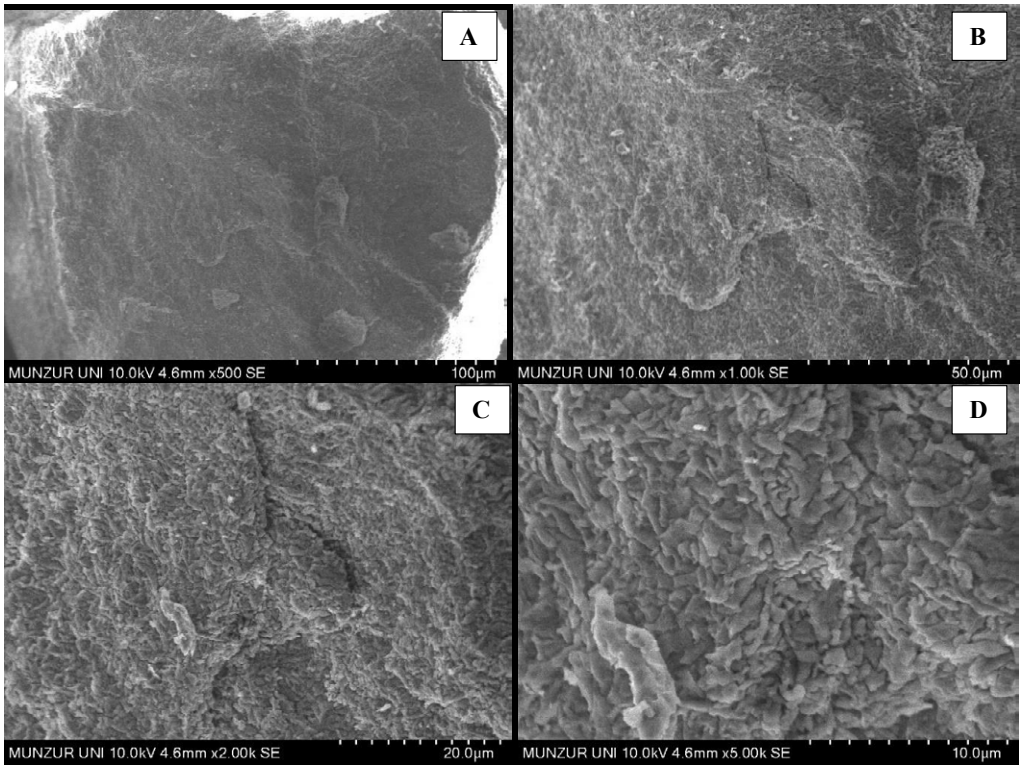

Fig. 4 SEM micrographs of cross-sectional view of shells of *Chamelea gallina* at different magnifications, A) scale bar = 100 µm, B) scale bar = 50 µm, C) scale bar = 20 µm, D) scale bar = 10 µm.

