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Exploring the functional properties and utilisation potential of mollusca shell by-products through an interdisciplinary approach

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Molluscan shellfish aquaculture contributes to 42.6% of global aquaculture production. With a continued increase in shellfish production, disposal of shell waste during processing is emerging as an environmental and financial concern. Whilst major commercial species such as *Crassostrea* spp. has been extensively investigated on usage of its shell, with information that are crucial for valorisation, e.g. safety and crystal polymorphs, evaluated. There is currently little understanding of utilisation opportunities of shell in several uprising Australian commercially harvested species including Akoya Oyster (*Pinctada fucata*), Roe's Abalone (*Haliotis roei*) and Greenlip Abalone (*Haliotis levigata*), making it challenging to identify ideal usages based on evidence-based information. Therefore, in this study, an interdisciplinary approach was employed to characterise the shells, and thereafter suggest some potential utilisation opportunities. This characterisation included crude mineral content, elemental profiling and food safety evaluation. As well, physical, chemical, and thermal stability of the shell products was assessed. TGA result suggests that all shells investigated have high thermal stability, suggesting the possibility of utilisation as a functional filler in engineering applications. Subsequent FTIR, SEM and XRD analyses identified that $CaCO_3$ was the main compositions with up to 77.6% of it found to be aragonite. The spectacular high aragonite content compared to well-investigated *Crassostrea* spp. suggested an opportunity for the utilisation of refined abalone shell as a source of biomedical engineering due to its potent biocompatibility. Additionally, safety evaluations on whole shell also outlined that all investigated samples were safe when utilised as a crude calcium supplement for populations > 11 years old, which could be another viable options of utilisation. This article could underpin abalone and akoya industries actions to fully utilise existing waste streams to achieve a more sustainable future.

Keywords Shell waste, Blue economy, Seafood, Full utilisation, Upcycling, Supplement safety

Mollusca is a species-rich[1] phylum, including well-known subgroups such as gastropods, bivalves, and cephalopods. These animals contribute a significant portion of human seafood consumption[2]. According to 2024 statistics, 26% of global seafood consumed were shellfish species including mussels, oysters, clams, scallops, abalone, sea snail, cockle and whelks[3–6].

The majority of these consumption species are characterised by external hard shells. Whilst generally consumers are interested in consuming minimally processed shellfish, there is also considerable processing of molluscan shellfish into ready-to-eat items, with Loo[7] evidenced that rarely investigated species, such as abalone often produce piles of shell by-product in abalone processing[3,8]. This processing includes "shucking" to separate the shell from the edible meat, a process which generates a large quantity of shell waste, as the shell accounts for 35–90% of the animal weight[9–12]. Unregulated disposal of these shells is reported to create strong noxious smells and cause environmental pollution by having shells piling on shoreline, subsequently obstruct the usage of coastal area which ultimately require dredging to remove, further damaging existing marine habitat[4,12–15].

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Regulated disposal does occur but may incur a sizable operational fee that may be burdensome for small to medium enterprises[12]. Although in the past and present, there has been cases the shell waste were being utilised in Australia, such as utilised as decorative[7] and materials for reef restoration[16]. Existing strategies either have minimal impacts on reducing vast amount of shell waste produced or shown to have counter effective impact towards the environment. With the production of shellfish increasing, these existing shell disposal issues, are also expected to increase. Hence identifying potential strategies in utilising shell waste is important in improving the sustainability of current shellfish industries[12].

In systematic reviews conducted by Zhan et al.[4] and Morris et al.[12], the major valuable component in shell waste were found to be calcium carbonate ($CaCO_3$) which consist of three different polymorphs—aragonite, calcite and vaterite. These polymorphs occur in various ratios, depending on the location and species. Currently, the majority of molluscan shell valorisation activities are based on the overarching $CaCO_3$ composition and involve minimal processing of the shell. Such activities include use in poultry feed, animal nutritional supplementation, biofiller, soil liming, concentre aggregate and human supplements (details referred to Morris et al.[12] and Chang et al.[17]).

Further characterisation of the shell $CaCO_3$, can be undertaken to understand the relative percentages of the different polymorphs (aragonite, calcite and vaterite), which vary according to species and location. Such characterisation can potentially lead to more specialised valorisation applications. As an example, aragonite is often considered an optimal polymorph in the field of biomedical science due to high biocompatibility and promising osteo-bioactivity[18,19].

Additionally, despite the fact that various human calcium supplements derived from molluscan shell are currently on the market globally, branded as "Os-Cal" (US), "Hi-Calcium" (US), "Oyster Ca" (Netherlands), and "Nature Made Oyster Shell Calcium" (Japan)[17], understanding of safety regarding calcium supplements from multiple seafood species is mostly unexplored. Due to chemical similarity of calcium and some heavy metal, deposition of toxic heavy metal could occur during the biological formation process of shell, therefore, if shells were harvested from location with great anthropogenic activities, this could lead to potential adverse health effects if shell-based calcium supplements are to be consumed orally[17,20–23]. Hence, safety characterisation should be explored to understand the viability of utilising shell waste as a human supplement to fill the existing knowledge gap.

Although characterisation studies on shell have been conducted on some of the more commonly consumed shellfish, for instance, oyster, clam and mussel of various species[4], characterisation on uprising commercial edible species such as Akoya oyster (*Pinctada fucata*), Roe's abalone (*Haliotis roei*) and Greenlip abalone (*Haliotis levigata*) has not been conducted, which has been evidenced in Loo[7] study that has led to various pressing sustainability concern due to lacking in know-how and fundamental understanding of shell characteristics. This study was therefore initiated to investigate the compositional, stability and safety aspects of shell from these species, which aim to explore suitable valorisation formats through interdisciplinary techniques that have been applied in other shellfish by-product valorisation study. The findings of this study would be valuable to identify usage of shell waste from uprising commercial species, as well, providing a systematic methodological framework to be utilised in the investigation of other shell waste.

Methodology
Raw materials
Shells of Akoya oyster (*Pinctada fucata*) was provided by Harvest Road Group (Albany, WA, AU), Roe's Abalone (*Haliotis roei*) and Greenlip Abalone (*Haliotis levigata*) were provided by Rare Foods Australia (Augusta, WA, AU). For each sample, more than 10 kg of pooled samples were collected from the end of processing line. Three shells from each species were randomly selected using a quartering technique. Shell samples were soaked in double deionized water overnight to desalinize. Shells were then sundried and stored in ambient environment until analysis. Chemicals used in this study were sourced from Sigma-Aldrich Ltd. (Macquarie Park, NSW, AU).

Ash content
AOAC[24] standard method was utilised to determine the ash content of the samples. In brief, samples were heated at 550 °C in Thermolyne muffle furnace model 48000 (ThermoFisher Scientific Inc, IA, USA) until complete removal of organic matter as indicated with white ash.

