



## Investigation of the Chemical Composition of the Shell Structure of *Mytilus galloprovincialis* Mussel From Kefken, Türkiye

Bayram Kızılkaya<sup>1</sup> • Harun Yıldız<sup>1</sup> • Sefa Acarlı<sup>1</sup> • Pervin Vural<sup>2</sup>

<sup>1</sup> Çanakkale Onsekiz Mart University, Faculty of Marine Sciences and Technology, Department of Aquaculture, Çanakkale, Türkiye, bayram342001@yahoo.com; harunyildiz@comu.edu.tr; sefaacarlı@comu.edu.tr

<sup>2</sup> Çanakkale Onsekiz Mart University, Bayramiç Vocational School, Department of Aquaculture, 17700, Çanakkale, Türkiye, pervinvural@comu.edu.tr

✉ Corresponding Author: bayram342001@yahoo.com

### Please cite this paper as follows:

Kızılkaya, B., Yıldız, H., Acarlı, S., & Vural, P. (2024). Investigation of the Chemical Composition of the Shell Structure of *Mytilus galloprovincialis* Mussel From Kefken, Türkiye. *Acta Natura et Scientia*, 5(1), 57-68. <https://doi.org/10.61326/actanatsci.v5i1.7>

### ARTICLE INFO

#### Article History

Received: 27.02.2024

Revised: 04.05.2024

Accepted: 15.05.2024

Available online: 22.05.2024

#### Keywords:

*Mytilus galloprovincialis*

Bivalve shells

Calcium carbonate

The zero charge points

### A B S T R A C T

In this study, the chemical composition of *Mytilus galloprovincialis* shells was examined. As known, the main component of shell composition in bivalves is calcium carbonate, which constitutes approximately 94% of the shell. The zero charge points ( $P_{zc}$ ) of the shells were determined in the study. The  $P_{zc}$  value indicates the surface charge state of the shells. The  $P_{zc}$  value of the shells was determined to be 8.39. The  $P_{zc}$  value of the shells provides important information for the characterization and potential applications of the shells. SEM images and EDS analyzes of the shells were made. According to the EDS results, calcium, carbon, and oxygen atoms belonging to the main structure of calcium carbonate ( $CaCO_3$ ) appeared in the highest proportions. FT-IR analysis was supported to the calcium carbonate ( $CaCO_3$ ) structure. XRD analyses were performed within the scope of the study, and it was determined that the shell structures mainly consist of a mixture of calcium carbonate and aragonite. In conclusion, this study on the chemical composition of *M. galloprovincialis* shells provides a detailed analysis of shell composition. The analyses conducted provide important information about the chemical composition, structural properties, and potential applications of the shells. This study contributes to research on the biological and chemical properties of marine organisms and is considered to form the basis for future studies.

### INTRODUCTION

Mollusk shells (bivalves, gastropods, and monoplacophorans) evolved during the earliest Cambrian period (starting around 541 million years ago) to protect their soft tissues from abiotic and biotic

stresses. These shells belong to the Mollusca class, which is a diverse group of invertebrates found worldwide and forms an important part of marine life. Mollusk shells not only protect the soft tissues of mollusks from external factors but also serve as food sources and help them defend against predators (Qian

& Bengtson, 1989; Qian, 1999; Li et al., 2017). All mussels (Mollusca: Bivalvia) share unique morphological features, namely the presence of two symmetric calcareous valves that are connected by a calcified structure, forming their shells. This underscores the significance of these organisms as important components of the natural world. Mussels play a critical role in maintaining the ecological balance of oceans, seas, and even freshwater sources. Moreover, the economic importance of mussels cannot be overlooked. Therefore, research on the morphological features and shell structure of mussels holds great importance both scientifically and economically (Bogan, 2007; Graf, 2013; Chakraborty et al., 2020). Mollusk shells evolution is a result of the adaptation process to various environmental pressures during the early stages of life. These shells help protect the internal organs of the organism, aiding in their defense against predators and other threats (Li et al., 2017). Mussels belong to the subclass Bivalvia of the mollusk class Mollusca, which are recognized by their shells. These organisms are protected by two symmetric calcareous valves that make up their shells. These shells not only protect the bodies of mussels from external factors but also provide them with the ability to move and feed. Additionally, mussel shells help balance the chemical composition of the surrounding water, thus contributing to the healthy continuation of the ecosystem (Chakraborty et al., 2020). In bivalves, shells represent a significant portion, typically ranging from 56-61% by weight. The main component of shell composition is calcium carbonate (approximately 94%). Shells serve as the hard outer structures used by marine organisms for protection and structural support. They are produced and utilized by many organisms such as bivalves, gastropods, and other marine invertebrates. These shells are formed by the aggregation of calcium carbonate crystals and are usually covered by a thin organic membrane (Hamester et al., 2012; Ituen, 2015; Mititelu et al., 2022). The main component of shells is typically calcium carbonate, but small inorganic trace elements are also present in different bivalve species. These trace elements can influence the physical properties of shells and play an important role in the