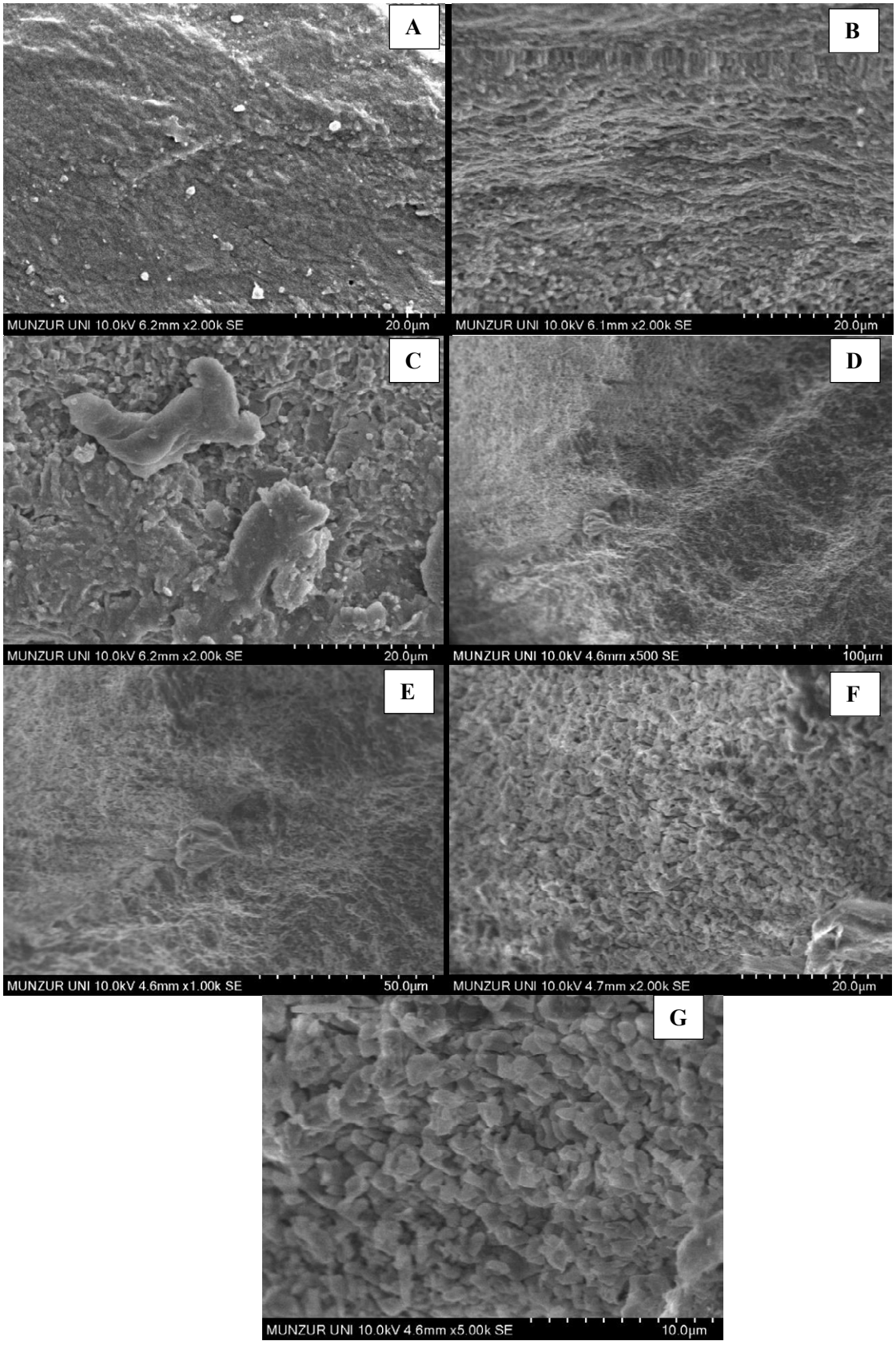

Fig. 5 A. Outer (A), inner (B) and nacre structure of shell, D-G. SEM micrographs of cross-sectional view of shells of *Ruditapes decussatus* at different magnifications.

