Rapid and Application-Tailored Assessment Tool for Biogenic Powders from Crustacean Shell Waste: Fourier Transform-Infrared Spectroscopy Complemented with X-ray Diffraction, Scanning Electron Microscopy, and Nuclear Magnetic Resonance Spectroscopy

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ABSTRACT: Due to their chemical composition, richness in calcium carbonate, chitin, proteins, and pigments, and nanoporous structure, crustacean shell waste shows great potential for a wide variety of applications. Large quantities of waste shells are produced annually, meaning that they can be considered a renewable source of ecofriendly biogenic materials, which can be turned into value-added byproducts. In this paper, an IR-based technique is developed to differentiate various biogenic powders originated from crude or food-processed crustacean shells. The validity of the method is supported by cross-checking with XRD, NMR, and SEM−EDX analyses. Our goal was to determine changes in properties of waste crab shells after the two most common treatments, deproteinization and milling. We discovered that deproteinization with NaOH could be tracked from the IR absorbance intensity ratio of the $\nu$(CH$_2,3$) and $\nu_{asym}$(CO$_3^{2-}$) bands while milling time less influenced this ratio but induced changes in powder particle size distribution and morphology. The relative organic/inorganic ratio was different for different colored shells. Unexpectedly, waste shells stored for an average of 6 months or more were found to contain hydrated calcium carbonate (monohydrocalcite), which was absent in equivalent fresh shell composition. Deproteinization caused changes in mechanical properties of shells, making them more brittle, which resulted in a larger fraction of fine particles after powdering.

1. INTRODUCTION

The global trends of sustainability and waste reduction call for more efficient recycling of waste biogenic materials resulted from aquaculture exploitation and seafood industry. Crustacean shells are currently viewed as food waste. These shells are rich in valuable natural compounds, most notably chitin, proteins, amino acids, and carotenoid pigments,$^1$ along with the well-known calcite biomineral. Proper and efficient management and further reuse of this biogenic raw material require a solid knowledge-based and well-developed analytical methodology, including data on structural and compositional analysis of crab shells resulted from aquaculture exploitation, seafood industry, and domestic seafood consumption. It is well-known that the blue crab, Callinectes sapidus, like any other crab or lobster species, turns red when cooked (immersed in boiling water) due to the breakage of the crustacyanin carotenoprotein and release of the free pigment, astaxanthin, but it is not known if fine powdering of waste from cooked crustacean shells preserves their chemical properties similar to those from raw (uncooked) materials.

The widely present blue crab is a valuable resource for commercial fisheries with its global catch reaching 95,456 tons in 2015.$^5$ The green crab, Carcinus aestuarii, is a locally exploited species in the Mediterranean, while its congenerous species Carcinus maenas is being exploited worldwide.$^6$ Because of large global consumption, discarded crab shells present an unexploited recurrent resource with potential advanced applications of their compositional and ultrastructural features, which were earlier described by Roer and Dillaman$^7$ and Santos$^8$ and recently by Nekvapil et al.$^2$ For instance,
chitosan extracted from crab shells was used for construction energy storage units (batteries) and electrical double layer capacitor (EDLC) cells.9 The crab shell carbonate biocomposite was considered as a filler for automotive brake pads,10 as a steel coating material,11 as an organochlorine pesticide and textile industry dye removal agent,12 and as a template for high-performance capacitors.13 With global trends shifting toward a circular economy and with the emphasis on reusing and recycling waste, it is expected that a wider use will be found for crab shells in multiple industries.2,4,14

Depending on the final aim of reusing, the steps in shell waste processing can roughly be divided into demineralization (removal of CaCO3) and deproteinization (removal of proteins), which are applied depending on the class of the compound to be extracted.15

An emerging concept of "shell biorefinery", which comprises sets of methods for crustacean shell refining, is pushing its way toward perspective applications. Refining refers to fractionation of the shell biomass, comprising recovery of CaCO3, proteins, and chitin,16 with the latter compound receiving the greatest attention by merit of the high content (up to 7%) of biologically incorporated nitrogen.17,18 Chemical methods have been reviewed for most economic and sustainable chitin recovery17 and its processing into a range of N-containing compounds, such as amino sugars, amino alcohols, amino acids, and heterocyclic compounds.18