Elemental profiling
Elemental quantification via microwave plasma atomic emission spectroscopy (MP-AES)
Individual whole shell was coarsely grounded with 150 g of subsample pulverised using TissueLyser II (Qiagen, Hilden, Germany) to obtain powder with fine flour-like consistency and oven dried at 105 °C for 48 h. 0.5 g of homogenized sub-sample was then transferred to a 50 mL Pyrex screw cap culture tube. Due to potential reactivity, digestion was conducted in an ice slurry bath with 2 mL concentrated nitric acid (70%) and 0.5 mL hydrogen peroxide (30%). Digestion was deemed complete when digestate was transparent, solution was then standardized to 20 mL in 1% nitric acid. K, Na, P, Mg, Ca, Fe, Zn, Cu, Cd, Cr, Pb and Ni were determined using Microwave plasma atomic emission spectroscopy 4100 (MP-AES) (Agilent Technologies, SC, US). At least 7 Multi-element calibration solutions were prepared in the range of 0–20 mg/L in 1% nitric acid. All glassware used in this experiment was cleaned twice with 2% RBS-25 solution to prevent chemical retention and improve accuracy of elemental determination. Analyses were conducted on three shell per species.

Total and inorganic arsenic quantification via inductively coupled plasma mass spectroscopy (ICP-MS)
ICP-MS was used to determine total (tAs) and inorganic arsenic (iAs) content of shell due to its sensitivity towards elements with high ionization potential. Digestates were prepared using the method described in

the previous MP-AES section and analysed via inductively coupled plasma mass spectroscopy ELAN DRC II (ICP-MS) (PerkinElmer, MA, US). Details of the method is listed in AOAC[24] Method 986.15 and Matsumoto-Tanibuchi et al.[25]. All samples were measured in duplicate.

Mercury quantification via cold vapor atomic fluorescence spectroscopy (CV-AFS)
Cold vapour technique was used to assess mercury (Hg) content due to the potential of mercury being in free atomic state at room temperature and expectation of detecting it at a low level. Pulverised sample was digested according to AOAC 974.14[24]. The quantity of mercury was then determined using cold vapor atomic fluorescence spectroscopy PSA Millennium (CV-AFS) (PS Analytical, LDN, UK).

Safety evaluation
Risk assessment of the shells was conducted to investigate the risk of utilising seashell as an oral calcium supplement. The method used was the Hazard Index (HI) equation developed by USEPA[26] and reproduced below.

$$HI = \sum_{i=1}^{n} \left[\frac{(m_i \cdot DC)/BW}{RfD_i} \right]_i \tag{1}$$

With m_i being ith metal in mg/kg Ca, dry basis. *DC* being recommended dietary allowances (RDAs) of Ca for dedicated age and gender group, due to low percentage of population consumed Ca supplement dosage exceeding level of recommended intake ($<1\%$)[27,28]. *BW* represent body weight (kg) of adult male and female based on ABS[29] or body weight (kg) of children based on ABS[30]. RfD_i utilised data based on the assumption that existing metals including Cr[31,32], Cd[33,34], Hg[35,36], Cu[37,38] and iAs[39,40] are in the most toxic forms.

Thermogravimetric analysis (TGA)
The thermal stability of cured samples was studied from ambient to 800 °C using a temperature ramp of 10 °C per min under a nitrogen atmosphere flowing at 50 mL/min on SDT 2960 simultaneous DTA-TGA (TA Instruments, DE, US). Approximately 10–20 mg of solid sample was weighed accurately into a 110 μL platinum crucible with a matched empty crucible as a reference. The temperature scale of the instrument was calibrated using the melting points of 99.999% indium (156.5985 °C), 99.99+% tin (231.93 °C), 99.99+% zinc (419.53 °C), and 99.99% silver (961.78 °C). The balance was calibrated over the temperature range used with alumina mass standards provided by the instrument manufacturer.

X-ray crystallography (XRD)
The shell waste from *Pinctada fucata*, *Haliotis laevigata*, and *Haliotis roei* were roughly ground using TissueLyser III (Qiagen, HI, DE) to obtain coarse shell powder. The three coarse powdered samples were then further pulverized in a micronizing container with ethanol using a McCrone Micronizing Mill (The McCrone Group, WE, IL) with agate pellets. Sand was used to wash out the container and pellets between the micronization process for each sample. Approximately 10 weight percent (wt. %) of corundum (as an internal standard) powder was added to each sample before micronization. The micronized samples were dried for 24 h in a fume hood, followed by being loaded onto PMMA specimen holders. Diffraction data were collected using a D8 Advance diffractometer (Bruker Corporation, MA, US) with Cu Kα radiation over a range of 5–80°. Phase identification was carried out in Bruker EVA 6.0 (Bruker Advanced X-ray Solutions) (Bruker Corporation, MA, US) using the PDF4+ ICDD database. Crystalline phases were quantified by the Rietveld refinement using Bruker Topas 6.0 (Bruker Corporation, MA, US). An assessment of the amorphous fraction of each specimen was made using the known addition of corundum via the internal standard method.

Scanning electron microscope (SEM)
For morphological investigation, SEM imaging was performed on a Zeiss Neon 40EsB (Zeiss, BW, GE) operating in a dual-beam field emission configuration. The images were taken using an accelerating voltage of 10 kV with an aperture size of 30 μm. The crushed shell powders were mounted on carbon tape that was adhered to SEM pin stubs. The samples were then coated with platinum with a thickness of 7 nm to prevent charging on non-conductive samples.

Fourier-transform infrared spectroscopy (FTIR)
The FT-MIR spectrum was also recorded for each shell powder in the range of 4000–450 cm^{-1} on a Nicotel™ iS50 FTIR spectrometer (ThermoFisher Scientific, MA, US) operating in ATR sampling mode with a diamond ATR crystal. The fingerprint region (1200–450 cm^{-1}) was analysed and the calcium carbonate phases was identified.

Statistical analysis
Graphical components and statistical analyses in this manuscript were generated using Origin version 2023b (OriginLab Corporation, Northampton, MA, US) and IBM SPSS version 28 (IBM Australia Ltd, St Leonards, NSW, AU). In terms of statistical analysis for mineral composition and ash content, Levene's tests were first performed to determine whether the data set fulfilled the assumption of classic ANOVA. In the case of equal variance, classic one-way ANOVA was then carried out with Tukey's post-hoc test. When unequal variance was noted, to reduce likelihood of Type I error, Welch's ANOVA with Games-Howell post hoc were carried out to increase robustness of statistical analysis. Independent t-test was carried out to compare iAs content due to <3 interventions. To investigate similarity between seashell samples, a principal component analysis (PCA) was

subsequently conducted with a targeted model of $\geq 60\%$ cumulative scores and inclusion of all components with eigenvalue ≥ 1. Significance value of statistical analysis was set at $P \leq 0.05$ and quantitative data was presented as mean $\pm$ standard deviation.