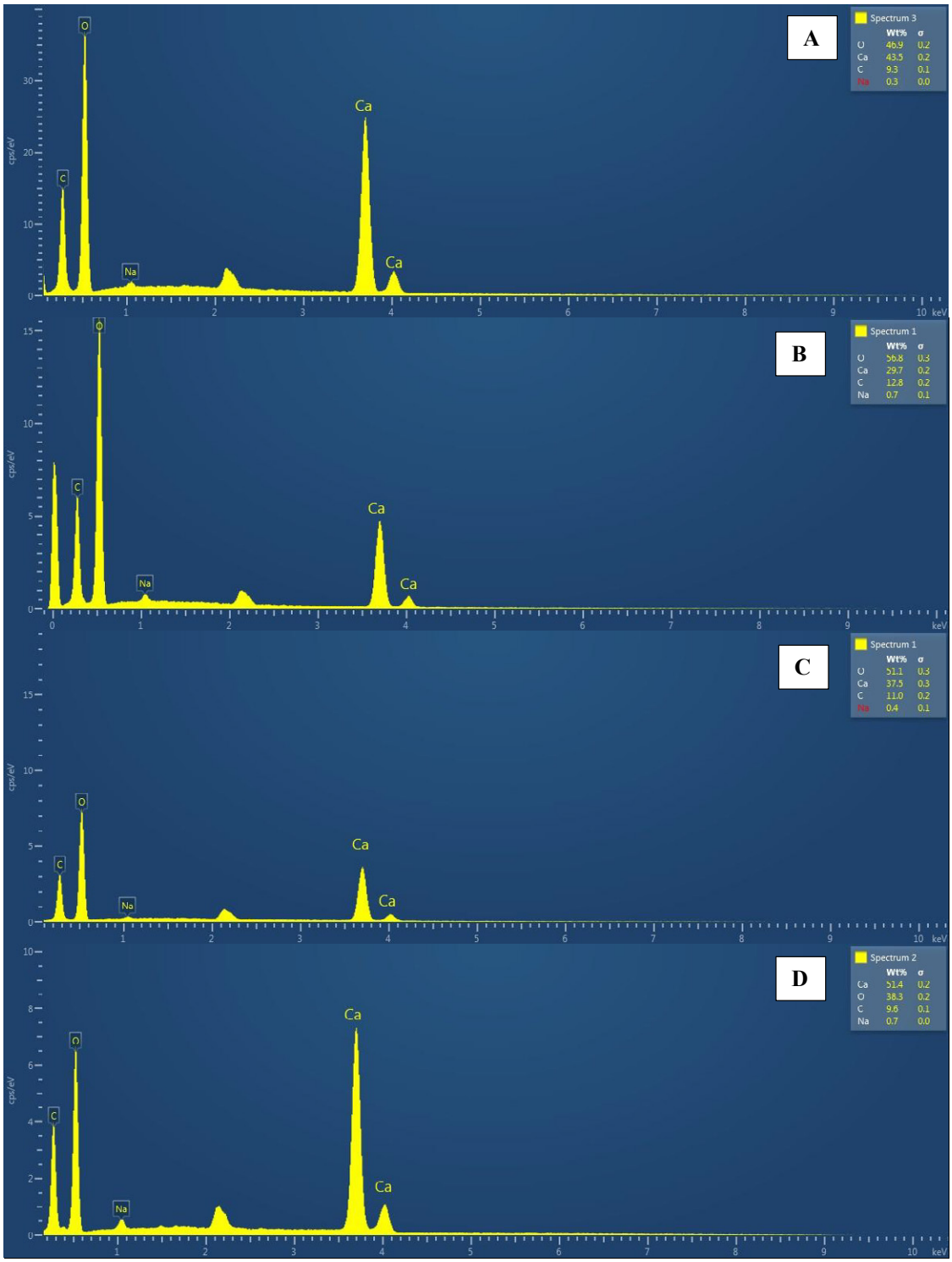

Fig. 6 The SEM-EDS of cross sectioned surface of shells A) *Donax trunculus*. B) *Cerastoderma edule*, C) *Chamelea gallina* and, D) *Ruditapes decussatus.*