life of shelled organisms (Chakraborty et al., 2020). Shells dominant chemical component, calcium carbonate, is typically found in the form of calcite or aragonite crystals. These crystals provide the hardness and durability of the shell. However, other trace elements present in the shells also play a significant role. For example, elements like magnesium, manganese, iron, copper, and zinc can contribute to the coloration and pattern formation of shells. Additionally, these elements can enhance the durability of the shells and help shelled organisms defend against predators (Carroll & Romanek, 2008; Spann et al., 2010; Nakamura et al., 2014; Agbaje et al., 2017, 2018a, 2018b; Chakraborty et al., 2020). This study was conducted to obtain information about the chemical structure and contents of *Mytilus galloprovincialis* mussel shells. Mussel shells consist of a combination of calcium carbonate and organic matrix, and this structure provides the strength and durability of mussel shells. The study determined how the chemical composition of mussel shells is affected by environmental factors such as seawater temperature, pH level and nutrients. These findings may help us understand how mussel shells respond to environmental changes.

## MATERIAL AND METHODS

### Sampling Stations and Time

In this study, 100 samples of *M.galloprovincialis* were obtained from the Kefken station. The obtained shells were first passed through pure water. After that, these shells were homogenized by grinding.

### Determination of Zero Charge Point (P<sub>ZC</sub>) in Shells

The zero point of charge (P<sub>ZC</sub>) was determined based on pH measurements of homogenized and ground shell samples (Mahmood et al., 2011). To determine the pH<sub>PZC</sub>, 100 ml of 0.01 M KNO<sub>3</sub> solutions were first prepared in an Erlenmeyer flask. The initial pH values (pH<sub>i</sub>) of these solutions were adjusted between pH 4 and 10 using 0.1 M HCl and NaOH. Then, the modified samples were added to these solutions. Subsequently, the solution was stirred at a constant temperature using a magnetic stirrer for 48 hours. After 48 hours, the final pH (pH<sub>f</sub>) of the

solution was measured and recorded. The difference between the initial pH and final pH ( $\Delta\text{pH} = \text{pH}_i - \text{pH}_f$ ) was plotted against the initial pH ( $\text{pH}_i$ ). The point at which the curve intersects the x-axis was determined as the  $P_{zc}$ .

### Chemical Structure Analyzes in Shells

SEM-EDS (Scanning Electron Microscope-Energy Dispersive X-Ray Spectroscopy, model JEOL JSM-7100F) analyses were conducted at the Central Laboratory of Çanakkale Onsekiz Mart University. The device has a magnification capacity ranging from  $\times 40$  to 300,000 and an accelerating voltage between 0.2 and 30 kV. To enhance the conductivity properties of the samples, a vacuum of  $8 \times 10^{-1}$  mbar/Pa was first applied using a Quorum coating device, followed by a gold-palladium (80-20%) coating process with a voltage of 10 mA (The coating thickness is approximately 2-3 nm). SEM images were captured using a voltage of kV (the applied voltage is indicated in the lower right corner of the images). Images in the SEM device were acquired using secondary electrons. Surface characteristics of the ground shells were examined using a Scanning Electron Microscope (SEM), and differences were identified. Additionally, EDS analysis of the shells was performed to analyze carbon, oxygen, nitrogen, calcium, and sulfur content.

FT-IR spectra were measured in the range of  $650\text{-}4000\text{ cm}^{-1}$  using the ATR technique on a Perkin-Elmer Spectrum One device. FT-IR analyses of ground and homogenized *M. galloprovincialis* shells were performed to determine the chemical bonding properties. Thus, changes in chemical bonding properties and bond strengths of shell structures were investigated concerning species, time, and area dependencies.

Additionally, XRD analyses were conducted to obtain information about the chemical structures and compositions. The analyses were performed using the PANalytical Empyrean X-RD device located at the Central Laboratory of Çanakkale Onsekiz Mart University.

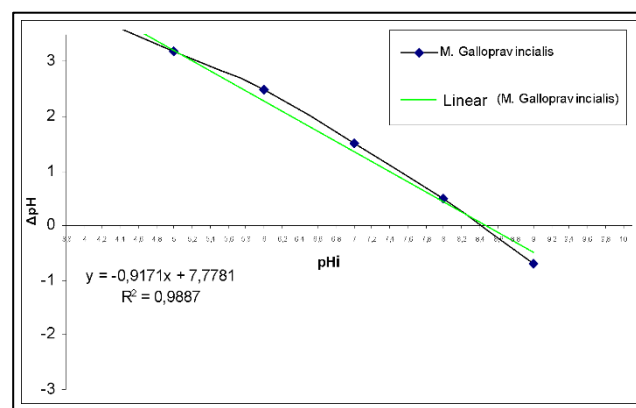
## RESULTS AND DISCUSSION

*M. galloprovincialis* is an economically important species as it is commercially cultivated and consumed in the seafood industry. They are also known for their filter-feeding behavior in ecosystems, where they filter nutrients from the water, contributing to the improvement of water quality. These mussels are typically identified by their blue-purple colored shells and usually measure between 5 to 8 cm in length. Their hard shells provide protection against waves and predators. Additionally, it is important to recognize the ecological impact of this species as they can regulate water quality through their filter-feeding behavior, thereby influencing ecological balance (Yıldız & Berber, 2010; Yıldız et al., 2013; Acarlı et al., 2011, 2015; Acarlı et al., 2018, 2023).