However, based on our previous observations on the structural and chemical features of crustacean shells,3 we advocate the use of the shell natural composite as a whole, with minimal structural alterations. Thus, a development of analytical methodology capable of comprehensive and non-destructive assessment of powder composition resulted from the whole shell waste is highly desired. Such methodology would enable prompt optimization of the processing steps (chemical and/or mechanical treatment) to obtain desired powder composition and particle morphology.

In this study, we explored the spectral information obtained from the biogenic waste resulted from the crustacean’s exploitation using Fourier-transform infrared spectroscopy (FTIR) and propose it as one of the cost-effective and fast assessment methods for waste shell composition tracking along the processing steps, such as grinding, fine powdering, and deproteinization. We adopted two approaches: (1) separate consideration of the native shell counterparts from a blue crab, according to their distinct ultrastructural properties,20 which are suitable for targeted applications requiring specific porosity, and (2) consideration of the bulk, unselected shell waste leftover from the seafood industry, which usually appears thermally treated (cooked) and thus, its applicability route as a cooked bulk material is different. The FTIR results were validated with other robust methods like XRD, SEM–EDX, and NMR. The simple IR absorbance ratios are exploited and proposed for the powdered shell composition assessment (Figure 1) along with the processing steps.

2. MATERIALS AND METHODS

2.1. Waste from Cooked Crab Shells and Their Treatments. 2.1.1. Raw Crab Waste. The crab specimens belonging to species C. sapidus (Atlantic blue crab) and C. aestuarii (Mediterranean green crab) were caught by traps in the brackish waters of the Neretva River estuary, Croatia (southeastern Adriatic Sea). The crabs were kept frozen at −20 °C until analysis. As the blue crab exhibits distinct blue, red, or white shell counterparts, they were separately considered, due to their distinct pigment composition and morphology, as previously described.3 Separated shell type samples, isolated and thoroughly washed in warm water (30 °C) and air dried, from the blue crab were (i) a blue carapace, (ii) blue claws and legs, (iii) white ventral shell, and (iv) red claw shells. The green crab, C. aestuarii was comparatively used for (v) green colored shells.

2.1.2. Cooked Crab Waste. A 10 kg stock of unselected shell waste leftover after domestic cooking/frying was accumulated over a period of 6 months. These shells represent a relevant bulk sample for experimental upscaling of the crab inedible food waste. Prior to analysis, the shells were rinsed in warm water (50 °C), mixed with a small amount of detergent to remove the bulk of adhering organic matter from the soft tissue, and left to dry at room temperature.

The deproteinization step was accomplished by heating 100 g of fragmented crab shells to 80 °C in 400 mL of distilled water containing 4 g of dissolved NaOH (4% by weight), following the generalized procedure summarized by Kandra et al.15

Powder preparation from native crab shell counterparts, waste mixed shells, or deproteinized waste was done by grinding the shell fragments in an agate mortar with a pestle, and subsequently milling it using a Fritsch Pulverisette 4 planetary ball mill, following the procedure by Nekvapil et al.2 The resulting powder was sieved through a 100 micron sieve to eliminate larger particles. Milling cycles of both 3 and 5 min were applied separately to test the effect of milling time on waste shell powder morphology and composition.

2.2. Methods. Fourier-transform infrared absorption spectroscopy was used to quickly determine the chemical composition of the resulted powders. The powders were KBr pellets (1% w/w ratio) and analyzed using a Spectrum BX II (PerkinElmer, USA) spectrometer. The sample compartment was rinsed with N2 between measurements.

