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RESEARCH ARTICLE

Shell composition analysis of European flat oyster (*Ostrea edulis*, Linnaeus 1758) from Marmara Sea, Türkiye: Insights into chemical properties

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ABSTRACT

The chemical structure of *Ostrea edulis* (*O. edulis*) shells was investigated in this work. The study determined zero charge points (PZC) of *Ostrea edulis* shells. The shell surface charge status is indicated by the PZC value. It was found that the shell PZC value was 8.30. The shells were subjected to Energy Dispersive X-Ray Spectroscopy (EDS) analyses and scanning electron microscope (SEM) pictures. The main structure of calcium carbonate ($CaCO_3$) is made up of carbon, oxygen, and calcium atoms, which were found in the largest quantities based on the EDS data. The structure of $CaCO_3$ was supported by Fourier Transform Infrared Spectroscopy (FT-IR) analysis. As part of the study, X-Ray Diffraction (XRD) investigations were conducted, and it was found that the shell structures are primarily composed of an aragonite and $CaCO_3$ mixture. As is well known, $CaCO_3$, which makes up roughly 94% of the shell, is the primary constituent of bivalves' shells. This research offers a thorough examination of the chemical makeup of *O. edulis* shells. This study is thought to serve as the foundation for further research on the biological and chemical characteristics of marine species.

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Introduction

Oyster species are significant organisms of the seas and hold great economic importance. Oysters, which are a fundamental resource for mollusk fishing, are subject to various operations worldwide. These operations encompass activities such as the collection, cultivation, and trade of oysters (Yildiz et al., 2011; Acarli et al., 2011, 2015). Oysters are an integral part of marine ecosystems, serving as a vital food source for many marine organisms (Grabowski et al., 2012). Additionally, they are

known as a high-value seafood for humans. The proteins, vitamins, and minerals contained within oysters are highly beneficial for human health, and their regular consumption is recommended (Ulagesan et al., 2022). The shells of molluscs, which inhabit the seas, have evolved to protect and support their bodies. These shells not only provide resilience against external forces but also serve as a defense mechanism against predators. Similarly, the shells of bivalves and gastropods fulfill similar protective functions, while the soft tissues of monoplacophorans have also evolved for a similar purpose of providing protection. Belonging to the Mollusca class, these shells are integral to the diverse array of invertebrates found globally, playing crucial roles in marine ecosystems. Beyond safeguarding soft tissues, mollusk shells serve as sustenance and provide defense mechanisms against predators (Yi & Bengtson, 1989; Qian, 1999; Li et al., 2017). All oysters (Mollusca: Bivalvia) exhibit distinctive morphological characteristics, notably the presence of two symmetric calcareous valves interconnected by a calcified structure, which form their shells. This underscores the importance of these organisms as integral components of natural ecosystems. Oysters play a pivotal role in preserving the ecological equilibrium of oceans, seas, and freshwater bodies. Additionally, their economic significance cannot be overstated. Therefore, scientific and economic research on the morphological features and shell structure of bivalve is of paramount importance (Bogan, 2008; Graf, 2013; Chakraborty et al., 2020). The process of early life adaptation to diverse environmental stresses led to the evolution of mollusk shells. The interior organs of the creature are shielded by these shells, which also help in their protection against predators and other dangers (Li et al., 2017). Oysters are classified within the subclass Bivalvia of the mollusk class Mollusca, distinguished by their characteristic shells. These organisms are shielded by two symmetrical calcareous valves comprising their shells. Beyond mere protection from external elements, these shells afford oysters mobility and feeding capabilities. Furthermore, bivalve shells play a crucial role in stabilizing the chemical composition of the surrounding water, thereby fostering the sustainable functioning of the ecosystem (Chakraborty et al., 2020). Shells make up a large percentage (about 56–61%) of bivalves' weight. About 94% of the makeup of a shell is made up of $CaCO_3$. Marine species employ their shells as their hard outer structures for protection and structural support. Numerous creatures, including gastropods and other marine invertebrates, make and use them. Crystals of $CaCO_3$ aggregate to form these shells, which are often protected by a thin organic membrane (Hamester et al., 2012; Ituen, 2015; Mititelu et al., 2022). The

primary constituent of shells is commonly $CaCO_3$, yet various small inorganic trace elements are also found in different bivalve species. These trace elements have the potential to impact the physical characteristics of shells and play a significant role in the life cycles of shelled organisms (Chakraborty et al., 2020). Calcite or aragonite crystals are the most common forms of $CaCO_3$ that makes up shells. The shell resilience and hardness are derived from these crystals. Other trace elements found in the shells, nevertheless, are also quite important. Examples of elements that can affect the color and pattern creation of shells are magnesium (Mg), manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn). These substances can also make the shells more resilient and aid in the defense of species with shells against predators (Carroll & Romanek, 2008; Spann et al., 2010; Nakamura et al., 2014; Agbaje et al., 2017, 2018a, 2018b; Chakraborty et al., 2020). The aim of this study was to investigate the chemical structure and composition of *O. edulis* shells. These shells comprise a blend of $CaCO_3$ and organic matrix, crucial for providing strength and durability. The research sought to elucidate how environmental factors such as pH levels, and the chemical composition of *O. edulis* shells. These findings offer insights into how *O. edulis* shells respond to environmental fluctuations, facilitating a deeper understanding of their adaptive mechanisms.