The zero point of charge ( $P_{zc}$ ) is a parameter in physicochemistry related to the adsorption phenomenon, defining the condition when the electrical charge on a surface is zero. In other words,  $P_{zc}$  is typically the pH value at which the net electrical charge on the surface of a solid immersed in an electrolyte solution is zero. This concept not only defines a specific condition where the surface charge is zero but is also closely associated with adsorption phenomena.  $P_{zc}$  is generally defined as the pH value at which the net electrical charge on the surface of a solid in contact with an electrolyte is zero (Somasundaran & Agar, 1967; Sverjensky, 1994; Babić et al., 1999; Hou et al., 2001; Kosmulski, 2002; Fiol & Villaescusa, 2008; Mahmood et al., 2011). To understand the meaning of this definition, it is important to first grasp what adsorption is. Adsorption is the process by which one substance is held onto the surface of another substance. This process typically occurs between solid and liquid or gas phases, and the electrical charge on the surface plays a significant role in the occurrence of adsorption. In many fields where adsorption phenomena are studied, determining and understanding the  $P_{zc}$  is crucial (Fiol & Villaescusa, 2008; Mahmood et al., 2011). This concept is used to determine at which pH values a surface is electrically neutral, which is a critical step in understanding how adsorption occurs. The determination of  $P_{zc}$  is typically carried out

experimentally, and different pH values can be obtained for different surfaces. This can vary depending on the chemical composition, structure, and other properties of the surface. Therefore, determining and understanding  $P_{ZC}$  is a fundamental step in understanding the adsorption properties of a surface. Understanding  $P_{ZC}$  is important not only in physical chemistry but also in various industrial applications. Particularly, in materials science, colloid chemistry, environmental engineering, and other fields where surface chemistry and adsorption phenomena are studied, determining and understanding  $P_{ZC}$  plays a critical role in the design and application of materials. Consequently,  $P_{ZC}$  is an important parameter in physical chemistry related to adsorption phenomena, defining the condition when the electrical charge on a surface is zero.  $P_{ZC}$  is typically the pH value at which the net electrical charge on a surface immersed in an electrolyte solution is zero.

$P_{ZC}$  of *M. galloprovincialis* shell particles was determined based on the surface characteristics. The  $P_{ZC}$  value of the shells was found to be 8.39 (Figure 1). Depending on the surface characteristics of the shells, the  $P_{ZC}$  value can vary according to the ability of groups on the molecule to gain or lose hydrogen or electrons. When the ambient pH is lower than the  $P_{ZC}$  value, the system is said to be “below  $P_{ZC}$ .” In this case, acidic water attracts more protons from hydroxyl groups, resulting in a positively charged adsorbent surface, and negative ions are adsorbed. Conversely, when the pH is greater than the  $P_{ZC}$  value, the system is said to be “above  $P_{ZC}$ ,” and the surface becomes negatively charged, with positively charged groups adsorbing. Therefore, it was determined that in environments where the pH of the shells is greater than 8.0, positively charged shells will be significant adsorbents for the adsorption of negatively charged groups, or anionic species. Figure 1 shows the curves of initial pH ( $pH_i$ ) versus  $\Delta pH$  for the shells. The experimental  $P_{ZC}$  values obtained at the end of the study are also shown along with the values obtained by linear regression.



**Figure 1.** Zero charge point of *Mytilus galloprovincialis* shell particles

SEM-EDS is a microscopy technique used for surface imaging and elemental analysis. This technique allows the electron beam to scan the sample surface to visualize surface topography and simultaneously determine the chemical composition of elements in the sample. SEM-EDS is a widely used analysis method in many industrial and scientific applications. The broad application of SEM-EDS stems from its effectiveness in examining the surface properties and chemical composition of samples. Another significant feature of SEM-EDS is its capability for high-resolution surface imaging. This allows for the examination of surface morphology and observation of microstructures on the surface of the sample. Additionally, regional elemental analysis can be performed to obtain information about the chemical composition of the sample surface, providing detailed insights into the surface properties of the sample. The SEM images of the shells are shown in Figure 2. The SEM image illustrates the microscopic structure of the ground mussel shell. As can be seen from the image, the main component of the mussel shell consists of calcium carbonate crystals. The majority of the image is composed of irregularly shaped and sized plates of calcium carbonate ( $CaCO_3$ ) crystals, which are the main component of the mussel shell. The small particles on the plates may consist of organic matter or other elements. This image provides valuable information about the microscopic structure of the mussel shell, which can be utilized to understand its mechanical properties, chemical composition, and biological activity.