The mineral composition and crystallinity of the experimental waste shell stock were checked by X-ray powder diffraction (XRD) using a Bruker D8 Advance diffractometer in Bragg–Brentano geometry, with a Cu tube with λKα = 0.15418 nm, 0.0125 mm Ni filter, and a LynxEye detector. Corundum (NIST SRM1976a) was used as an internal standard. The data was collected on a 10–70° 2θ interval at a 0.02° 2θ step, measuring each step for 0.5 s. The identification of mineral phases was performed with the
NMR spectra were calibrated relative to the CH₃ line in TMS presumably structural water O patterns were processed in Origin 8.5 software (OriginLab, adamantane as an external reference. (tetramethylsilane), through an indirect procedure that uses overlap with the bands arising from the bulk CaCO₃ phase at the experimental treatments are presented in Figure 2. In from the shell powders (SP) of various origins and subjected to from Waste Crustacean Shells. or extrinsic process determined by the processing steps needed tion triggered the present study to evaluate if this is an intrinsic or extrinsic process determined by the processing steps needed for biogenic powder reuse.

3. RESULTS AND DISCUSSION

As for their mineral composition, blue crab shells are known to consist of low-Mg calcite and amorphous calcium carbonate. Powdered shells stored for an average of 6 months or more were found to contain hydrated calcium carbonate (monohydrocalcite, MHC), which was absent in equivalent fresh shell composition previously reported. Observation triggered the present study to evaluate if this is an intrinsic or extrinsic process determined by the processing steps needed for biogenic powder reuse.

3.1. Chemical Composition of Different Powders from Waste Crustacean Shells. The FTIR spectra recorded from the shell powders (SP) of various origins and subjected to the experimental treatments are presented in Figure 2. In untreated samples, infrared absorption bands of two carbonate phases could be detected from powders: (i) the low Mg-calcite, revealed by the absorption bands around 710–714, 874–876, and 1407–1411 cm⁻¹; and (ii) monohydrocalcite (MHC), revealed by the band around 1485–1487 cm⁻¹, and presumably structural water O–H bending modes around 3436 cm⁻¹ (Figure 2a). The assignments of the vibrational modes observed in the FTIR spectra of both native and waste materials are summarized in Table 1, along with the characteristic FTIR bands of geogenic calcite, chitin, and monohydrocalcite.

The bands arising from shell chitin and protein contents are revealed in the 1030–1155 cm⁻¹ range, the shoulders around 1420 and 1660 cm⁻¹, the wide band peaking in the 2924–2935 cm⁻¹ range corresponding to the C–H stretching, and the N–H stretching band at 3264 cm⁻¹ (Table 1). It should be noted that the dominant bands of chitin and proteins, at 1420 and 1660 cm⁻¹, are difficult to differentiate due to a very strong overlap with the bands arising from the bulk CaCO₃ phase at 1407–1411 and 1485–1487 cm⁻¹. The intensity ratio of the υ(CH₂,₃) band and the υ₅(SO₄²⁻) band (Figure 1a, inset 1) suggests that the green SP contains the highest amount of organic phase, followed by the white, blue SP, carapace powder, and red SP in decreasing order. As expected, the SP from mixed waste exhibited intermediate content of the organic material.
To validate our FTIR results, we considered our previous study where the chemical composition of fresh and untreated crab shells has been extensively investigated using various Raman spectroscopy techniques. The crystallinity ratio (CR), calculated as the ratio of the amorphous calcium carbonate (ACC) band area at 710–715 cm\(^{-1}\) versus the crystalline CaCO\(_3\) band area at 874–877 cm\(^{-1}\) (Figure 2a, inset 2), shows that the SP resulted from the natural red shell (claws) contains the lowest amount of ACC relative to the crystalline CaCO\(_3\), followed by white, green, and blue SP. This observation is in agreement with Nekvapil et al.,\(^3\) where the crystallinity was estimated via the full width at half maximum (FWHM) of the \(\nu_4\) band from the FT-Raman spectra of the respective shells. Additionally, the CR revealed here from the IR data that the blue crab carapace powder contains the highest relative amount of ACC, hence it exhibits the lowest crystallinity. As expected, the powder obtained from mixed shell waste (Waste SP) exhibited an intermediate CR value.