Material and Methods

Study Area and Sampling

One hundred samples of *O. edulis* were collected from the coasts of the Fener Island, Bandırma Gulf in the Marmara Sea. First, the collected shells were run through clean water. These shells were then ground to a homogeneous texture.

Determination of Zero Charge Point (PZC) in Shells

The zero point of charge (PZC) was established through pH assessments conducted on homogenized and ground samples of mollusk shells (Mahmood et al., 2011). Firstly, 100 ml of 0.01 M KNO_3 solutions were made in an Erlenmeyer flask in order to calculate the pH_{PZC}. Using 0.1 M HCl and NaOH, the starting pH values (pH_I) of these solutions were changed from pH 4 to pH 10. These solutions were then supplemented with the altered samples. After that, the mixture was agitated for 48 hours at a steady temperature with a magnetic stirrer. The solution's ultimate pH (pH_F) was tested and noted after 48 hours. Plotting $\Delta pH = pH_I - pH_F$, the difference between the initial and final pH, was done against the initial pH (pH_I). The

PZC was identified as the point where the curve contacts the x-axis.

Chemical Structure Analyzes in O. edulis Shells

Analysis using the SEM-EDS (Scanning Electron Microscope-Energy Dispersive X-Ray Spectroscopy, model JEOL JSM-7100F) was carried out at Science and Technology Application and Research Center, Çanakkale Onsekiz Mart University. The instrument offers a magnification range spanning from ×40 to 300,000, coupled with an accelerating voltage varying between 0.2 and 30 kV. This was followed by a gold-palladium (80-20%) coating procedure employing a voltage of 10 mA. The resulting coating thickness typically measures approximately 2-3 nm. Secondary electrons were utilized in the SEM apparatus to obtain images. Using a scanning electron microscope (SEM), surface features of the ground shells were analyzed, and discrepancies were found. Additionally, the carbon (C), oxygen (O), nitrogen (N), calcium (Ca), and sulphur (S) contents of the shells were examined using EDS analysis. FT-IR spectra were recorded in the 650–4000 cm^{-1} range on a Perkin-Elmer SpectrumOne instrument. FT-IR investigations of ground and homogenized *Ostrea edulis* shells were conducted. To learn more about the chemical structure of *O. edulis* shell, XRD investigations were also carried out. The PANalytical Empyrean XRD instrument, housed in the Science and Technology Application and Research Center of Çanakkale Onsekiz Mart University, was utilized to conduct the analyses.

Results and Discussion

The zero point of charge (PZC) is a crucial parameter in physicochemistry, particularly concerning adsorption. It signifies the state where the electrical charge on a surface reaches zero. Essentially, PZC corresponds to the pH value at which the net electrical charge on the surface of a solid, immersed in an electrolyte solution, becomes neutral. This concept not only delineates a specific condition of PZC but also holds significant relevance to adsorption processes. PZC represents the pH at which the net electrical charge on the surface of a solid, in contact with an electrolyte, equals zero (Somasundaran & Agar, 1967; Sverjensky, 1994; Babić et al., 1999; Hou et al., 2001; Kosmulski, 2002; Fiol & Villaescusa, 2008; Mahmood et al., 2011). Understanding the significance of the definition provided requires a grasp of the concept of adsorption. Adsorption is the phenomenon where one substance adheres to the surface of another substance. This

interaction commonly transpires between solid and liquid or gas phases, with the surface's electrical charge playing a pivotal role in facilitating adsorption. In numerous fields where adsorption processes are investigated, the determination and comprehension of PZC hold paramount importance (Fiol & Villaescusa, 2008; Mahmood et al., 2011). Understanding the pH levels at which a surface is electrically neutral is a crucial first step in comprehending the process of adsorption, and this idea helps with that. Different pH values can be obtained for different surfaces when determining PZC, which is normally done experimentally. The determination and comprehension of PZC are important steps in understanding the adsorption properties of a surface. The variability in PZC across different surfaces, stemming from factors such as chemical composition, structure, and other surface properties, underscores its fundamental importance. This understanding is not only crucial in the realm of physical chemistry but also holds significant relevance across various industrial applications. Especially in materials science, colloid chemistry, environmental engineering, and related fields focusing on surface chemistry and adsorption phenomena, the determination and comprehension of PZC are crucial for material design and application. In conclusion, the PZC is a significant parameter in physical chemistry pertaining to adsorption phenomena, indicating the state where the electrical charge on a surface is zero. Typically, PZC refers to the pH value at which the net electrical charge on a surface submerged in an electrolyte solution equals zero. The PZC of *O. edulis* shell particles was established through analysis of their surface characteristics. The determined PZC value for the shells was 8.30 (Figure 1). Depending on the surface properties of the shells, PZC value may fluctuate based on the capability of groups within the molecule to either gain or lose hydrogen or electrons. The hydroxyl groups in the acidic water draw additional protons, creating a positively charged adsorbent surface on which negative ions are adsorbed. The surface becomes negatively charged and positively charged groups adsorb. The shells' initial pH (pHi) vs ΔpH curves are displayed in Figure 1. Along with the values derived using linear regression, the experimental PZC values acquired at the conclusion of the investigation are also displayed.