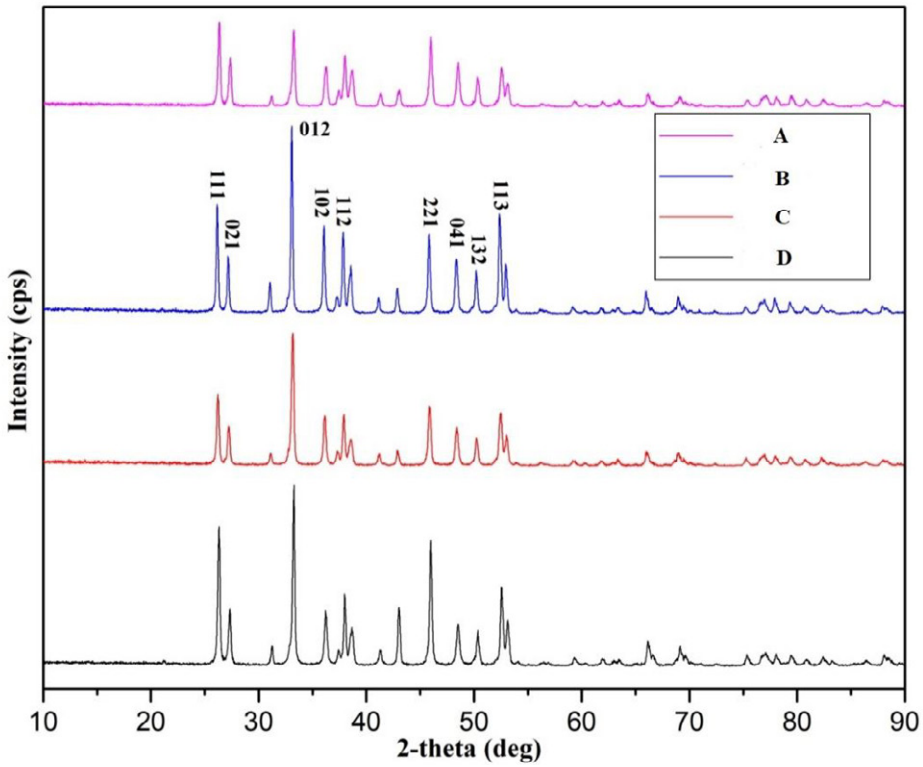

Fig. 7 XRD pattern of A) *Ruditapes decussatus,* B) *Chamelea gallina* C) *Donax trunculus*, and, D) *Cerastoderma edule*.

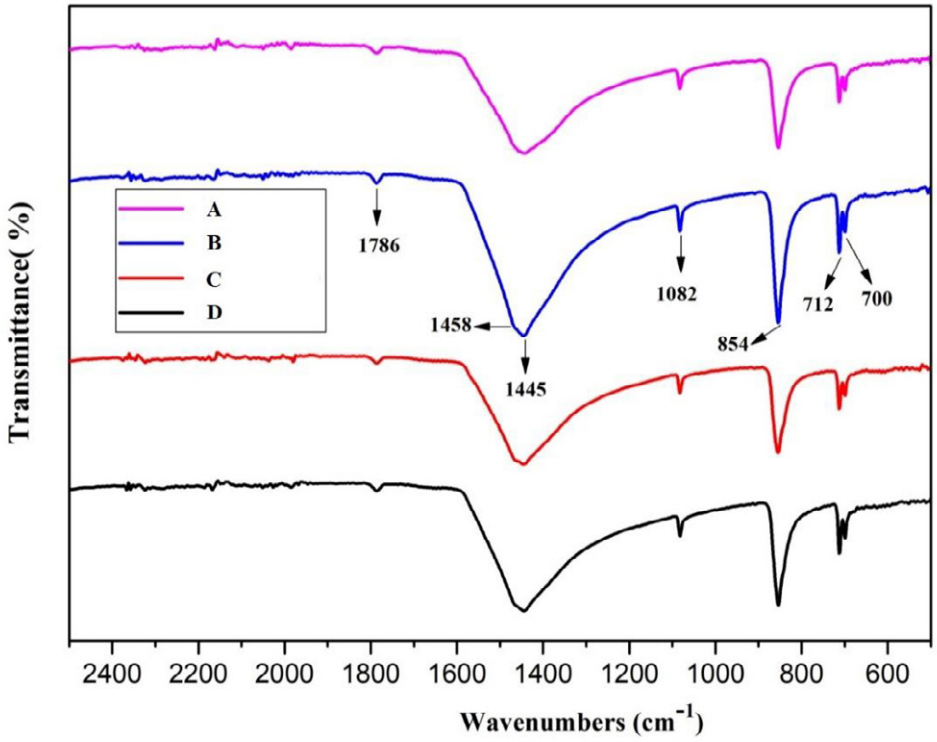

Fig. 8 The representative FTIR spectra of the prepared shells of A) *Donax trunculus*, B) *Ruditapes decussatus,* C) *Cerastoderma edule* and, D) *Chamelea gallina*.

characteristic carbonate band at 700, 712 and 854 cm^{-1} showed the aragonite form of calcium carbonate in the shell.