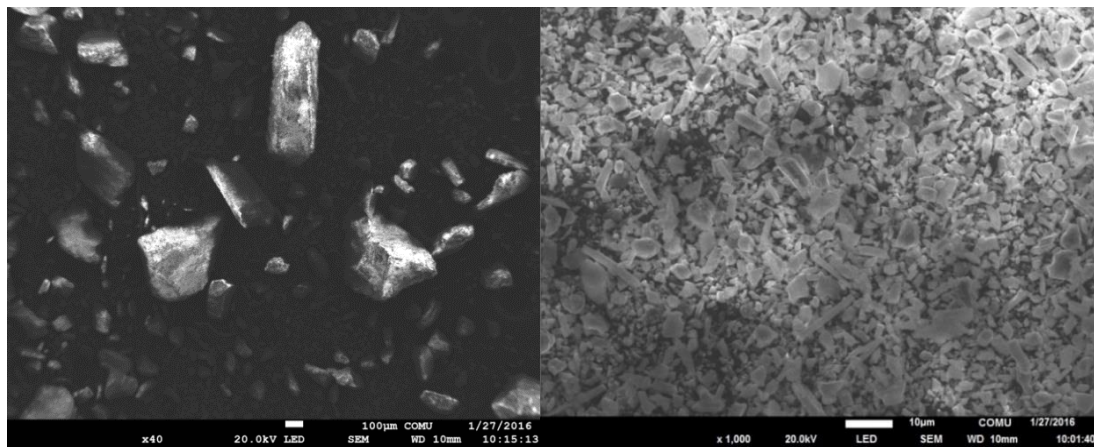


Figure 2. SEM images of *Mytilus galloprovincialis* shells

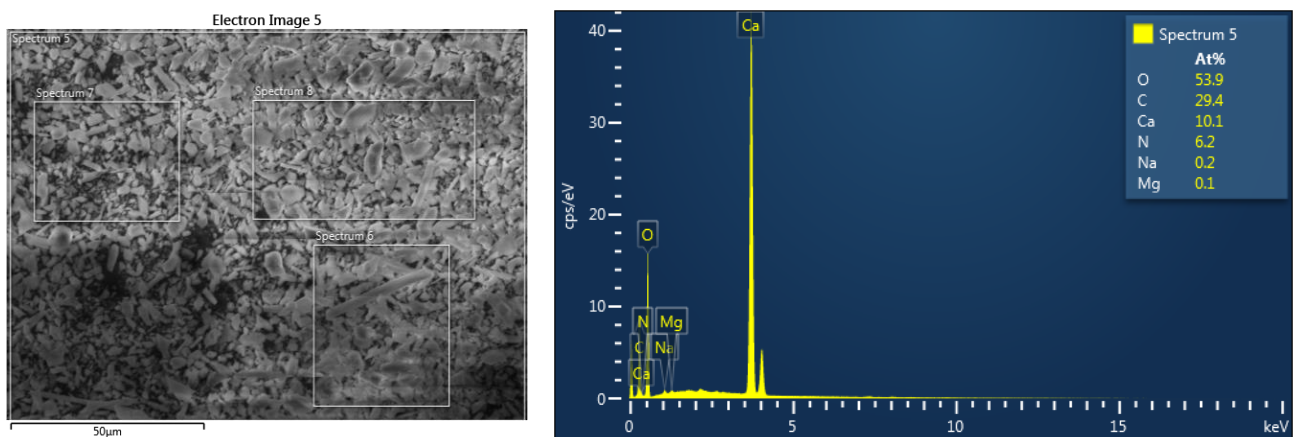


Figure 3. EDS spectrum of *Mytilus galloprovincialis* shell

Figure 3 shows the EDS analysis result of the *M. galloprovincialis* shell. EDS (Energy Dispersive X-ray Spectroscopy) analysis is a technique used to determine the chemical composition of a material. In the spectrum, elements such as C (Carbon), O (Oxygen), Ca (Calcium), Mg (Magnesium), Na (Sodium), and N (Nitrogen) are evident. In the spectrum, it can be observed that calcium (Ca), carbon (C), and oxygen (O) are the predominant and fundamental elements, which constitute the main structural elements of  $\text{CaCO}_3$ . Carbon indicates the presence of calcium carbonate, the main component of the mussel, as well as organic matter. Mussel shells contain proteins and other organic molecules. Oxygen originates from the oxygen atoms in calcium carbonate, the compound present in the mussel shell. Calcium, on the other hand, comes from calcium carbonate, which is the main component of the mussel shell. Magnesium is thought to come from magnesium carbonate, which is present in small amounts in the mussel shell. Sodium can be attributed to sodium