Using the X-Ray Diffraction (XRD) method, the shells of *O. edulis* were examined and their crystal structures were compared to those of calcium carbonate ($CaCO_3$) and aragonite. This research, which looks at the atomic and molecular structures of the shells, is based on the X-ray pattern that is specific to each crystal and is produced by the crystalline

phase of the structure. The crystal structures of $CaCO_3$ constituting these shells are shaped through biomineralization processes influenced by diverse factors such as species characteristics, growth conditions, and environmental factors. To comprehend the alterations in molecular structures of shells contingent upon crystal formation, it becomes imperative to advance these techniques. Thus, understanding the mechanism by which organic tissue interacts with the mineral phase within tissues becomes crucial. This insight is pivotal for elucidating the intricate processes underlying biomineralization and facilitating advancements in fields such as materials science, biology, and environmental studies (Pokroy et al., 2007). The references employed in this work were aragonite and calcium carbonate, which share the same chemical components but have distinct crystal forms. Aragonite is a carbonate ($-CO_3^{-2}$) mineral that primarily develops naturally in freshwater or marine environments. It differs from calcite (calcium carbonate) in that it has a distinct crystal lattice structure. Figure 2 is a comparison with our samples using XRD information provided by US Geological Survey (USGS, 2001). Every peak in the picture represents the X-rays that a particular atomic plane in a mineral crystal structure diffracted. The peak intensity reflects the quantity of diffracted X-rays, while the peak angle indicates the direction of the diffracted X-rays. Due to their distinct crystal structures, calcite and aragonite exhibit varying peak positions. This image illustrates how XRD enables the determination of a mineral chemical composition and crystal structure. By analyzing peak positions and intensities, valuable insights into the crystalline nature of the material can be gained, facilitating a deeper understanding of its properties and characteristics. After analyzing the spectra presented in Figure 1, it was ascertained that the principal constituents of *O. edulis* shell structures are aragonite and $CaCO_3$. The samples are represented by the blue portion of the spectrum, calcite by the red section, and aragonite by the green component. As a result, recognizing the aragonite and calcium structures in the composition was simpler. An effective method for classifying minerals and analyzing crystal structures is XRD.

SEM-EDS is a microscopy method employed to image surfaces and analyze elemental composition. By directing an electron beam onto the sample surface, this technique visualizes surface topography while also identifying the chemical elements present. SEM-EDS is a prevalent analysis technique utilized across various industrial and scientific domains. Its extensive application derives from its efficacy in scrutinizing both surface characteristics and chemical composition of samples. Another notable aspect of SEM-EDS is its capacity for

high-resolution surface imaging. This capability enables the inspection of surface morphology and the observation of microstructures present on the sample surface. Moreover, it facilitates regional elemental analysis, offering valuable information regarding the chemical composition of the sample surface. These capabilities provide intricate insights into the surface properties of the sample, contributing to a comprehensive understanding of its characteristics. Figure 3 displays the shells SEM photos. The ground *O. edulis* shell's tiny structure is depicted in the SEM image. The image shows that $CaCO_3$ crystals make up the majority of the mussel shell. The principal component of the mussel shell, plates of unevenly formed and sized $CaCO_3$ crystals, make up the majority of the image. There could be other elements or organic materials among the tiny particles on the plates. The mechanical characteristics, chemical makeup, and biological activity of the *O. edulis* shells can all be understood by using the important information this image provides on its tiny structure.
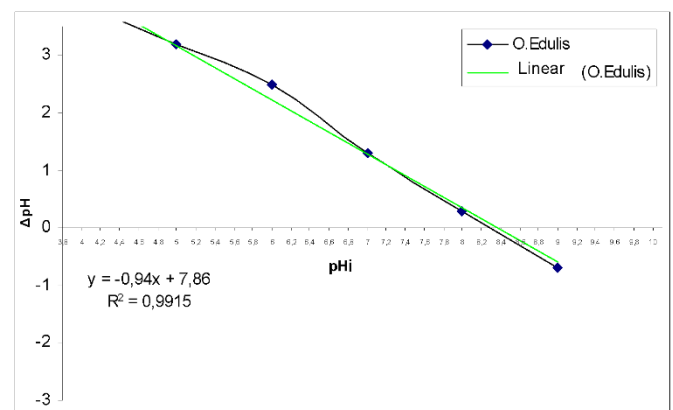

Figure 1. Zero charge point of *O. edulis* shell particles

The salinity values of sea water in the sampling location were measured between 24 and 26 ‰. In addition, temperatures were measured between 6°C and 30°C. The Energy Dispersive X-ray Spectroscopy (EDS) analysis result of *O. edulis* shell is displayed in Figure 4. The method of EDS analysis is used to ascertain a material's chemical makeup. Elements including C, O, Ca, Mg, sodium (Na), and N are clearly visible in the spectrum. The primary structural constituents of $CaCO_3$ are represented by the elements that are most prevalent and fundamental in the spectrum: Ca, C, and O. C is a sign of organic stuff and $CaCO_3$, which is the oyster primary component. Proteins and other organic compounds can be found in oyster shells. The oxygen atoms in $CaCO_3$, a substance found in oyster shells, are the source of oxygen. Ca is derived from $CaCO_3$, the principal constituent of the oyster shell. Magnesium is believed to originate from magnesium