The means values of microhardness in the shell of *C. edule, C. gallina, D. trunculus, R. decussatus* are shown in Table 1. Considerable variations in the mechanical features exhibited depending upon species ($P < 0.05$).

Discussion

The species-specific differences cause the variations in minerals contents of the shells (Parveen et al. 2020). Previous studies by Jacob et al. (2008) and Lakshmanna et al. (2018) reported that the structure of bivalve and Gastropoda shells composed of carbon and oxygen and on the surface of the material of Cardita also adsorbed to elements Ca, Na, Si and Al. Our data obtained EDS spectra indicated that the shell in freshwater *C. edule, C. gallina, D. trunculus* and *R. decussatus* shells composed of atomic elements C 12.8%, 11.0%, 9.3% and 9.6%, O 56.8%, 51.1%, 46.9% and 38.3%, Ca 29.7%, 37.5%, 43.5% and 51.4%, and Na 0.7%, 0.4%, 0.3% and 0.7%. As the major mineralogical components, C, O, and Ca are found in most of the carbonated biominerals as reported by Vu et al. (2019).

As a shell arrangement, the crossed-lamellar structure has been reported in Bivalvia by earlier works (de Paula and Silveira 2009; Jiao et al. 2016; Milano et al. 2017a). A complex crossed-lamellar microstructure in the inner shell layer, simple crossed-lamellar microstructure forms the inner portion of the outer shell layer and a non-denticular composite prismatic microstructure has been reported in *C. edule* by Carter et al. (2012), Milano et al. (2015) and Milano et al. (2017b). The morphology of the vertical section in middle layer has been demonstrated as a crossed-lamellar structure in *Ruditapes philippinarum* by Mu et al. (2018). The granular crystals have been stated in inner layer by Taylor and Layman (1972) and Mu et al. (2018). Our results obtained from *R. decussatus* and *C. edule* were in accordance with the structural type of the outer and inner layer reported by Shimamoto (1986), Chen and Huo (2015), Mu et al. (2018), Carter et al. (2012), Milano et al. (2015) and Milano et al. (2017b).

The aragonite is metastable, rigid and relatively fragile (Chakraborty et al. 2020). In mollusk shells, the aragonite polymorph of $CaCO_3$ is dominant as a primary stabilized phase besides calcite form (Carter et al. 2012; Agbaje et al. 2017; Chakraborty et al. 2020). Aragonite occurs as single phase in some species including Veneridae family (Shimamoto 1986; Pokroy et al. 2006; Zhu et al. 2006; Yang et al. 2010; Mu et al. 2018). Medakovic et al. (2003) stated aragonite form of crystal in an outer inorganic layer of two freshwater snails (*Belgrandiella fontinalis* and *B. kuesteri*). Dauphin et al. (2017) demonstrated aragonite crystals in the prismatic layer of *U. pictorum*. Nearly pure biogenic aragonite (98% $CaCO_3$) in freshwater snail shell has been reported by Vu et al. (2019). Our observations are consistent with the findings of previous works. Concerning mineralogy of shells analyzed in this study, an inorganic layer of shells of four Molluscan species entirely consisted of aragonite. The mineralogy of in the inner inorganic layer is influenced by the environmental conditions, phylogenetic effects, locality and species (Taylor and Layman 1972; Medakovic et al. 2003; Mu et al. 2018).

The organic matter contents show variations the species differences (Chakraborty et al. 2020). In current study, the organic matrix of four mussel species indicated C–O stretching, C═H stretching, Alkyl Halide C═C stretching and alkyl Halide C–Cl stretching. Shells showed a complex organic and inorganic matrix as reported previous studies (Lee et al. 2011; Chakraborty et al. 2020; Parveen et al. 2020; Yarra et al. 2021).