chloride in seawater. Nitrogen is generally a significant component of proteins and other organic molecules. Therefore, the presence of nitrogen in the mussel shell indicates the presence of proteins or other organic molecules. It is observed that nitrogen is present in the shell at a rate of 6.8 %. The relatively high concentration of nitrogen compared to other elements in the mussel shell indicates that the shell is rich in proteins or other organic molecules. Possible sources of nitrogen in the mussel shell include proteins from the mussel's diet, proteins secreted by the mussel itself, and bacteria or other microorganisms adhering to the mussel shell. This spectrum provides valuable information about the chemical composition of the mussel shell. This information can be used to determine whether the mussel shell is pure, what elements it contains, and what impurities it may contain. The source of trace elements in shells generally comes from seawater. Seawater contains various minerals and elements, which shell-bearing organisms can utilize in the

formation of their shells. The presence of these elements in shells can affect their chemical composition and physical properties. For example, the integration of magnesium into calcium carbonate crystals can make the shells more durable and flexible. The importance of the elements obtained from seawater by shell-bearing organisms in forming their shells is significant. Seawater contains calcium, magnesium, carbonate, phosphate, and other minerals. These elements are the materials shell-bearing organisms use to form their shells. Especially calcium carbonate is the main component of shell-bearing organisms shells, and shells are formed with calcium and carbonate ions taken from seawater. The chemical composition of shells can vary depending on the ratio of the elements they contain. For example, calcium carbonate crystals can become more durable and flexible with the integration of magnesium. This allows the shells of shell-bearing organisms to be more resistant to environmental impacts. Additionally, it is believed that other elements can also influence the color and texture properties of the shells (Carroll & Romanek, 2008; Nakamura et al., 2014; Chakraborty et al., 2020). The elemental content of the shells of shell-bearing organisms can carry traces of geological and climatic changes. For example, changes in the elemental ratios in seawater can lead to differences in the formation and composition of shells. Therefore, the shells of shell-bearing organisms can be an important source for understanding the history of geological and climatic changes. The source of elements in shells and the processes of shell formation are important research topics for scientists. Understanding how elements taken from seawater are used in shell formation provides information about the evolutionary processes of shell-bearing organisms. Additionally, the elemental content of shells can serve as an indicator for understanding the effects of environmental changes (Carroll & Romanek, 2008; Nakamura et al., 2014; Agbaje et al., 2017, 2018a; Chakraborty et al., 2020). Consequently, trace elements in shells generally originate from seawater and play a significant role in the formation of shell-bearing organisms shells. The presence of elements in shells can affect their chemical composition and physical properties. This situation can provide

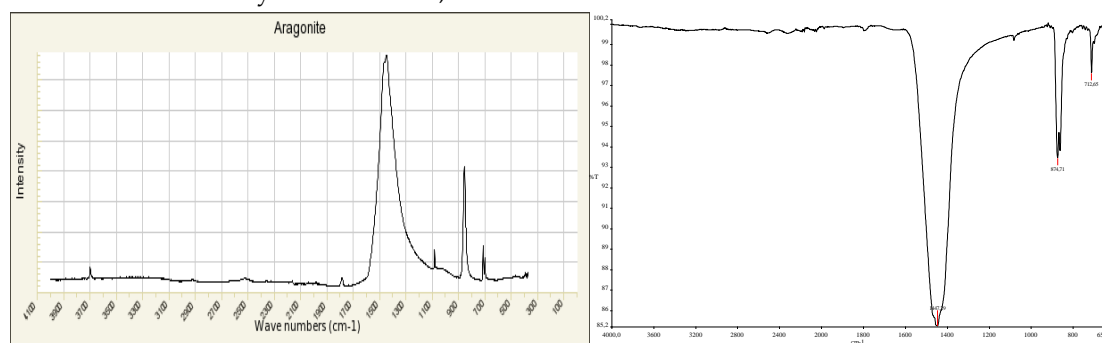
important insights into the evolutionary processes of shell-bearing organisms and their adaptation to environmental changes. This topic is an important area of research for scientists, and the shells of shell-bearing organisms can serve as a valuable resource for understanding the history of geological and climatic changes.

Fourier Transform Infrared Spectroscopy (FT-IR) is a spectroscopic technique used to determine the bond structures of molecules. Infrared light is directed onto a sample and absorbed by the molecules of the sample. The amount of absorbed light depends on the wavelength of the light. By measuring the absorption of light at different wavelengths, information about the chemical composition of a sample can be obtained. It is a highly effective tool for determining the chemical bonds, functional groups, and molecular structures of the substance under investigation. This analysis method is widely used in various industries, biological research, drug development processes, food analysis, and materials science. FT-IR spectroscopy is a powerful and versatile technique because it provides information at the molecular level. Therefore, the aim of the conducted (FT-IR) analysis is to determine the structure of the main component of the shells. Below, in Figure 4A, the FT-IR spectra of Aragonite, namely  $\text{CaCO}_3$  mineral crystal, from spectral data characterized within the RRUFF Project are provided (Lafuente et al., 2015). In the spectrum in Figure 4A, the peaks at  $1420\text{ cm}^{-1}$  represent the C-O stretching, at  $870\text{ cm}^{-1}$  represent the C-O stretching, and at  $710\text{ cm}^{-1}$  represent the C-O stretching of the carbonate molecule. The FT-IR spectra of *M. galloprovincialis* shells are provided in Figure 4B. When examining the FT-IR data of the shells, it was determined that there are vibration bands of  $\text{CO}_3^{2-}$  at  $1420\text{ cm}^{-1}$ . The moderately intense in-plane bending band at  $710\text{ cm}^{-1}$  emerges as an in-plane bending band (V4) of the carbonate ( $-\text{CO}_3^{2-}$ ) molecule, which constitutes the shell's main chemical structure. This band is attributed to carbonate, clearly indicating that the main chemical structure of the shells is carbonate. Generally, in the spectrum of the sample, the peaks are observed at  $1420\text{ cm}^{-1}$ : C-O stretching (carbonate),  $870\text{ cm}^{-1}$ : C-O stretching (carbonate),  $710\text{ cm}^{-1}$ : C-O stretching (carbonate),  $3400\text{ cm}^{-1}$ : N-H stretching