### 3.2. Effect of Deproteinization and Milling Time on Shell Micropowder Chemical Composition

#### 3.2.1. Effects Observed Using FTIR

The FTIR spectra of powders obtained from the raw waste and deproteinized material are shown in Figure 2b. The difference spectra between untreated and deproteinized waste SP FTIR spectra showed that the NaOH treatment caused a notable reduction in band intensities at 865, 1008, 1137, 1377, 1544, and 1670 cm\(^{-1}\) assigned to MHC, chitin, and proteins (Table 1). The loss of C–H, N–H, and O–H in the 2800–3600 cm\(^{-1}\) range is almost nonexistent, indicating that the milling time less influenced the chemical composition of powders described by the FTIR spectra.

To objectively describe the ratio of the inorganic-to-organic material content, the asymmetric CO\(_3\)\(^{2−}\) stretching band intensity was taken as an approximation of total inorganic carbonate, while the intensity of the band at 2935 cm\(^{-1}\) was taken as an approximation of total organic carbonate. This ratio \(R_0 = I_0\) \(\nu_3\) [CH\(_3\)] / \(\nu_3\) [\(\nu_8\) CO\(_3\)\(^{2−}\)] shows that the SP produced from untreated shells milled for 3 min exhibits the highest organic matter content (Figure 3), while other samples exhibit a progressively smaller ratio with the increase of treatment time. It is worth noting that the treatment of shells

### Table 1. Observed FTIR Bands of Powders from Crab Shell Parts Compared to the FTIR Bands of Calcite CaCO\(_3\), Chitin, and Monohydrocalcite

| observed IR band positions | reference bands\(^{20–22}\) | vibrational assignment |
|---------------------------|-------------------------------|-----------------------|
| blue claws                | red claws                      | green claws           | white shell                | carapace (blue) | mixed shell waste (cooked) | ACC | calcite | chitin | MHC | vibrational assignment |
| 584                       | 575                           | 580                   | 580                        | 575                  | 576                  | 576                      | 590 | 700 | \(\nu_4\) [CO\(_3\)\(^{2−}\)] \text{ out of plane bending} |
| 713h                      | 710h                          | 700h                  | 700h                       | 713                  | 714                  | 725                      | 712 | 776 | \(\nu_4\) [\(\nu_8\) CO\(_3\)\(^{2−}\)]; ACC |
| 765                       | 765                           | 765                   | 765                        | 764                  | 764                  | 764                      | 766 | MHC | \(\delta\text{C–H)}\ chitin |
| 875                       | 875                           | 874                   | 874                        | 875                  | 874                  | 864                      | 877 | 892 | \(\nu_3\) [\(\nu_8\) CO\(_3\)\(^{2−}\); \(\delta\text{C–H)}\ chitin |
| 1033h                     | 1033h                         | 1029h                 | 1035h                      | 1025h                | 1026h                | 1026h                    | 1026 | 1026 | C–O asym. stretch in the phase ring |
| 1071                      | 1071                          | 1071                  | 1071                       | 1071                 | 1068                 | 1068                     | 1068 | 1068 | ACC \(\nu_3\) [CO\(_3\)\(^{2−}\)]; C–O–H, C–O–C, and CH\(_2\)CO stretch chitin |
| 1154                      | 1154                          | 1154                  | 1154                       | 1154                 | 1154                 | 1155                     | 1155 | 1155 | \(\nu_6\) [\(\delta\text{CH}_3\)] chitin and \(\nu_6\) [\(\delta\text{CH}_2\)] ACC |
| 1409                      | 1411                          | 1408                  | 1411                       | 1414                 | 1414                 | 1414                     | 1414 | 1414 | \(\nu_6\) [\(\delta\text{CH}_2\)] chitin and \(\nu_6\) [\(\delta\text{CH}_3\)] ACC |
| 1419                      | 1419                          | 1419                  | 1419                       | 1419                 | 1419                 | 1419                     | 1419 | 1419 | \(\nu_6\) [\(\delta\text{CH}_2\)] chitin and \(\nu_6\) [\(\delta\text{CH}_3\)] ACC |
| 1485sh*                   | 1451sh*                       | 1489sh*               | 1489sh*                    | 1489sh*              | 1472                 | 1472                     | 1472 | 1472 | \(\nu_3\) [\(\nu_8\) CO\(_3\)\(^{2−}\)]; \(\delta\text{C–H)}\ chitin |
| 1661sh                    | 1661sh                        | 1661sh                | 1661sh                     | 1661sh               | 1661sh               | 1661sh                   | 1661sh | 1661sh | \(\nu_3\) [\(\nu_8\) CO\(_3\)\(^{2−}\)]; \(\delta\text{C–H)}\ chitin |
| 2924sh                    | 2924sh                        | 2924sh                | 2924sh                     | 2924sh               | 2924sh               | 2924sh                   | 2924sh | 2924sh | \(\nu_3\) [\(\nu_8\) CO\(_3\)\(^{2−}\)]; \(\delta\text{C–H)}\ chitin |
| 3264                      | 3264                          | 3264                  | 3264                       | 3264                 | 3264                 | 3264                     | 3264 | 3264 | \(\nu(\text{C}=\text{O)} \text{amide I} |
| 3437sh                    | 3436sh                        | 3438sh                | 3437sh                     | 3437sh               | 3436sh               | 3436sh                   | 3436sh | 3436sh | \(\nu(\text{C}=\text{O)} \text{amide I} |
| 2700–3600                 |                               |                      |                            |                     |                      |                           | 2700–3600 | 2700–3600 | \(\nu(\text{O)} \text{structural water |