Result and discussion

Ash content

Ash contents of investigated molluscan shell in this study were found to be 93.71 ± 0.37 (*Pinctada fucata*), 95.88 ± 0.38 (*Haliotis roei*), and 96.40 ± 0.25 (*Haliotis laevigata*). Similar values were identified in studies conducted by Elegbede et al.[41] where ash content of bloody cockle (*Anadara senilis*), mangrove oyster (*Crassostrea gasar*), and blue mussel (*Mytilus edulis*) were reported at the range of 92–98%.

In the field of food compositional analysis, ash content is often use as an indicative matrix to estimate the quantity of inorganic matter, the majority of which is usually presumed to be mineral[41]. For molluscan shells, 95–99% of the mass is reported to be $CaCO_3$ with as low as 1–5% organic matter[42,43]. When combining results from the ash analyses with the TGA result reported in a later section (Fig. 6), it can be determined that $CaCO_3$ is the major component in ash content for the shells from all three species.

Whilst abalone shell is a valuable commodity that is sometimes marketed and utilized for decorative purposes, substantial portions may be discarded to landfill due to the inability of individual processor to accumulate sufficient volume for efficient commercial outcomes such as exporting and refinery[7]. The current findings in this study identifying that abalone shells have similar major component—$CaCO_3$ compared to the other molluscan shells, this could indicate that there could be possibility of a including investigated shell to existing $CaCO_3$ refinery and diverted from entering landfill.

In fact, in Galicia, Korea, US and Peru, there are already existing industrial or government-initiated facilities that refine seashell into lime (CaO derived from $CaCO_3$) or $CaCO_3$ for uses such as soil conditioning, liming[44] and prevention of river eutrophication[42,45,46]. Shell-derived $CaCO_3$ has also been shown to be a possible replacement for cement or filler in construction[47–49]. This product can also act as a natural-sourced food additive that could replace synthetic phosphate in meat products, to offer crucial functions such as pH adjustment, buffering, improved production yield and improved sensory characteristics[50,51]. CaO transformed from shell's $CaCO_3$ through simple reactions has also been found to exhibit strong antibacterial activity that can extend shelf-life of food such as tofu[52] and kimchi[53].

Elemental profile

Results of mineral analysis are shown in Table 1. All samples had calcium as the highest level components, reaching 37.84–38.53%. Similar results were also found in a previous study on Black-lip Pearl Oyster (*Pinctada margaritifera*) (39.7%)[17], Akoya Oyster (35.6%)[54], Pacific Oyster (*Crassostrea gigas*) (41.55–48.27%)[55], Bloody Cockle, Mangrove Oyster, and Blue Mussel (12.44–51.00%)[41]. The high Ca result suggests that all samples investigated could be utilised as an human calcium supplement that are comparable to existing oyster shell-based supplements produced in the US, Netherland and Japan (summarised in[17]). In Australia, ABS[28] identified that 21% of females and 15% of males above 2 years old consume calcium supplements, product thereby reaching 3.8 million population in Australia alone. With the increasing demand of "natural" human product due to consumer

	Greenlip Abalone (*Haliotis laevigata*)	Roe's Abalone (*Haliotis roei*)	Akoya Oyster (*Pinctada fucata*)
Macro-minerals (mg/kg dry basis)			
K	409.47 ± 110.62^a	314.85 ± 27.66^a	237.01 ± 7.45^b
Na	1097.50 ± 168.82^b	1230.76 ± 7.83^a	1239.20 ± 121.90^{ab}
P	22.56 ± 8.46^b	29.15 ± 3.09^b	35.79 ± 3.90^a
Mg	35.34 ± 0.75^c	90.60 ± 0.86^b	191.20 ± 14.20^a
Ca	$385,307.10 \pm 67.6^a$	$382,781.63 \pm 15.69^b$	$378,400.79 \pm 58.05^c$
Trace elements (mg/kg dry basis)			
Fe	N.D	25.06 ± 3.10	N.D
Zn	N.D	N.D	5.77 ± 0.80
Cu	1.29 ± 0.42	1.64 ± 0.35	2.06 ± 0.78
Cd	13.21 ± 0.65^a	11.51 ± 1.68^b	11.80 ± 0.86^a
Cr	1.98 ± 0.07^b	2.33 ± 0.13^a	1.87 ± 0.11^b
Pb	N.D	N.D	N.D
tAs	0.08 ± 0.01^b	1.10 ± 0.00^a	0.06 ± 0.01^c
iAs	N.D	0.85 ± 0.11^a	0.04 ± 0.06^b
Hg	0.01 ± 0.01	N.D	N.D
Ni	N.D	N.D	N.D

Table 1. Mineral content of Greenlip abalone, Roe's abalone and Akoya oyster shell. Welch's ANOVA was conducted on K, Na, P, Mg, Ca, Cd, tAs and independent *t*-test was carried out on iAs. Values are expressed in mean $\pm$ standard deviation from three individual seashell with triplicate analytical measurement. Significant differences ($P \leq 0.05$) are indicated with different uppercase letters.

perception of enhanced health benefits and safety, the current findings could be used to propose a potential business opportunity in valorisation of such shells into high value nutraceuticals[56,57].

The high Ca content in the tested samples also highlighted the potential of utilisation as a supplement for animals, especially egg-laying poultry. Previous studies by Ahmad and Balander[58] and Wang et al.[59] found that by supplementing poultry with seashell, eggshell quality improved, which reduce food loss due to breaking of egg shell during post-harvest handling.

In considering the use of the Ca of the shell as nutraceutical, it is noteworthy that all samples investigated were found to have relatively high Cd content (11.51–13.21 mg/kg, dry basis) in comparison to previous studies (< 6.21 mg/kg) on Pacific Oyster, Black-lip Pearl Oyster and Hairy Cockle (*Anadara antiquata*), Eared Horse Mussel (*Modiolus auriculatus*), Pacific Jewel-box (*Chama pacifica*), Fluted Giant Clam (*Tridacna squamosa*), and Cockle (*Periglypta reticulata*)[17,22,55]. In comparison, in the studies of Ha et al.[55] and Nour[22], other heavy metals such as Ni, Pb or Cu (up to 87.19, 67.41, 67.74 mg/kg, respectively) were found to be more concerning in molluscan shell in comparison to the Cd levels. The variation in heavy metal findings was not surprising as marine invertebrate shells have previously been suggested to be used as marine pollution indicators due to their ability to accumulate environmental pollution, especially heavy metals[60,61].

Heavy metal content in seashell has previously been hypothesised to be incorporated from the surrounding environment during shell formation[62]. However, a recent study conducted to understand Cd appearance on oyster shell has identified that Cd can be attached to shell via precipitation and chemisorption outside of shell formation[63]. Although this ability seems to be undesirable as it could impact heavy metal safety if shells were to be utilised as nutraceuticals, Lee et al.[63] has suggested that this mechanism is the principle behind the reported strong ability of seashells as a biosorbent in removing heavy metals (see also review by Tamjidi and Ameri[64]).