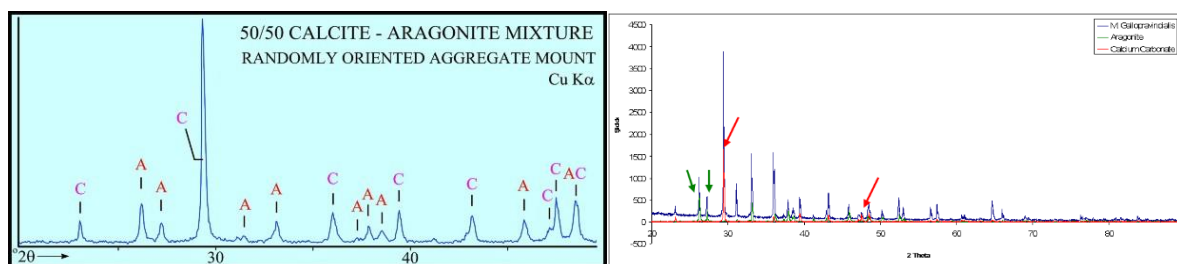
(amides),  $1650\text{ cm}^{-1}$ : C=O stretching (amides), and  $1540\text{ cm}^{-1}$ : N-H bending (amides). Based on the peak positions in the spectrum, we can conclude that the mussel shell consists of calcium carbonate and also contains proteins. This spectrum provides valuable information about the chemical composition of calcium carbonate structure. This information can be used to determine whether the calcium carbonate structure is pure, what crystal form it is in, and what impurities it contains. The basic Raman peaks of carbonate ( $-\text{CO}_3^{2-}$ ) that constitute the main structure of *M. galloprovincialis* shells in this study and aragonite, analyzed by FT-IR under the RRUFF Project, complement each other.

The shells of *M. galloprovincialis* were analyzed using X-Ray Diffraction (XRD) technique and compared with the crystal structures of calcium carbonate and aragonite ( $\text{CaCO}_3$ ). This analysis, which examines the atomic and molecular structures of the shells, relies on the characteristic pattern of X-rays scattered by the crystalline phase of the structure, which is unique to each crystal. The crystal structures of calcium carbonate that make up these shells are formed through biomineralization processes influenced by various factors such as species characteristics, growth conditions, and the environment. To understand the changes in the molecular structures of shells depending on the formation of crystals, it is necessary to develop these techniques. Therefore, it is important to understand how the interaction between organic tissue and mineral phase is carried out within tissues (Pokroy et al., 2007). In this study, calcium carbonate and aragonite, which are composed of the same chemical molecules but have different crystal structures, were

used as references. Aragonite, with a crystal lattice structure different from calcite (calcium carbonate), is actually a carbonate mineral that typically forms biologically in marine or freshwater environments. Therefore, the crystal structures of the shells within the scope of the study were examined to investigate which structure is more suitable for the shells (Figure 5). Figure 5A shows the XRD spectrum of a mixture of calcite (C) and aragonite (A) produced by the US Geological Survey (USGS, 2001). This spectrum is suitable data for comparing the XRD results of the shells. Calcite and aragonite are two different crystal forms of calcium carbonate ( $\text{CaCO}_3$ ) mineral. Both belong to the trigonal crystal system but have different geometric structures. The most intense peak in the image is the calcite peak. Each peak in the image corresponds to the diffraction of X-rays by a specific atomic plane in the crystal structure of a mineral. The intensity of the peak indicates the number of diffracted X-rays. The angle of the peak indicates the angle of the diffracted X-rays. Because calcite and aragonite have different crystal structures, they have different peak positions. From this image, we can see how the chemical composition and crystal structure of a mineral can be determined using X-ray diffraction. Upon examining the spectra in Figure 5A, it was determined that the shell structures of *M. galloprovincialis* predominantly consist of calcium carbonate and aragonite. The blue part of the spectrum represents the samples, the red part represents calcite, and the green part represents aragonite. Thus, it was easier to identify the calcium and aragonite structures in the composition. X-ray diffraction is a powerful tool used to identify minerals and examine the structure of crystals.