carbonate, present in trace amounts within the oyster shell. Na, meanwhile, can be attributed to sodium chloride present in seawater. Most organic compounds, including proteins, contain a sizable amount of nitrogen. Consequently, the presence of proteins or other organic molecules is indicated by the presence of N in the oyster shell. It has been noted that the shell contains N at a 5.8% rate. The elevated N concentration in the oyster shell, compared to other elements, suggests an abundance of proteins or other organic molecules. Potential N sources in the shell encompass proteins from the oyster's diet, proteins secreted by the oyster itself, and bacteria or other microorganisms adhering to the oyster shell. This range of sources offers valuable insights into the chemical composition of the oyster shell. This data aids in assessing the purity of the oyster shell, identifying its elemental composition, and discerning potential impurities. Trace elements in shells typically originate from seawater, which harbors diverse minerals and elements utilized by shell-bearing organisms in shell formation. These components have the potential to alter the chemical structure and physical characteristics of shells. For instance, adding Mg to $CaCO_3$ crystals can increase the shells elasticity and durability. The components that organisms that bear shells acquire from seawater play a crucial role in the formation of their shells. Seawater indeed comprises Ca, Mg, -CO_3^{-2}, phosphate, and various other minerals. These elements serve as the building blocks for shell-bearing organisms in the formation of their shells. $CaCO_3$, in particular, stands out as the primary component of shells, with shell formation facilitated by the uptake of Ca and -CO_3^{-2} ions from seawater. Depending on the ratio of the elements they contain, shells can have different chemical compositions. For instance, the incorporation of Mg

can enhance the strength and pliability of $CaCO_3$ crystals. As a result, shell-bearing species' shells can withstand larger environmental stresses. Furthermore, it is thought that additional factors may potentially affect the shells color and texture (Carroll & Romanek, 2008; Nakamura et al., 2014; Chakraborty et al., 2020). Indeed, the elemental composition of shells from shell-bearing organisms can contain traces reflecting geological and climatic variations. Alterations in elemental ratios within seawater can result in discrepancies in shell formation and composition. Consequently, the shells of these organisms serve as valuable archives for deciphering the history of geological and climatic transformations. Scientists are interested in researching the origins of materials found in shells as well as the mechanisms involved in shell creation. It is possible to learn more about the evolutionary processes of creatures that bear shells by comprehending the materials derived from saltwater and their role in the production of shells. In addition, understanding the consequences of environmental changes can be achieved by using the elemental content of shells as an indication (Carroll & Romanek, 2008; Nakamura et al., 2014; Agbaje et al., 2017, 2018a; Chakraborty et al., 2020). In summary, trace elements found in shells primarily derive from seawater and exert a substantial influence on the formation of shells in shell-bearing organisms. The presence of these elements can impact both the chemical composition and physical characteristics of shells. This phenomenon offers valuable insights into the evolutionary dynamics of shell-bearing organisms and their capacity to adapt to environmental changes. For scientists, this is a vital field of study, and knowledge of the past geological and climatic changes can be gained from studying the shells of creatures that carry them.
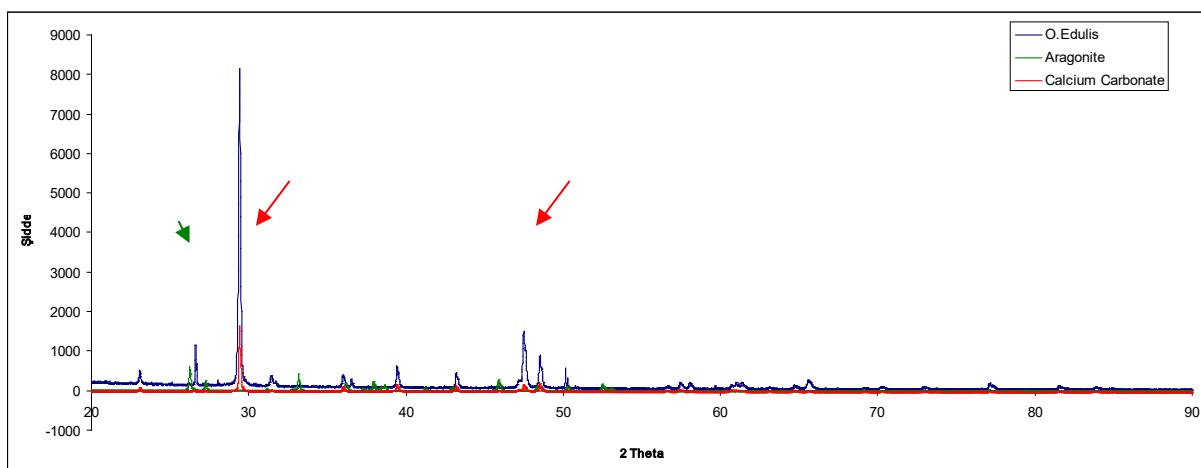