When statistically comparing the trace element content across samples, there is seemingly a lack of distinctive genus (*Haliotis* spp.) similarity between Greenlip and Roe's Abalone. In comparison, the trace elements profile of Roe's Abalone shell appeared to be closer to the Akoya Oyster, the same phylum but a different genus. Hence, a PCA was conducted and the distances across all three species was shown to be evenly spaced (Fig. 1), confirming the earlier observation. Previously, taxonomic differences has been hypothesized to play an important role in trace elements content in seashell[17,21]. However, in this case, perhaps other factors such as variation in ontogenetic stages, fluctuation in salinity and temperature of surrounding water, seasonal factors, similarity of sediments and proximity of growing locations have overridden the previously observed taxonomical similarity[17,21,22]. The findings also suggest that, since trace element content seems to be unpredictable, if such shells are to be utilised as a human supplement, trace elements content should be inspected regularly to ensure product safety.

Safety evaluation

As suggested in previous sections on trace-elements results, investigated samples could pose safety concerns if the shell is to be consumed as a nutraceutical or calcium supplements. Hence, safety evaluations of the
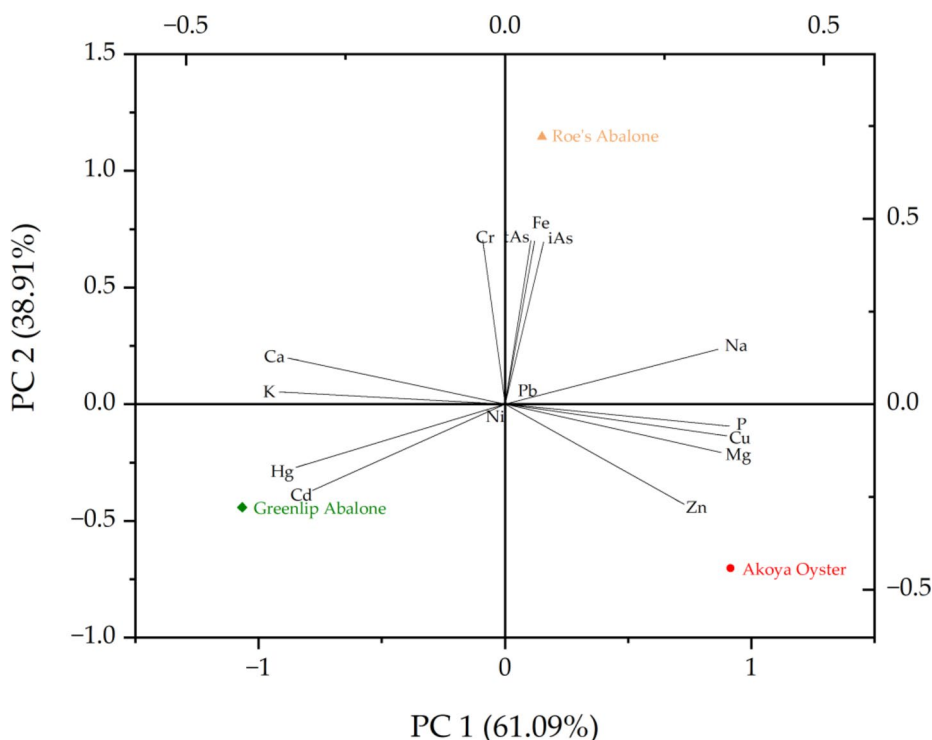
Fig. 1. Principal component analysis (PCA) biplot generated via elemental composition of Greenlip abalone, Roe's abalone and Akoya oyster shell.

different shells using the USEPA[26] HI equation were conducted to evaluate safety holistically, in considering the appropriate dosage aligned with all investigated toxic trace elements.

Result of HI analysis was presented in Fig. 2, with all investigated seashells being unsuitable and potentially harmful to populations below 11 years of age (HI > 1). This result is likely a consequences of low body mass and high calcium needs for growth in this age group[30,65]. Cd was found to contribute to the majority of the HI score. Cd exposure to children at this < 11 development stage is particularly detrimental, with the potential to cause chronic and severe adverse health outcomes[66]. However, it should be noted that the current investigation was conducted with a relatively small sample size and number of tests species. As mentioned previously, the trace element composition of seashell can vary depending on multiple factors. It is therefore recommended to undertake further investigation to contributory factors for Cd accumulation in molluscan shell.

However, using the cumulative HI, a "worse-case scenario" hazard analysis as described by Bandara et al.[67], the results showed that the shell products posed no concern for any age groups > 12. These results suggest that the investigated shells could be safely valorised for calcium supplements for adolescents and adults. Yet, as stated previous, it is recommended that ongoing monitoring of heavy metal contamination and investigation of potential contributing factors using a larger sample cohort be completed to allow a more rigorous examination of the valorisation of seashell, for safe supplement utilisation.

Scanning electron microscopy (SEM)

The shell from the three species of molluscs namely Greenlip abalone (Fig. 3a, *Haliotis laevigata*), Roe's abalone (Fig. 3b, *Haliotis roei*) and Akoya oyster (Fig. 3c,d, *Pinctada fucata*) were prepared for SEM characterisation. SEM imaging of all three pulverised powder samples show similar and distinctive features of molluscan shell morphology. Based on Fig. 3a–d, it was apparent that the investigated shell powders are a mixture of large lamellar structures (b1) and column-like pieces (d1); the origins of which are aragonite platelets originating from nacre[68] and calcite columnar prismatic crystal arising from the prismatic layer of the shell, respectively[69]. Layers of nacre was observed and measured with a mean thickness of about 500 nm as shown in Fig. 3e. This result was consistent with those previously reported[70]. The particle size distribution estimated from the SEM images after crushing the shell was broad with particle size ranging from < 1 μm to 1 mm.

