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Hydroxyapatite (HAp) Extracted from Cockle Shells with Polylactic Acid (PLA) for Bone Implant Application

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ABSTRACT

Cockle shell waste has been utilized to produce HAp powders for biomedical applications. This research involves creating HAp powder through a precipitation method and analyzing the properties of $CaCO_3$, CaO, and HAp. A composite PLA/HAp material was produced with varying ratios of 10%, 20%, and 30% HAp for tensile test samples. The results show that HAp derived from cockle shells contains calcium and phosphorus, similar to commercial HAp. Samples with different weight percentage of HAp provides Young's modulus values comparable to cortical bone (7-30 GPa), with the sample containing 30% HAp achieving the highest value (8.17 GPa), where High Young's modulus values indicate increased stiffness. In summary, cockle shell-derived HAp is a promising material for biomedical applications.

1. Introduction

Natural waste products have a great deal of promise as resources for the recovery and extraction of valuable chemicals. The transformation of these waste products into useful commodities requires the application of certain methods and procedures. Hydroxyapatite, HAp ($Ca_{10}(PO_4)_6(OH)_2$), is a known biomaterial that may be obtained from natural waste sources and, when exposed to body fluids, maintains its thermodynamic stability in its crystalline state. Additionally, it closely mimics the chemical makeup of bone minerals [1]. The unique quality of HAp is its ability to blend in perfectly with bone tissue without causing any negative reactions, such as toxicity, inflammation, or reactivity to foreign bodies [2]. Its outstanding osteoconductive qualities, amazing bioactivity, and outstanding biocompatibility are all credited with this quality. As a result, HAp is becoming more and more well-known for a variety of biomedical uses, especially in the fields of dental materials and orthopaedic implants [3-5]. HAp has been widely employed as a catalyst or adsorbent due to its porous structure and chemical and heat resistance [6].

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HAp powders can be produced from natural sources or chemicals utilizing a variety of synthesis processes and reactants. Precipitation, hydrothermal, hydrolysis, and the sol-gel process are examples of processing processes [1]. Thus, in this study cockle shells would be employed as a source of calcium in the production of HAp powders. Cockle shells, which are common in Malaysia, are a common by product of food production and are abundant along coastal shorelines [7]. Recycling wasted seashells is known to have a similar composition of minerals to corals which makes the use of cockle shells as alternative biomaterials for bone substitute is seen as very suitable besides helping to contribute to a cleaner environment [8].

Furthermore, Malaysia adheres to Mazhab Shafi'i which emphasizes that impurities present in non-halal products, even if they are for medical applications, are considered to be persistent. This includes animals that are permissible for consumption, such as bovine, if they are not slaughtered in accordance with Islamic law, there is a compulsory requirement regarding issue for halal product to cater the needs of Muslim around the world, which also the main concern of this research [9]. Additionally, the use of products derived from porcine sources is strictly prohibited under Islamic law. Therefore, there is awareness to produce HAp that are halal and safe for use by consumers, especially Muslims and other religious believers who are prohibited from using illegal sources.

Polylactic acid (PLA) is notable for its biodegradability and thermoplastic characteristics. PLA has gained prominence as a material of choice in the field of bioengineering, in addition to its modern industrial uses in industries such as textiles and packaging. Utilization of PLA in medical applications encompasses a wide range of uses, including tissue engineering for regenerative medicine as well as orthopedic, cardiac, and dental procedures [10]. PLA in conjunction with HAp has demonstrated significant potential in the field of bone tissue engineering. HAp, known for its osteoinductive properties, independently stimulates the formation of bone tissue by activating osteoblasts and pre-osteoblastic cells, as indicated by prior research studies [11,12]. The combination of PLA and HAp brings about a synergistic effect, impacting the unique properties of each constituent.

PLA has a recognized influence on the physical and mechanical attributes of HAp, while concurrently, HAp contributes to the enhancement of the flexural strength of PLA, as demonstrated in earlier studies [13,14]. Previous investigations have explored the applications of PLA/HAp blends, with researchers like Hatano *et al.,* [15] conducting a comprehensive analysis of the mechanical properties of a PLA/HAp composite. This composite material was synthesized using a cost-effective and user-friendly hot-pressing technique [15]. Based on the results indicated that PLA/HAp blend consisting of 80% HAp by weight exhibited an elastic modulus similar to that observed in human cortical bone, approximately measuring 10 GPa [15].