Figure 2. XRD spectrum of calcite, aragonite and *O. edulis* shell using the USGS data (USGS, 2001)
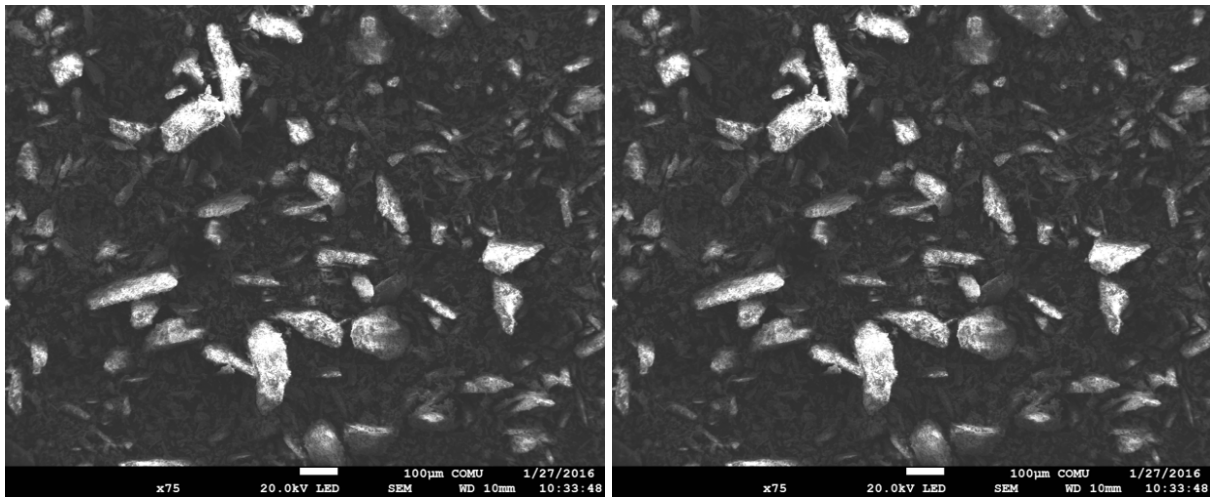
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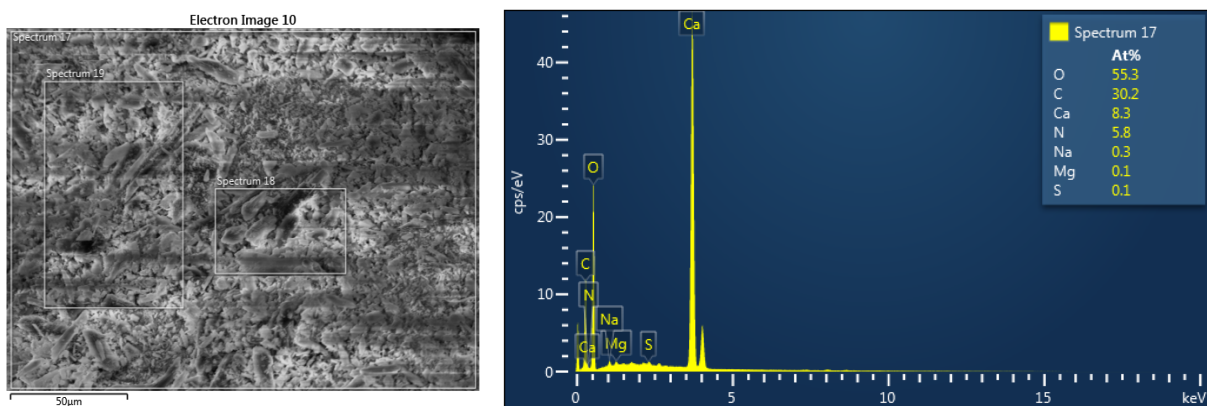**Figure 3.** SEM images of *O. edulis* shells