The morphological and particle size distribution results can be an important consideration for potential utilisation of the shell as a filler in polymer composites. Both morphological characteristics and particle size distribution can influence the particle packing behaviour, and consequently, the loading capacity[71]. Additionally, morphological differences such as spherical, lamellar (flat-shaped) or irregular shapes can have a significant impact on the polymer composite properties such water vapor transmission rate. In particular, lamellar morphological shapes can act as a physical barrier, and increase tortuosity for various permeants, such as water
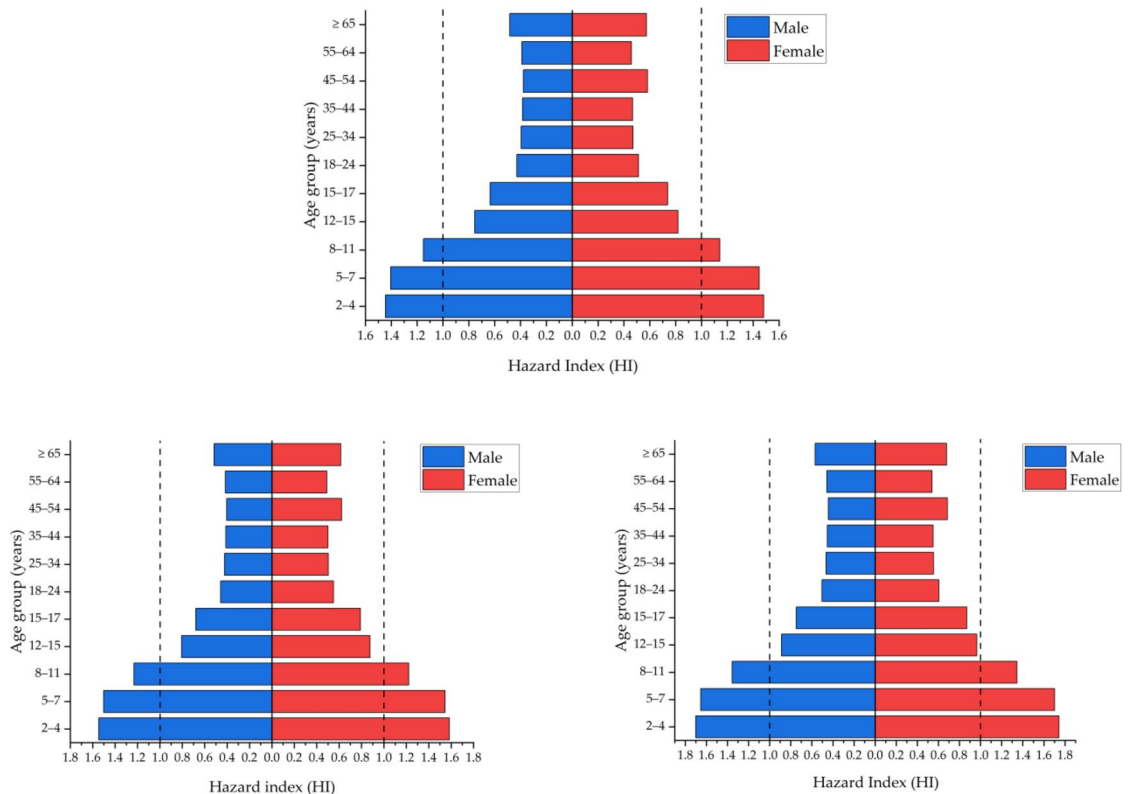
Fig. 2. Safety evaluation of greenlip abalone (left), Roe's abalone (right) and akoya oyster (top) shell as a human calcium supplement.
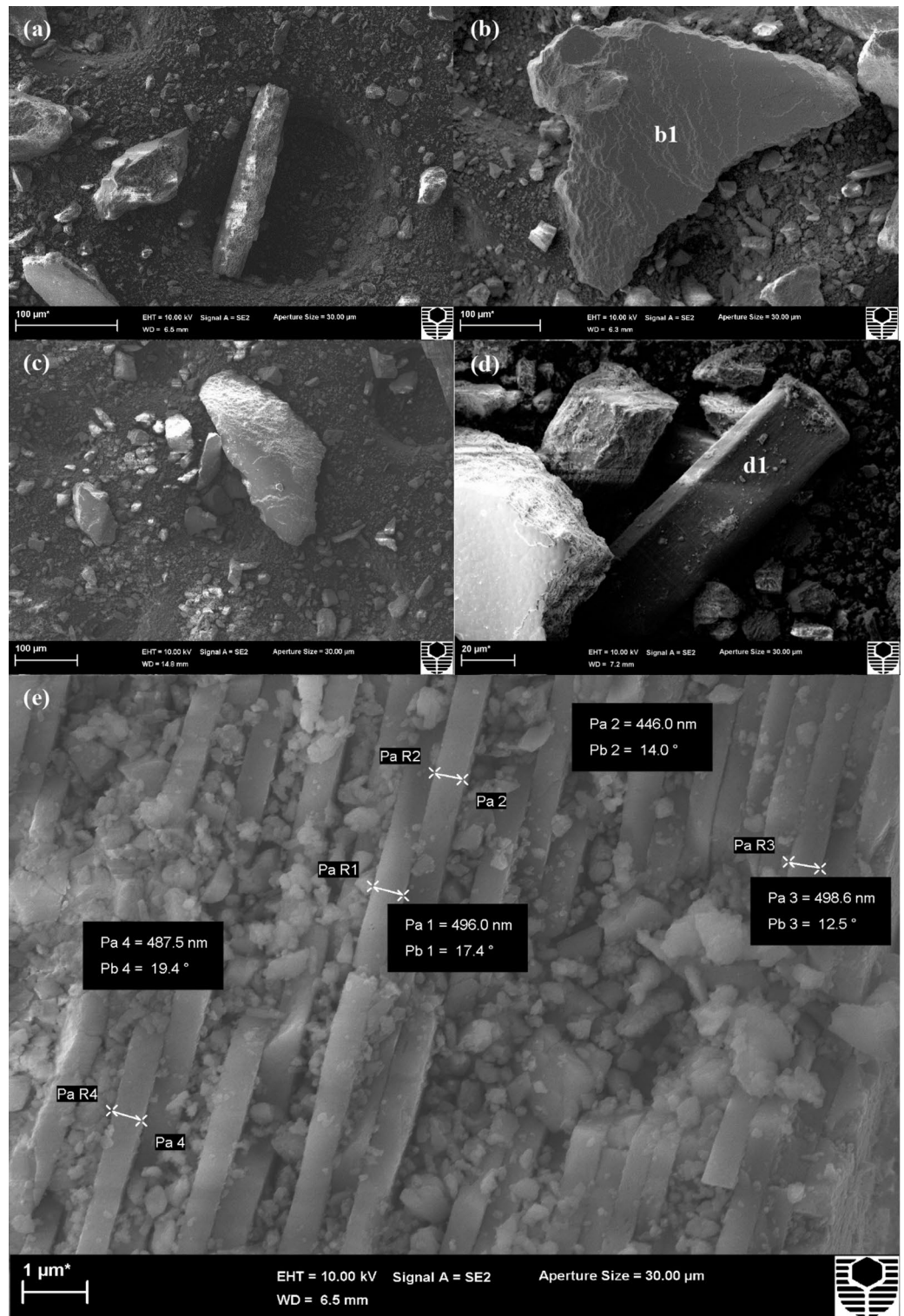
Fig. 3. SEM micrograph of shell powder mixture obtained from three pulverised shell per sample. (**a**) Greenlip abalone; (**b**) Roe's abalone; (**c**) Akoya oyster which originates from the nacre layer of the shell due to its plate-like morphology. (**d**) Akoya shell powder shows an intact columnar prismatic calcite crystal originating from the prismatic layer of the molluscs' shell after crushing. (**e**) Nacre layer with a thickness between 400–500 nm. (**b1**) indicate lamellar structure of aragonite platelets and (**d1**) highlight column-like morphology of calcite columnar prismatic crystal. All SEM images were taken with a Zeiss Neon 40EsB (Zeiss, BW, GE) operating in a dual-beam field emission configuration using a 10 kV accelerating voltage and an aperture size of 30 μm.