**Figure 4.** FT-IR spectra of **A)** Aragonite ( $\text{CaCO}_3$ ) mineral crystal characterized within the scope of the RRUFF Project (Lafuente et al., 2015) and **B)** *M. galloprovincialis* shells



**Figure 5.** A) X-RD spectrum of the mixture consisting of calcite and aragonite given by the US Geological Survey (USGS) (USGS, 2001): Aragonite (A), Calcite (C); B) X-Ray Diffraction (XRD) spectrum of *M. galloprovincialis* shell

The shells of shelled organisms are structures formed by the accumulation of minerals such as calcium carbonate. These shells not only protect the organism's body but are also influenced by environmental factors. It is known that trace elements in the shells are affected by environmental factors. For example, the temperature, acidity, and salinity level of seawater can affect the chemical composition and trace element content of the shells. Therefore, environmental changes can affect the composition and properties of shells in shelled organisms (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 level of seawater can directly affect the life of shelled organisms. These factors can also influence the growth and composition of their shells. For example, as the acidity of seawater increases, the utilization of calcium carbonate in the formation of shells by shelled organisms may decrease, resulting in thinner and more fragile shells. Similarly, the salinity level of seawater can also affect the composition of the shells. High salinity levels can alter the mineral content of the shells and reduce their durability. The influence of environmental factors on the shells of shelled organisms can also affect the trace element content. Trace elements in seawater play a significant role in the composition of shells. For instance, elements like iron, magnesium, and strontium can be influential in the formation of shells. The levels of these elements can vary depending on environmental changes, thereby impacting the composition of the shells. The effect of environmental factors on the trace elements in the shells of shelled organisms has been a focus of scientific research. Researchers examine the chemical composition of seawater and the effects of environmental changes on the composition of shells,

uncovering new findings in this area. The results of these studies can help us understand the life and evolutionary processes of shelled organisms (Spann et al., 2010; Nakamura et al., 2014; Agbaje et al., 2017, 2018b; Chakraborty et al., 2020). Consequently, the shells of shelled organisms are influenced by environmental factors. The temperature, acidity, and salinity level of seawater can affect the chemical composition and trace element content of the shells. Therefore, environmental changes can impact the composition and properties of the shells of shelled organisms. This topic continues to be the focus of scientific research and can have significant implications for the evolutionary processes of shelled organisms.

## CONCLUSION

Bivalves are a group of shelled organisms that inhabit both marine and freshwater environments. The growth of these organisms is influenced by various factors, including environmental conditions, nutrition, water temperature, water quality, light, and the presence of other organisms. The effects of these factors on the growth of bivalves are significant, and research on this topic provides important insights into the life cycle and population dynamics of bivalves. Environmental factors play a crucial role in the growth of bivalves. Factors such as seawater temperature, salinity level, oxygen levels, and acidity directly influence the growth of bivalves. For example, some species may grow faster within specific temperature ranges, while others may thrive under different temperature conditions. Similarly, the salinity level of the water also affects the growth of bivalves. Therefore, studying environmental factors provides important information about the habitats and populations of bivalves. Nutrition is also a significant



factor in the growth of bivalves. The availability of plankton and other organisms that bivalves feed on affects their growth rate. Additionally, water quality also influences nutrition. Pollution and other factors can adversely affect the food sources of bivalves, thereby negatively impacting their growth. Calcium carbonate, the main component of shells, allows marine organisms to build their shells using dissolved minerals from the water. These minerals are excreted from the organisms' bodies and used to form shells. Calcium carbonate crystals provide the shell with strength and hardness, helping organisms to protect themselves from predators and survive in the marine environment. The calcium carbonate content in shells is part of the calcium cycle in marine ecosystems. Marine organisms use calcium and carbonate ions from the water to build their shells. These ions play a crucial role in the chemical composition of seawater and are released back into the water after being utilized by marine organisms to form shells. This process helps maintain the chemical balance of seawater and contributes to the overall health of marine ecosystems. The source of trace elements in shells is generally seawater. Seawater contains various minerals and elements that shell-bearing organisms can use in the formation of their shells. The presence of these elements in shells can influence their chemical composition and physical properties. For example, the integration of magnesium into calcium carbonate crystals can make shells more durable and flexible. Additionally, it is known that trace elements in shells are influenced by environmental factors. For instance, factors such as seawater temperature, acidity, and salinity can affect the chemical composition and trace element content of shells. Therefore, environmental changes can impact the composition and properties of shells in shell-bearing organisms. This study provides a detailed examination of the chemical composition of shells belonging to *M. galloprovincialis* mussels. Calcium carbonate, which constitutes a significant portion of the shell composition in bivalves, accounts for 94% of the shells. The study analyzed the surface charge status of the shells by determining their zero charge points ( $P_{zc}$ ). The findings revealed that the  $P_{zc}$  value of the shells is 8.39, providing valuable insights into the characterization of the shells and their