Figure 4. EDS spectrum of *O. edulis* shell

Fourier Transform Infrared Spectroscopy (FT-IR) is a spectroscopic method employed to elucidate the bond structures of molecules. In this technique, infrared light is directed onto a sample, where it interacts with the molecules present. The extent of light absorption by the sample molecules is contingent upon the wavelength of the incident light. The chemical makeup of a sample can be ascertained by measuring the absorption of light at various wavelengths. It is a very useful method for figuring out the molecular structures, functional groups, and chemical bonds of the material being studied. FT-IR spectroscopy is extensively utilized across diverse industries, including biological research, drug development, food analysis, and materials science. Its widespread application stems from its effectiveness in providing molecular-level information. Consequently, the primary objective of FT-IR analysis is to ascertain the structure of the main component present in the shells. The FT-IR spectra of Aragonite (the mineral crystal $CaCO_3$) from spectral data described during the RRUFF Project are given below in Figure 5A (Lafuente et al., 2015). In Figure 5A, the peaks observed at 1420 cm^{-1} correspond to the C-O stretching vibration, while those at 870 and 710 cm^{-1} represent additional C-O stretching vibrations within the -CO_3^{-2} molecule. The FT-IR spectra of *Ostrea edulis* shells are depicted in Figure 5B. FT-IR analysis of the shell data revealed the presence of CO_3^{-2} vibration bands at 1420 cm^{-1}. The -CO_3^{-2} molecule, which makes up the majority of the shell's chemical structure, has an in-plane bending band (V_4) that is moderately intense and appears at 710 cm^{-1}. The presence of the CO_3^{-2} bands, particularly at the observed peaks of 1420, 870, and 710 cm^{-1}, unequivocally indicates that the primary chemical structure of the shells is CO_3^{-2}. Additionally, in the spectrum of the sample, peaks are detected at 3400 (N-H stretching of amides), 1650 (C=O stretching of amides), and 1540 cm^{-1} (N-H bending of amides). Based on the positions of these peaks in the spectrum, it can be inferred that *O. edulis* shells comprises $CaCO_3$ and also contains proteins. This spectrum offers important insights on the chemical makeup of the structure of $CaCO_3$. The purity of the calcium carbonate structure, its crystal form, and the contaminants it contains may all be ascertained using this information. The primary Raman peaks of -CO_3^{-2}, which make up the shells of *Ostrea edulis* in this work, and aragonite, which was subjected to FT-IR analysis as part of the RRUFF Project (Lafuente et al., 2015), are complementary to one another.
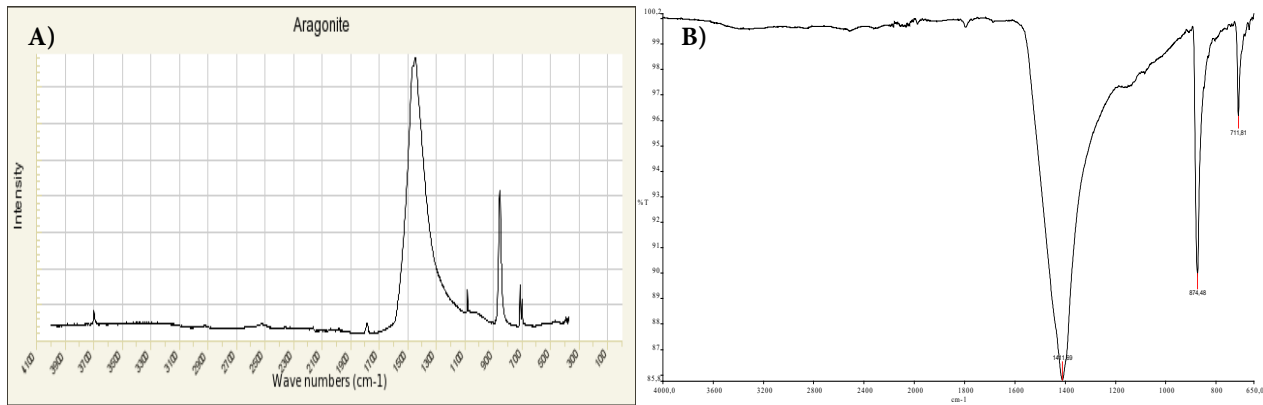
Figure 5. FT-IR spectra of **A)** Aragonite (CaCO₃) mineral crystal characterized within the scope of the RRUFF Project (Lafuente et al., 2015) and **B)** *O. edulis* shells

The structures found on shelled organisms are the result of the buildup of minerals like CaCO₃. In addition to shielding the organism's body, these shells are impacted by their surroundings. It is well recognized that environmental influences have an impact on the trace elements found in shells. For instance, the chemical makeup and trace element content of the shells can be impacted by the temperature, acidity, and salinity of the saltwater. As a result, changes in the environment can have an impact on the characteristics and makeup of shells in species with shells (Carroll & Romanek, 2008; Spann et al., 2010; Nakamura et al., 2014; Agbaje et al., 2017, 2018a; Chakraborty et al., 2020). The temperature, acidity, and salinity levels of seawater have a direct impact on the survival of shelled organisms. Additionally, these factors play a crucial role in shaping the growth and composition of their shells. For instance, as seawater acidity rises, shelled organisms may reduce their utilization of CaCO₃ during shell formation, potentially leading to the production of thinner and more fragile shells. These environmental changes pose significant challenges to the life and sustainability of shelled organism populations within marine ecosystems. Likewise, the salinity level of seawater can impact the composition of shells. Elevated salinity levels have the potential to modify the mineral content of shells, thereby decreasing their durability. Furthermore, environmental factors exert influence on the trace element content of shells in shelled organisms. Trace elements present in seawater significantly contribute to the composition of shells. For example, elements such as strontium, Mg, and Fe can have an impact on how shells develop. The composition of the shells can be affected by variations in the levels of these components due to changes in the surrounding environment. Scientific research has focused on how environmental conditions affect the trace elements found in the shells of organisms that have shells. New discoveries are made in the study of the chemical

makeup of saltwater and the impact of environmental changes on shell composition. The findings of these investigations can aid in our comprehension of the life and evolutionary mechanisms of species with shells (Spann et al., 2010; Nakamura et al., 2014; Agbaje et al., 2017, 2018b; Chakraborty et al., 2020). In summary, the shells of shelled organisms are subject to the influence of environmental factors. Variations in seawater temperature, acidity, and salinity levels can directly impact the chemical composition and trace element content of these shells. Consequently, environmental changes have the potential to alter the composition and properties of shelled organism shells. This area remains a focal point of scientific investigation, with profound implications for the evolutionary trajectories of shelled organisms.