or oxygen molecules, thereby, reducing permeability[72]. This strategy is commonly employed in the formulation of anti-corrosion protective coatings in which ceramic or mineral filler such as glass flakes[73] and micaceous iron oxide[74] both of which have plate-like morphology are often used to decrease the permeability of the protective polymer film. A similar strategy was implemented in food packaging technology using hexagonal boron nitride (BN) fillers as reported in previous literature[75]. Based on the shell morphology, evident in the SEM images, it may also be worthwhile to consider identification of a commercially viable method to isolate homogenous lamellar shape aragonite crystal which can serve as viable alternative to the more exotic hexagonal BN fillers. For instance, a mechanical, chemical and heating method has been previously shown to be useful in separating calcite and aragonite crystals[76,77].
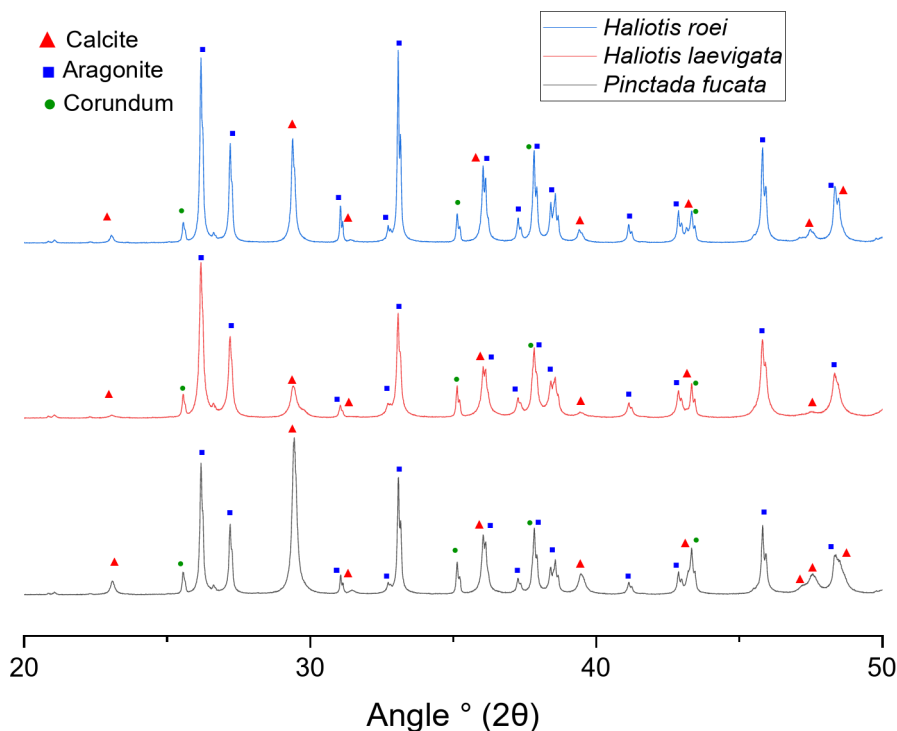
Powder X-ray diffraction (XRD)

In the scientific literature, there are multiple qualitative investigations into identifying crystalline phases of various molluscan shells, however, there is a paucity of studies on the exact composition of crystalline phases in the shells of Akoya oyster (*Pinctada fucata*) and Australian abalone species including Greenlip (*Haliotis laevigata*) and Roe's abalone (*Haliotis roei*) species. As mentioned previously, composition of the inorganic phases in molluscan shell is generally comprised of two crystalline phases—calcite and aragonite[78] which are the commonly observed polymorphs of calcium carbonate.

In this investigation, a comprehensive and novel approach was adopted in the quantification of polymorphic phases of $CaCO_3$ in the tested shell powders to obtain the relative and absolute quantities of the different phases. This was achieved by powdered X-ray diffraction (XRD) which was used to identify the $CaCO_3$ polymorphs and within the same characterisation technique, the Rietveld refinement was employed for the quantification of the crystalline and non-crystalline (amorphous) phases.

From the powdered XRD, two main polymorphs of calcium carbonate ($CaCO_3$), calcite and aragonite, were identified in the abalone shell powder from both the Roe's Abalone, Greenlip Abalone and Akoya Oyster. The most abundant polymorph in both the abalone and oyster shell were the aragonite phases (49.8–77.7%) which was followed by calcite (10.3–32.9%). The remaining phases were attributed to the amorphous content (1–15.5%). In part, this amorphous content can be attributed to amorphous calcium carbonate (ACC) as evident in the IR spectrum (Fig. 4) and other miscellaneous organic compounds such as polysaccharide like chitin, protein and others[79]. A small amount of contaminant i.e., quartz was observed in all powder XRD diffraction pattern which comes from the cleaning of the container in the micronizer with sand. The summary of the phase analysis via powder XRD is presented in Table 2.

Based on the composition obtained from three different species of molluscs commonly harvested in Australia, the shell powder obtained from *Haliotis roei* and *Haliotis laevigata* have one of the highest aragonite contents of about 75–77 w/w% whilst the *Pinctada fucata* shell has about 50 w/w% of aragonite. Drawing on the

Fig. 4. Powder X-ray diffraction pattern of *Haliotis roei*, *Haliotis laevigata* and *Pinctada fucata* shell powder obtained from three homogenized seashell. All samples were spiked with corundum (10% w/w) for quantification purposes. Quantification was performed via Rietveld refinement with all fitting having a R_{wp} value less than 10.

Phases	Greenlip Abalone (*Haliotis laevigata*)		Roe's Abalone (*Haliotis roei*)		Akoya Oyster (*Pinctada fucata*)	
	Relative	Absolute	Relative	Absolute	Relative	Absolute
Corundum* (internal reference)	10.00%	–	10.00%	–	10.00%	–
Aragonite	68.15%	75.72%	69.89%	77.66%	44.84%	49.82%
Calcite	9.26%	10.29%	16.63%	18.48%	29.61%	32.90%
Quartz	2.58%	2.87%	1.91%	2.12%	1.56%	1.73%
Amorphous content	10.01%	11.12%	1.57%	1.74%	13.99%	15.54%

Table 2. Quantitative powder X-ray diffraction analysis using Rietveld refinement showing the relative and absolute phase content. *Corundum was added as internal standard for quantification purposes and quartz is a residual contamination from the mechanical cleaning of the micronizer container with silica in between sample preparation. Values were obtained from three micronized seashell.

current findings, the data suggests that shell mineralogy is dependent, to a certain extent, on the mollusc's genus. However, a previous report, based on historical and resample data, established a relationship between climate change and its profound impact on biomineralization in *Mytilus californianus* (common name, California mussel) leading to a decrease in aragonite content due to anthropogenic ocean acidification[80]. Previous reports on shell mineralogy in other cultivated molluscs species such as Blood cockles (*Anadara granosa*) reveal that its shell composition is mostly comprised of aragonite, although quantitative contribution was not determined[81]. Whilst in other molluscan species such as California mussel (*Mytilus californianus*), the aragonite content can be up to 48% by weight[80].