potential applications. Additionally, scanning electron microscopy (SEM) images and energy-dispersive X-ray spectroscopy (EDS) analyses of the shells were conducted. According to the EDS results, calcium, carbon, and oxygen atoms, which are characteristic of calcium carbonate ( $\text{CaCO}_3$ ) structures, were found in the highest proportions, indicating the main structure of the shells. These findings contribute to a more detailed understanding of the chemical composition of the shells. Furthermore, Fourier-transform infrared (FT-IR) analysis supported the presence of calcium carbonate ( $\text{CaCO}_3$ ) structures. Within the scope of the study, X-ray diffraction (XRD) analyses were also conducted, and the results indicated that the shell structures mainly consist of a mixture of calcium carbonate and aragonite. These analyses provide important information about the structural properties of the shells. While the predominant chemical component of the shells is calcium carbonate, it is known that small inorganic trace elements are commonly found in different bivalve species. These trace elements can affect the physical properties of the shells, determine their colors and patterns, and play a significant role in the lives of bivalve organisms. Therefore, the conservation of bivalve habitats and the investigation of the effects of environmental changes can help understand the effects on the composition and properties of the shells. Research in this area is considered to be an important step for the conservation of bivalve organisms and the sustainability of marine ecosystems.

## ACKNOWLEDGEMENTS

This study was funded by TÜBİTAK, Project number: FHD-113O381.

## Compliance with Ethical Standards

## Authors' Contributions

BK: Conceptualization, Writing – original draft, Data curation, Formal Analysis, Visualization, Writing – review & editing

HY: Conceptualization, Investigation, Methodology, Writing – review & editing, Supervision, Funding acquisition, Project administration

SA: Conceptualization, Methodology, Investigation

PV: Investigation, Methodology, Data curation

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.

### Funding

Not applicable.

### Data Availability

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

### REFERENCES

- Acarlı, S., Lok, A., Acarlı, D., & Kucukdermenci, A. (2018). Gamogenetic cycle, condition index and meat yield of the Noah's Ark shell (*Arca noae* Linnaeus, 1758) from Gerence Bay, Aegean Sea Turkey. *Ege Journal of Fisheries and Aquatic Sciences*, 35, 141-149. <https://doi.org/10.12714/egejfas.2018.35.2.06>
- Acarlı, S., Lök, A., Kirtik, A., Acarlı, D., Serdar, S., Kucukdermenci, A., & 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>
- Acarlı, S., Lok, A., Küçükdermenci, A., Yıldız, H., & Serdar, S. (2011). Comparative growth, survival and condition index of flat oyster, *Ostrea edulis* (Linnaeus 1758) in Mersin Bay, Aegean Sea, Turkey. *Kafkas Üniversitesitesi Veterinerlik Fakültesi Dergisi*, 17(2), 203-210.
- Acarlı, S., Vural, P., & Yıldız, H. (2023). An assessment of the cultivation potential and suitability for human consumption of Mediterranean mussels (*Mytilus galloprovincialis* Lamarck, 1819) from the Yalova coast of the Marmara Sea. *Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi*, 9(1), 12-24. <https://doi.org/10.58626/menba.1282775>
- 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., McInerney, 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](https://doi.org/10.1016/s0008-6223(98)00216-4)
- Bogan, A. E. (2007). 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>
- 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. *Materials Research*, 15(2), 204-208. <https://doi.org/10.1590/S1516-14392012005000014>
- Hou, W., Yan-Lei, S., D, S., & Zhang, C. (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 mollusc shells in Nigeria. *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](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 T. Armbruster & R. M. Danisi (Eds.), *Highlights in mineralogical crystallography* (pp 1-30). De Gruyter. <https://doi.org/10.1515/9783110417104-003>
- 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, 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, 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<sub>3</sub> and microstructure of the shell of a Brazilian invasive mollusc (*Limnoperna fortunei*). *Materials Research-ibero-american Journal of Materials*, 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 Y. Qian (Ed.), *Taxonomy and Biostratigraphy of Small Shelly Fossils in China* (pp. 216-219). Science Press.
- Qian, Y., & 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>
- 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](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](https://doi.org/10.1016/0016-7037(94)90184-8)
- USGS. (2001), U. S. USGS OFR01-041: X-Ray Diffraction Primer, U. S. Geological Survey Open-File Report 01-041, Retrieved on January 28, 2024 from <https://pubs.usgs.gov/of/2001/of01-041/htmldocs/xrpd.htm>
- Yildiz, H., & Berber, S. (2010). Depth and seasonal effects on the settlement density of *Mytilus galloprovincialis* L. 1819 in the Dardanelles. *Journal of Animal and Veterinary Advances*, 9, 756-759.
- Yıldız, H., Acarlı, S., Berber, S., Vural, P., & Gündüz, F. (2013). A preliminary study on Mediterranean Mussel (*Mytilus galloprovincialis* Lamarck, 1819) culture in integrated multitrofik aquaculture systems in Çanakkale Strait. *Alinteri Journal of Agricultural Sciences*, 25(2), 38-44.