Conclusion

CaCO₃, the primary constituent of shells, enables marine organisms to construct their shells by utilizing dissolved minerals from the water. The bodies of the organisms expel these minerals, which are then utilized to create shells. A component of the calcium cycle in marine environments is the CaCO₃ found in shells. This study offers an in-depth analysis of the chemical composition of shells from *O. edulis* by identifying the zero charge sites (PZC) of the shells, the study examined their surface charge status. The results showed that the shells PZC value is 8.30, which offers important information about the shell characteristics and possible uses. The EDS results revealed that Ca, C, and O atoms, which are characteristic constituents of CaCO₃ structures, were present in the highest proportions, suggesting the predominant structure of the shells. The existence of CaCO₃ structures was confirmed by FT-IR research. XRD examinations were carried out as part of the study, and the findings showed that the shell formations are mostly composed of an aragonite and CaCO₃ mixture. These

analyses offer crucial insights into the structural characteristics of the shells. While $CaCO_3$ emerges as the predominant chemical component of the shells, it is recognized that various small inorganic trace elements are often present in different bivalve species. Understanding the presence and impact of these trace elements is paramount in unraveling the complexities of bivalve ecology and shell formation processes. The preservation of marine habitats and the conservation of bivalve organisms are thought to benefit greatly from research in this field.

Compliance With Ethical Standards

Authors' Contributions

BK: Investigation, Methodology, Data curation, Formal analysis, Writing - original draft, Writing - review & editing

HY: Investigation, Writing - original draft, Supervision, Funding acquisition

PV: Investigation, Data curation, Formal analysis, Writing - review & editing

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Acarli, S., Lök, A., Kirtik, A., Acarli, D., Serdar, S., Kucukdermenci, A., Yigitkurt, S., Yildiz, H., & Saltan, A. N. (2015). Seasonal variation in reproductive activity and biochemical composition of flat oyster (*Ostrea edulis*) in the Homa Lagoon, Izmir Bay, Turkey. *Scientia Marina*, *79*(4), 487-495. https://doi.org/10.3989/scimar.04202.16A

Acarli, S., Lok, A., Küçükdermenci, A., Yildiz, H., & Serdar, S. (2011). Comparative growth, survival and condition index of flat oyster, Ostrea edulis (Linnaeus 1758) in Mersin Bay, Aegean Sea, Turkey. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, *17*(2), 203-210. https://doi.org/10.9775/kvfd.2010.2806.

Agbaje, O. B. A., Shir, I. B., Zax, D. B., Schmidt, A., & Jacob, D. E. (2018a). Biomacromolecules within bivalve shells: Is chitin abundant? *Acta Biomaterialia*, *80*, 176–187. https://doi.org/10.1016/j.actbio.2018.09.009

Agbaje, O. B. A., Thomas, D., Dominguez, J. G., Mclnerney, B. V., Kosnik, M. A., & Jacob, D. E. (2018b). Biomacromolecules in bivalve shells with crossed lamellar architecture. *Journal of Materials Science*, *54*(6), 4952–4969. https://doi.org/10.1007/s10853-018-3165-8

Agbaje, O. B. A., Wirth, R., Morales, L. F. G., Shirai, K., Kosnik, M. A., Watanabe, T., & Jacob, D. E. (2017). Architecture of crossed-lamellar bivalve shells: The southern giant clam (*Tridacna derasa*, Röding, 1798). *Royal Society Open Science*, *4*(9), 170622. https://doi.org/10.1098/rsos.170622

Babić, B., Milonjić, S. K., Polovina, M., & Kaludierović, B. (1999). Point of zero charge and intrinsic equilibrium constants of activated carbon cloth. *Carbon*, *37*(3), 477–481. https://doi.org/10.1016/s0008-6223(98)00216-4

Bogan, A. E. (2008). Global diversity of freshwater mussels (Mollusca, Bivalvia) in freshwater. *Hydrobiologia*, *595*(1), 139–147. https://doi.org/10.1007/s10750-007-9011-7

Carroll, M., & Romanek, C. S. (2008). Shell layer variation in trace element concentration for the freshwater bivalve *Elliptio complanata*. *Geo-Marine Letters*, *28*(5–6), 369–381. https://doi.org/10.1007/s00367-008-0117-3

Chakraborty, A., Parveen, S., Chanda, D. K., & Aditya, G. (2020). An insight into the structure, composition and hardness of a biological material: the shell of freshwater mussels. *RSC Advances*, *10*(49), 29543–29554. https://doi.org/10.1039/d0ra04271d

Fiol, N., & Villaescusa, I. (2008). Determination of sorbent point zero charge: Usefulness in sorption studies. *Environmental Chemistry Letters*, *7*(1), 79–84. https://doi.org/10.1007/s10311-008-0139-0

Grabowski, J. H., Brumbaugh, R. D., Conrad, R. F., Keeler, A. G., Opaluch, J. J., Peterson, C. H., Piehler, M. F., Powers, S. P., & Smyth, A. R. (2012). Economic valuation of ecosystem services provided by Oyster Reefs. *BioScience*, *62*(10), 900–909. https://doi.org/10.1525/bio.2012.62.10.10

Graf, D. (2013). Patterns of freshwater bivalve global diversity and the state of phylogenetic studies on the Unionoida, Sphaeriidae, and Cyrenidae. *American Malacological Bulletin*, *31*(1), 135–153. https://doi.org/10.4003/006.031.0106