In terms of various $CaCO_3$ polymorph, calcite is the most stable in nature which has been applied in bone regenerative application such as bone grafts previously[82,83], however, in previous study, when compare to aragonite and vaterite, it is not as readily able to convert to hydroxyapatite under physiological condition due to its lower solubility, suggesting that those less stable polymorphs are perhaps superior in biomedical engineering[84]. Moreover, natural biogenic aragonite, i.e. nacre from shell, as well has additional favourable characteristics demonstrated[19], for instance, high porosity[85], solubility[86] and unique topographical features[87] which enables an environment conducive for bone tissue regeneration[88]. In comparison to another polymorph, vaterite, it has as well been utilised previously in hydrogel for bone regenerative medicine[89] due to it being metastable polymorph which makes it highly soluble under physiological condition providing calcium ions for conversion into hydroxyapatite[90]. However, while its metastability improve biocompatibility of crafted materials, since it is relatively unstable, it is difficult to identify natural source with abundant vaterite, hence, limited opportunities for valorisation due to its low abundance.

The current finding of spectacularly high aragonite content in abalone could indicate a promising, high value utilisation of the investigated shells, particularly, for bone tissue engineering. Previous research has already demonstrated the osteogenic, osteoinductive and osteoconductive property of nacre in various in vitro and in vivo human and animal models[91–94]. Especially, favourable outcomes have been identified in human clinical trials. For instance, Atlan et al.[95] have identified that direct usage of powdered aragonite-rich shell powder from *Pinctada maxima* could reconstruct damaged bone in human patients. As well, purified aragonite obtained from corals has also been used to construct commercial medical implants such as Agili-c scaffold (CartiHeal). These implants have been shown to be a viable option for use in patients with mild to moderate osteoarthritis, and aligned, reported to improve patient outcomes[94,96].

Since the osteoactivity of nacre are mostly attributed to aragonite[92,93], the high aragonite content in *Haliotis roei* and *Haliotis laevigata* could be used to establish a more efficient raw material conversion rate for aragonite refinement. Additionally, in comparison to corals which are used to construct Agili-C, abalone shells are more sustainable since it is currently being disposed of as a by-product. Refinery of aragonite from existing shell powder is thus recommended as the next step of research. Alternatively, direct utilisation of whole abalone shell for veterinary purposes could also be investigated.

Fourier transform infrared (FTIR) spectroscopy

To further support the crystalline phase analysis obtained via powdered XRD, Fourier transform infrared (FTIR) spectroscopy was performed on the shell powder derived from the three different mollusc species. While powder XRD was instrumental in the identification and quantification of crystalline phases, FTIR spectroscopy was appropriate for the elucidation of the amorphous phases. From the FTIR spectrum presented in Fig. 5, it is evident that $CaCO_3$ polymorphs are present. The key signals characteristic for aragonite can be observed at around 1 083 cm^{-1}, 713 cm^{-1} and 700 cm^{-1} whereas the vibrational band associated with calcite[97] are 871 cm^{-1} and 713 cm^{-1}. This reinforces the analysis performed for the identification of the crystalline phases of $CaCO_3$ via powder XRD.

As shown in Table 2, powder XRD analysis indicates that shell powder derived from these molluscs have certain percentage of amorphous phase with Akoya oysters at ~ 15.5% w/w (Table 2) having the highest amorphous content. An attempt was made to correlate the amorphous content to amorphous calcium carbonate (ACC) which has been reported in other molluscs such as *Mercenaria mercenaria*, *Crassostrea gigas* and *Hyriopsis cumingii* and *Diplodon chilensis patagonicus* [98,99]. Based on the FTIR spectrum presented in Fig. 5, the broad band from 1500–1400 cm^{-1} shows an apparent splitting, typically associated with the presence of ACC[100–102]. The function of ACC in molluscs has been proposed by Weiss, Tuross, Addadi and Weiner (2002) to
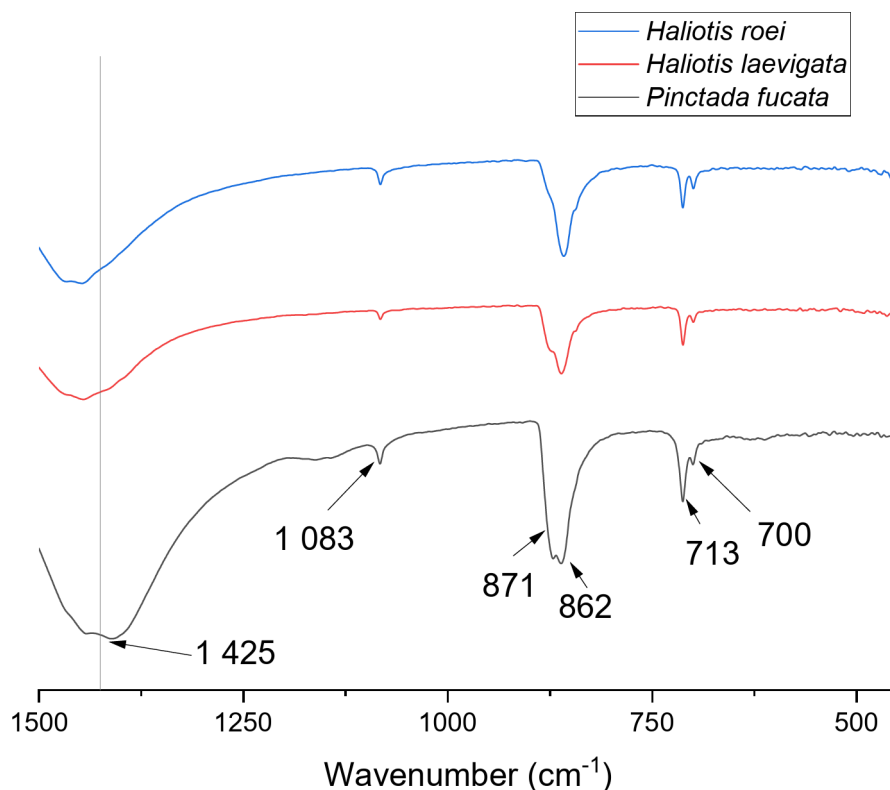
Fig. 5. Normalised transmission FTIR spectra of *Haliotis roei*, *Haliotis laevigata* and *Pinctada fucata* shell powder. Qualitative data on the presence of calcium carbonate polymorphs such as aragonite and calcite are apparent in the finger-print region in the FTIR spectrum. Amorphous calcium carbonate (ACC) has also been identified at around 1425 cm^{-1} and a line for illustration purposes was drawn to show the splitting in the signal which is characteristic when ACC is present. Micronized samples from XRD analysis were utilised.