*Abbreviations: ACC, amorphous calcium carbonate; MHC, monohydrocalcite.

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Figure 3. Relative intensity ratio \(R_0\) between \(\nu_3\) [\(\nu_8\) CO\(_3\)\(^{2−}\)] and \(\nu_3\) [CH\(_3\)] bands, indicating the inorganic-to-organic content of the crab shell powder produced from shells experimentally deproteinized and milled for 3 and 5 min. The ratio is calculated as \(R_0 = I_0\) \(\nu_3\) [CH\(_3\)] / \(I_0\) \(\nu_3\) [\(\nu_8\) CO\(_3\)\(^{2−}\)].
with NaOH promoted the R reduction to a greater extent than prolonged milling time, supporting the spectral comparison in Figure 2b,c.

The properties of the FTIR band around 875 cm\(^{-1}\) recorded from the native crab shells, including the position, width, and area, are shown in Table 2. The downshift of the band position and the increase in band width indicate that the red claw shell has the highest CaCO\(_3\) crystallinity, followed by the white and blue claw shells, the carapace, and the green claw shells, in that order. The variation in crystallinity among different native shell counterparts is shown in Figure 4. Different colors are also the cause of downshifting of all other CaCO\(_3\) bands in the spectra recorded from the shells relative to the positions of the pure calcite reference sample.

The properties of the band at about 875 cm\(^{-1}\) in the FTIR spectra acquired from the powder from the waste shell (cooked and processed seafood) show that different thermal, chemical, and mechanical treatments of shells affected the bulk mineral phase in the following ways (Table 3): (i) milling for 5 min consistently resulted in a lower FWHM, indicating higher CaCO\(_3\) crystallite uniformity; (ii) under equal milling time, the band was 1 to 2 cm\(^{-1}\) narrower when shells were deproteinized with NaOH, again indicating a higher number of micro-crystallites that contribute to the overall absorbance of carbonate modes; and (iii) the band position shifted to higher frequencies concomitantly with the reduction of the FWHM, supporting the slight change in the crystalline phase versus amorphous calcium carbonate.23,24

3.2.2. Effects Observed Using XRD. The XRD data showed that the rinsed shell waste not treated with NaOH contains a higher amount of MHC than the shells treated with NaOH, where MHC reflections are barely visible (peaks at 16.8, 20.5, 29.08, 31.60, and 41.8° 2\(\theta\)) (Figure 5a). Milling time (3 or 5 min) was not relevant regarding the biomineral phase composition, and the resulting samples exhibited almost identical XRD patterns (Figure 5b). However, a longer milling time yielded smaller particles, which results in a better XRD signal-to-noise ratio. A few reflections of thermonatrite and trona phases (Na\(_2\)CO\(_3\)·H\(_2\)O and Na\(_2\)CO\(_3\)·NaHCO\(_3\)·2H\(_2\)O) were occasionally recorded, which are most probably the result of NaOH treatment at high temperature for deproteinization purposes.