As an important engineering parameter, microhardness affects the fracture toughness (Fleischli et al. 2008; Mu et al. 2018). In Molluscan, the hardness of the shells provides the protection the inner organs

Table 1 Mean hardness values (±SD) of *Donax trunculus*, *Ruditapes decussatus*, *Cardium edule* and, *Chamelea gallina*.

Species	Microhardness (GPa)
Donax trunculus	2.68 ± 0.16^{a}
Ruditapes decussatus	3.18 ± 0.10^{b}
Chamelea gallina	3.59 ± 0.03^{c}
Cerastoderma edule	4.28 ± 0.32^{d}

*Different letters (a, b, c, d) indicate significant differences as determined via one-way-ANOVA ($P < 0.05$).

Table 2 Bending strengths of different shells obtained from the literature.

Class	Species	Layer	Hardness (GPa)	References
Gastropoda	Haliotis rufescens		3.7	Wang et al. (2001)
	Crassostrea gigas		4.1	Taylor and Layman (1972)
	Clinocardium californiense		3.0-6.0	Yan et al. (2007)
	Crassostrea gigas	inner	4	Lee et al. (2008)
	Saxidomus purpuratus	inner middle	1.657 0.725	Yang et al. (2010)
	Saxidomus purpuratus		0.59-1.48	Yang et al. (2011a)
	Saxidomus purpuratus		1.75-2.5	Yang et al. (2011b)
Bivalvia	Crassostrea gigas	inner	0.19 ± 0.27	Lee et al. (2011)
	Clinocardium californiense	inner	3.04	Ji and Li (2014)
	Lajonkairia lajonkairii		1.102	Liang et al. (2016)
	Ruditapes philippinarum		3.0	Mu et al. (2018)
	Donax trunculus		2.68±0.16	Present study
	Ruditapes decussatus		3.18±0.10	
	Chamelea gallina		3.59±0.03	
	Cerastoderma edule		4.28±0.32	

against the injury by the outside world and resistance to breakage (Liang et al. 2016; Parveen et al. 2020). The different microstructures of species cause variations in microhardness values (Liang et al. 2016). Shell hardness has also been affected seasonal microstructural changes (Milano et al. 2017a). Ivanina et al. (2013) reported that hypercapnia together with elevated temperature caused decrease in hardness of *Mercenaria mercenaria* and *C. virginica*. A lower shell hardness has been demonstrated as a result of a combination of acidified and hyposaline waters when juvenile *C. virginica* grown (Dickinson et al. 2012). The hardness values of some kinds of gastropoda and bivalvia shells are presented in Table 2. In current study, the lowest microhardness (2.68±0.16 GPa) was in *D. trunculus* while the highest microhardness (4.28±0.32 GPa) was in *C. edule.* The structure resulted in the variation of hardness significantly. Our results about microhardness are different from reported data in previous studies. Considering the results shown in Table 2, the variations may be related with differences in structural features of the shells (soft organic matrix and hard aragonite), layered structure and porosities.

To conclude, microstructure, microhardness, phase composition and elemental composition of *C. edule, C. gallina, D. trunculus* and *R. decussatus* shells were assessed with different analytical techniques in this study. Based on the above observations, aragonite crystals were by confirmed with XRD and FTIR. FTIR showed the diverse arrangement pattern in shells of four Molluscan species. The highest microhardness was in the shell of *C. edule.* The shells as a waste can be reused different applications. In addition, shells can be used as an eco-friendly construction material due to good mechanical performance. Knowledge about microhardness of mussels is important to decrease the breakage and mortality rate in dredging and processing machinery. Further studies are necessary to assess characterization of different Molluscan species for utilization in different applications as a novel biomaterial.

Authors' contributions Filiz Kutluyer kocabaş and Mehmet Kocabaş wrote the manuscript, designed and carried out the experiment, and conducted data analysis. Aykut Çanakçi and A. Hasan Karabacak were determined microhardness in shells.

Ethical approval The Molluscan species used in experimental work does not need approval from the Ethics Committee for Animal Use in Turkey.

Competing interest The authors have no competing interests to declare..

Availability of data and materials The data are available from the corresponding author based on reasonable requests.

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