be a precursor phase for the biomineralization of aragonite, usually found during the larval stage of a mollusc's life cycle.
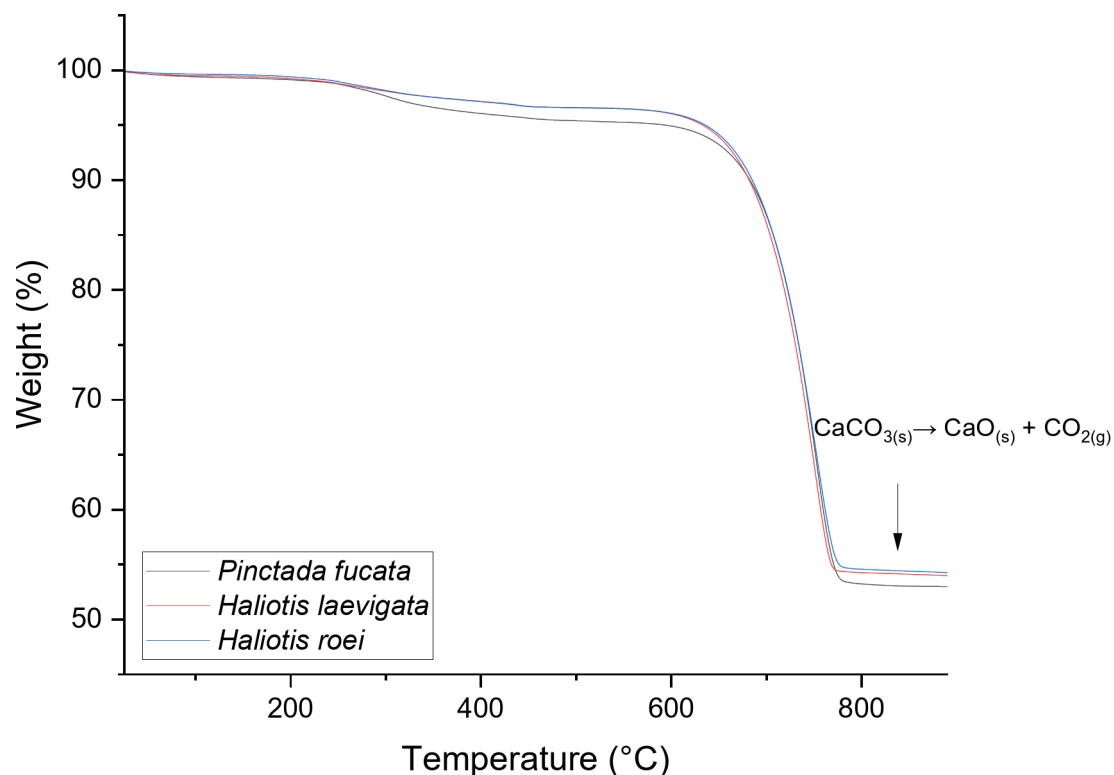
Thermogravimetric analysis (TGA)

As highlighted in previous sections, the mineralogy of molluscan shell powder is mainly inorganic $CaCO_3$. The chemical decomposition of pure $CaCO_3$ in response to heat is as shown in Eq. (2):

$$CaCO_3 \text{ (s)} \rightarrow CaO \text{ (s)} + CO_2 \text{ (g)} \qquad (2)$$

$CaCO_3$ found in biological systems is often embedded among organic molecules such as proteins, glycoproteins and polysaccharides[43,103] which make up a small percentage of the weight of the shell and are not stable to high temperature. In thermogravimetric analysis (TGA), any degradation of the organic material will culminate in a weight loss as a function of temperature. The TGA of shell powder demonstrated a small weight loss for both Roe's (-3.0%), Greenlip abalone (-2.9%) and Akoya shell powder (-4.1%) starting at 240 °C, which is the temperature associated with the decomposition of organic matter or moisture lost. A maximum weight loss of 42% was observed for all shell powder starting from 650 °C onwards which is attributed to the decomposition of $CaCO_3$ into calcium oxide (CaO) and carbon dioxide (CO_2). Overall, the thermal degradation behaviour based on the shape of the TGA curve presented in Fig. 6 of *Pinctada fucata* is consistent with previous report[104] while the TGA curves for *Haliotis laevigata* and *Haliotis roei* presents new TGA data for the thermal degradation behaviour of shell obtained from these Australian species.

The high thermal stability of the shell from all three species suggest that it could possibly be utilised to improve thermal stability of composite product. For instance, shell powder could be utilised as a functional bio-filler in biodegradable plastic to overcome the caveat of poor thermal stability in poly (lactic acid) (PLA) and polyhydroxyalkanoates (PHAs)[105]. As well the literature suggests that infusing shell powder which is mostly $CaCO_3$, with biodegradable plastic sources, can result in improvement on mechanical properties[106] and increase biodegradability[107]. Moreover, modified $CaCO_3$ has also been shown to potentially be able to slowly release bioactive substances into food product, consequentially, increasing shelf-life[108]. Therefore, future study of infusing shell by-products with biodegradable plastic could be valuable in reducing food waste, food loss and improving the current plastic pollution issues simultaneously.

Fig. 6. Thermogravimetric analysis of *Haliotis roei*, *Haliotis laevigata* and *Pinctada fucata* oyster shell powder in nitrogen atmosphere from 25–800 °C. Micronized samples from XRD analysis were utilised for this analysis.

Conclusions

In the first half of the study, ash content of shell powder from three different species of molluscs—*Haliotis laevigata*, *Haliotis roei* and *Pinctada fucata* was first determined, followed by characterisation of elemental profiling via MP-AES, CV-AFS and ICP-MS, and subsequently algorithmic safety evaluation was conducted using HI. The findings suggested that all investigated samples have similar $CaCO_3$ and Ca content which could allow centralized multi-species processing that might facilitate better use of shell, by overcoming commercial viability issues associated with disparate locations of low volumes of molluscan processing in Australia. Whilst Cd levels were demonstrated to be concerning in elemental analysis, HI analysis identified that shell waste could be safely utilised as Ca supplement for population > 12 years of age. However, since one of the limitations in the current study is small sample size, the authors were unable to determine factors contributing to Cd level in shells, as well as the potential fluctuation in the Cd level. Thus, if shell were to be valorised as a human nutraceutical, or for food applications, the authors urge further studies to investigate raw material for heavy metal contaminants in details.

In the second part of the study, physical and chemical properties of the shell powders were investigated. To the best of our knowledge, this is the first reported complete characterisation and quantification of the $CaCO_3$ polymorphs performed via powder XRD on powdered shell derived from *Haliotis laevigata* and *Haliotis roei*. FTIR spectroscopy was then completed as a secondary analysis to support the data obtained and TGA conducted to determine the thermal stability of the shell powder. The various potential applications of the tested shell waste were highlighted with a primary focus on bone regenerative medicine, as well as potential usage as a functional filler in food packaging materials, but also consideration as a biosorbent for oil spill remediation or wastewater treatment.

In conclusion, waste valorisation, is often a complex topic that requires collaborative efforts to fully understand the opportunities and possibilities of a commercially viable outcome. In this study, multiple authors with diverse knowledge and background have enabled exploration of undervalued resources—seashell, allowing derivation of multiple solutions that could possibly convert this product from exisiting linear economy to a circular economy. By integrating shell waste in any of the applications proposed earlier, it offers a pathway towards a more sustainable future, bridging the gap between sustainability and responsible resource management.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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