3.2.3. Effects Observed Using NMR. The NMR spectral difference between the powder produced from nondeproteinized (untreated) and deproteinized shells (Figure 6) shows that the NaOH treatment caused a notable reduction of the intensity of the bands ranging from 30 to 40 ppm. The methyl carbon from chitin has a clear peak at 23 ppm, which remains unshifted but becomes slightly weaker, a result that can be due to the partial deacetylation of chitin.25 The peaks and shoulders in the region of 170 ppm are specific to carbonyl

| sample                  | center (cm\(^{-1}\)) | FWHM (cm\(^{-1}\)) |
|-------------------------|-----------------------|--------------------|
| 3 min, untreated        | 873.45                | 19.90              |
| 5 min, untreated        | 873.76                | 17.76              |
| 3 min, deproteinized    | 874.4                 | 17.87              |
| 5 min, deproteinized    | 874.4                 | 16.83              |

Figure 5. XRD patterns acquired from (a) waste shell stock powdered after deproteinization (5 min milling) and (b) waste shell stock milled for 3 or 5 min. M marks monohydrocalcite, and C marks calcite reflections. Insets show the zoom of the base of the strongest calcite peak. Patterns are normalized to the strongest calcite reflection at 29.5°.

Figure 4. Zoom of the ∼875 cm\(^{-1}\) FTIR band, showing the difference in full width at half maximum (FWHM) between the sampled shell parts of the blue crab (C. sapidus), indicating the highest crystallinity of the red shell, followed by the white and blue shells, the carapace, and the green shell. All spectra are normalized to unit.
groups that here have three main sources: proteins, chitin, and calcium carbonate. The solid-state (ss) $^{13}$C($^1$H) NMR spectrum of deproteinized shell powder shows only the contribution of chitin and calcium carbonate. The peaks from 55, 61, 73, 76, 83, and 104 ppm belong to the skeleton of chitin nanofiber carbons and are all very little changed after the deproteinization process, which demonstrates the stability of the chitin nanofiber structure.

3.2.4. Effects Observed by SEM and EDX. EDX showed Ca, O, and C signals in all bulk shells and shell powder, consistent with the dominant CaCO$_3$ mineral phase, featuring also the P, Mg, and Na signals in the range of 1.5−11.5 wt % and occasional weak signals of Fe, Sr, and Cu near the limit of detection. Considering the average values of the Ca:P ratio, the highest value was observed in powder obtained from nondeproteinized shells milled for 5 min (34.85 ± 26.35 wt %; mean ± S.D.), followed by deproteinized shells milled for 3 min (29.25 ± 18.88 wt %), deproteinized shells milled for 5 min (22.67 ± 5.41 wt %), and nondeproteinized shells milled for 3 min 18.45 ± 7.17 wt %) (Figure 7). However, considering the large standard deviations, we conclude that the considered treatments of shells, including deproteinization and different milling durations, did not produce notably different Ca:P ratios in the subsequently milled powders.

The larger error bars in Figure 7 are due to two reasons: (1) EDX is less precise for light elements, such as those present in crustacean shells (Ca, P, Mg, C, and O), and (2) as a biological sample, crustacean shells naturally present a degree of variation of the quantitative composition, both within the same samples and between different specimens. Nevertheless, these issues do not affect our final analytical conclusions regarding FTIR analysis and interpretation of the shell micropowder composition.