Hamester, M. R. R., Balzer, P. S., & Becker, D. (2012). Characterization of calcium carbonate obtained from oyster and mussel shells and incorporation in polypropylene. *Material Research*, *15*(2), 204–208. https://doi.org/10.1590/S1516-14392012005000014

Hou, W. G., Su, Y. L., Sun, D. J., & Zhang, C. G. (2001). Studies on zero point of charge and permanent charge density of MG–FE hydrotalcite-like compounds. *Langmuir*, *17*(6), 1885–1888. https://doi.org/10.1021/la0008838

Ituen, E. U. (2015). Mechanical and chemical properties of selected mullusc shells in Nigeria. *The International Journal of Agricultural Policy and Research*, *3*(1), 53–59. https://doi.org/10.15739/IJAPR.026

Kosmulski, M. (2002). The significance of the difference in the point of zero charge between rutile and anatase. *Advances in Colloid and Interface Science*, *99*(3), 255–264. https://doi.org/10.1016/s0001-8686(02)00080-5

Lafuente, B., Downs, R. T., Yang, H., & Stone, N. (2015) The power of databases: The RRUFF project. In Armbruster, T., & Danisi, R. M. (Eds.), *Highlights in Mineralogical Crystallography* (pp. 1-30). De Gruyter.

Li, L., Zhang, X., Yun, H., & Li, G. (2017). Complex hierarchical microstructures of Cambrian mollusk *Pelagiella*: Insight into early biomineralization and evolution. *Scientific Reports*, 7(1), 1935. https://doi.org/10.1038/s41598-017-02235-9

Mahmood, T., Saddique, M. T., Naeem, A., Westerhoff, P., Mustafa, S., & Alum, A. (2011). Comparison of different methods for the point of zero charge determination of NIO. *Industrial & Engineering Chemistry Research*, *50*(17), 10017–10023. https://doi.org/10.1021/ie200271d

Mititelu, M., Stanciu, G., Drăgănescu, D., Ioniță, A. C., Neacșu, S. M., Dinu, M., Stefan-van Staden, R. I., & Moroșan, E. (2022). Mussel shells, a valuable calcium resource for the pharmaceutical industry. *Marine Drugs, 20*(1), 25. https://doi.org/10.3390/md20010025

Nakamura, A., De Almeida, A. C., Riera, H. E., De Araújo, J. L. F., Gouveia, V. J. P., De Carvalho, M. D., & Cardoso, A. V., (2014). Polymorphism of $CaCO_3$ and microstructure of the shell of a Brazilian invasive mollusc (*Limnoperna fortunei*). *Materials Research, 17*(suppl 1), 15–22. https://doi.org/10.1590/S1516-14392014005000044

Pokroy, B., Fieramosca, J. S., Von Dreele, R. B., Fitch, A. N., Caspi, E. N., & Zolotoyabko, E. (2007). Atomic structure of biogenic aragonite. *Chemistry of Materials*, *19*(13), 3244-3251. https://doi.org/10.1021/cm070187u

Qian, Y. (1999). Taxonomy and biostratigraphy of small shelly fossils in China. In Qian, Y. (Ed.), *Taxonomy and biostratigraphy of small shelly fossils in China* (pp. 216–219). Science Press.

Somasundaran, P., & Agar, G. (1967). The zero point of charge of calcite. *Journal of Colloid and Interface Science*, *24*(4), 433–440. https://doi.org/10.1016/0021-9797(67)90241-x

Spann, N., Harper, E. M., & Aldridge, D. C. (2010). The unusual mineral vaterite in shells of the freshwater bivalve *Corbicula fluminea* from the UK. *The Science of Nature*, *97*(8), 743–751. https://doi.org/10.1007/s00114-010-0692-9

Sverjensky, D. A. (1994). Zero-point-of-charge prediction from crystal chemistry and solvation theory. *Geochimica Et Cosmochimica Acta*, *58*(14), 3123–3129. https://doi.org/10.1016/0016-7037(94)90184-8

Ulagesan, S., Krishnan, S., Nam, T. J., & Choi, Y. H. (2022). A review of bioactive compounds in oyster shell and tissues. *Frontiers in Bioengineering and Biotechnology*, *10*, 913839. https://doi.org/10.3389/fbioe.2022.913839

USGS. (2001). USGS OFR01-041: X-Ray Diffraction Primer, U. S. Geological Survey Open-File Report 01-041. Retrieved on January 28, 2024, frpö https://pubs.usgs.gov/of/2001/of01-041/htmldocs/xrpd.htm

Yi, Q., & Bengtson, S. (1989). *Palaeontology and biostratigraphy of the Early Cambrian Meishucunian Stage in Yunnan Province, South China*. Universitetsforlaget. https://doi.org/10.18261/8200374157-1989-01

Yildiz, H., Berber, S., Acarli, S., & Vural, P. (2011). Seasonal variation in the condition index, meat yield and biochemical composition of the flat oyster *Ostrea edulis* (Linnaeus, 1758) from the Dardanelles, Turkey. *Italian Journal of Animal Science*, *10*(1), e5. https://doi.org/10.4081/ijas.2011.e5