3.3. Effect of Deproteinization and Milling Time on Shell Powdering. Particle size distribution in the deproteinized versus nondeproteinized material is clearly different, as shown in Figures 8 and 9. All four powders had the most particles in the 1−50 μm diameter range, while the particles larger than 150 μm in diameter account for less than 2% of all particles. The biggest effect on particle size distribution was made by the deproteinization treatment, which resulted in powders composed of a significantly higher ratio of fine particles (0−1 μm). The powder particle morphology was irregular in all cases, as observed in the comparative SEM images from Figure 9. However, the deproteinization treatment clearly affected the particle size distribution, resulting in finer particles, i.e., <1 μm diameter, in the respective samples.
(Figure 9b,d: red arrows). Deproteinization removed a substantial amount of chitin protein fibrils from the native structure. Such fibrils were abundantly observed in the SEM images of the native blue crab shells, previously shown by Nekvapil et al. These native fibrils are absent in the current SEM images of waste powders.

4. CONCLUSIONS AND PERSPECTIVES

The FTIR method has the potential to simplify the current biochemical characterization of shell waste and represents a potentially attractive tool for fast repetitive structural analysis of the starting material, intermediates, or final products during biogenic waste processing for reusing this valuable resource in value-added byproducts.

A deeper insight into the IR spectra associated with the structural changes or alterations of the considered biogenic waste following storage, deproteinization, and milling is provided. We show that the processing steps, such as fast and accessible chemical deproteinization using NaOH treatment, effectively reduced the organic content, while treatment of shells with a mild base, such as 4% NaOH used here, substantially decreased the protein content without damaging the contained chitin. Milling in a planetary ball mill also affected the organic matter content: a longer milling time (5 min) reduced the organic FTIR signal to a greater extent than a shorter milling time (3 min).

SEM images showed that the particles are of irregular shape, but generally spherical or polyhedral. A longer milling time resulted in a lesser proportion of the powder within the 1–50 μm size class but a greater proportion within <1 and >50 μm classes. Prior deproteinization of shells enabled better milling efficiency relative to untreated shell powder, as observed in twice as much particles from deproteinized shells in <1 μm compared to particles from untreated shells. This is presumably due to weakened organic fibers in shells following deproteinization.

On a semiquantitative basis, both FTIR and XRD showed a reduction of the MHC mineral phase (which was already present in the material prior to treatment with NaOH) after the deproteinization treatment. FTIR and NMR additionally indicated the decreased bands associated with organic compounds when deproteinization was applied. EDX showed the highest ratio of Ca and P in untreated shells milled for 5 min, while the experimental deproteinization treatments acted to increase this ratio. Variations of milling time between 3 and 5 min did not appear to influence crystallized phase composition according to XRD (Figure 3b), while FTIR showed a slight reduction of structural water bands around 3436 cm⁻¹ (Figure 2c).

FTIR comprehensive structural analysis of crab shell powder is validated using several highly sensitive techniques. We present here a scientific base for rapid structural characterization of the biogenic calcium carbonate powder originating from waste crab shells, which could be implemented between various industrial crab shell processing steps in view of reusing various powders for novel value-added products.

The proposed FTIR method can be easily used to evaluate the chemical composition of the waste biomaterial, which is in turn useful for decision-making regarding the development of advanced applications of biogenic powders such as pharmaceutical carriers, "loaded powders with various fluids" for new composite materials, adsorbents for environmental decontamination, and many others. Although this method cannot differentiate between crab species, it may be successfully applied in evaluating other categories of biogenic materials from seafood waste, such as oysters, snails, urchin shells, and shrimps, all consisting of calcite or calcite/aragonite, as main minerals and various amounts of organic materials. Hence, the proposed FTIR method can be a useful decision-making tool also for these types of waste, with minimal modifications in spectrum interpretation.

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■ REFERENCES
(1) Mancinelli, G.; Chainho, P.; Cilenti, L.; Falco, S.; Kapiris, K.; Katselis, G.; Ribeiro, F. On the Atlantic blue crab (Callinectes sapidus Rathbun 1896) in southern European coastal waters: Time to turn a threat into a resource? Fish. Res. 2017, 194, 1–8.
(2) Nekvapil, F.; Aluas, M.; Barbou-Tudoran, L.; Suci, M.; Bortnic, R.-A.; Glamuzina, B.; Pinzaru, S. C. From Blue Bioeconomy toward Circular Economy through High-Sensitivity Analytical Research on Waste Blue Crab Shells. ACS Sustainable Chem. Eng. 2019, 7, 16820–16827.
(3) Nekvapil, F.; Pinzaru, S. C.; Barbou-Tudoran, L.; Suci, M.; Glamuzina, B.; Tamaš, T.; Čišć, V. Color-specific porosity in double pigmented natural 3d-nanoarchitectures of blue crab shell. Sci. Rep. 2020, 10, 3019.
(4) Nekvapil, F.; Ganea, I.-V.; Cioriţă, A.; Hirian, R.; Tomiși, S.; Martonos, I. M.; Cintă Pinzaru, S. A New Biofertilizer Formulation with Enriched Nutrients Content from Wasted Algal Biomass Extracts Incorporated in Biogenic Powders. Sustainability 2021, 13, 8777.
(5) Bradley, G.; Stevens, T. J. M. Crab Fisheries. in Fisheries and Aquaculture; Ed, Lovrich, G.; Thiel, M. Oxford University Press 2021, 9.
(6) Glamuzina, L.; Conides, A.; Mancinelli, G.; Dobroslavić, T.; Bartulović, V.; Matić-Skoko, S.; Glamuzina, B. Population dynamics and reproduction of Mediterranean green crab Carcinus aestuarii in Parila Lagoon (Neretva estuary, Adriatic Sea, Croatia) as fishery management tools. Mar. Coast. Fish. 2017, 9, 260–270.
(7) Roer, R.; Dillaman, R. The Structure and Calcification of the Crustacean Cuticle. Am. Zool. 1984, 24, 893–909.
(8) Santos, V. P.; Marques, N. S. S.; Maia, P. C. S. V.; de Lima, M. A. B.; Franco, L. D. O.; de Campos-Takai, G. M. Seafood Waste as Attractive Source of Chitin and Chitosan Production and Their Applications. Int. J. Mol. Sci. 2020, 21, 4290.
(9) Azza, S. B.; Hamsan, M. H.; Noifal, M. M.; San, S.; Abdulwahid, R. T.; Saeed, S. R.; Brza, M. A.; Kadir, M. F. Z.; Mohammed, S. J.; Al-Zangana, S. From Cellulose, Shrimp and Crab Shells to Energy Storage EDLC Cells: The Study of Structural and Electrochemical Properties of Proton Conducting Chitosan-Based Biopolymer Blend Electrolytes. Polymer 2020, 12, 1526.
(10) Singaravelu, D. L.; Ragh, R. V.; Vijay, R.; Manoharan, S.; Kchaou, M. Development and Performance Evaluation of Eco-Friendly Crab Shell Powder Based Brake Pads for Automotive Applications. Int. J. Automot. Mech. Eng. 2019, 6502.
(11) Arulvel, S.; Elayarperumal, A.; Jagatheeshwaran, M. S. Discussion on the feasibility of using proteinized/deproteinized crab shell particles for coating applications: Synthesis and characterization. J. Environ. Chem. Eng. 2016, 4, 3891–3899.
(12) Lu, L. C.; Wang, C. I.; Sye, W. F. Applications of chitosan beads and porous crab shell powder for the removal of 17 organochlorine pesticides (OCs) in water solution. Carbohydr. Polym. 2011, 83, 1984–1989.
(13) Fu, M.; Chen, W.; Zhu, X.; Yang, B.; Liu, Q. Crab shell derived multi-hierarchical carbon materials as a typical recycling of waste for high performance supercapacitors. Carbon 2019, 141, 748–757.
(14) Rahman, M. A.; Hussain, M. M.; Samad, A.; Alam, A. M. S. Removal of Arsenic from Ground Water with Shrimp Shell. Dhaka Univ. J. Sci. 2012, 60, 175–180.
(15) Kandra, F.; Challa, M. M.; Jyothi, H. K. P. Efficient use of shrimp waste: present and future trends. Appl. Microbiol. Biotechnol. 2012, 93, 17–29.