2. Methodology

2.1 Materials

Cockle shells were obtained from local seafood restaurants near Pantai Sungai Lurus located at Senggarang, Batu Pahat. Ammonia solution and phosphoric acid purchased from supplier for the precipitation method of HAp, while distilled water was obtained from material laboratory.

2.2 Synthesize of $CaCO_3$

The work began with the extraction of $CaCO_3$ from cockle shell. Cockle shells were cleaned, rinsed and left to dry for overnight at room temperature. Dried cockle shells were crushed using a crushing machine until chip form was obtained. Chipped shells were loaded into planetary ball mill to produce a fine powder of calcium carbonate at 300rpm. $CaCO_3$ powder was sieved at 100µm.

2.3 Production of HAp

$CaCO_3$ powder was calcined at a temperature of 850℃ with desired heating rate of 10℃/min for 120 minutes before it was cooled using the furnace cooling rate to produced calcium oxide (CaO). Precipitation method was used for the conversion of CaO into HAp [16]. The method was thought to be suitable because of the low-cost production and easy to get materials [17]. CaO powder was added into a 100 ml beaker containing distilled water and later under a constant stirring using magnetic stirrer. After 10 min constant stirring, a mixture of ammonia, NH_4 (800ml) and phosphoric acid, H_3PO_4 (4 ml in 30 ml distilled water) was added gradually dropwise and stirred at room temperature for 1 hour. The solution was undergoing the aging treatment for 24 h and produced milky white HAp. The solution was dried in the drying oven at 100℃ for 24h, and then calcined at 900℃ for 2 hours [18].

2.4 Chemical Characterization

SEM, EDX, and FTiR analyses were performed to assure the purity of $CaCO_3$ that is synthesized from the cockle shells. CaO and HAp produced also was tested for its properties. The results from these analyses were compared with commercial HAp.

2.4.1 Scanning electron microscope (SEM)

This research uses SEM equipment with a model from Jeol, Japan. Sputter coating the samples before working with SEM is necessary to obtain a good-quality SEM image. The surface morphology of the powder sample was observed on SEM operated under a low vacuum at an accelerating voltage of 15kV.

2.4.2 Energy dispersive x-ray spectroscopy

EDX is an analytical technique used to identify and quantify the elemental composition of materials. It works by detecting X-rays emitted from a sample when it is bombarded with a high-energy electron beam, typically within a SEM. DX analysis provides valuable information about the chemical composition, distribution, and concentration of elements within the sample.

2.4.3 Fourier transform infrared spectroscopy

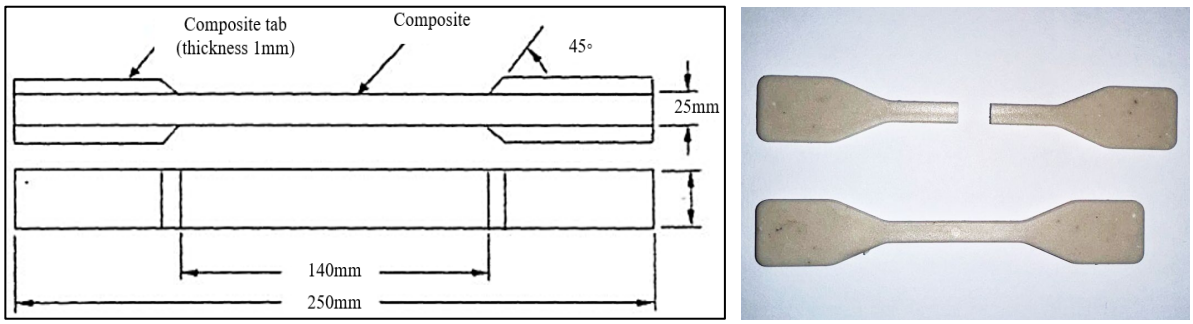
FTIR Analysis is an analytical technique for identifying organic, polymeric, and inorganic materials. This analysis identifies and characterizes unknown materials, contamination on or in a material, and additives after extraction from a polymer matrix, including identifying oxidation, decomposition, or uncured monomers in failure analysis investigations.

2.5 Sample Preparation

To make composite samples, HAp powder and PLA pellets were combined using a Brabender mixing machine at the temperature of 170°C. These samples were made in three different ratios using the following combinations: 1) 10% HAp with 90% PLA, 2) 20% HAp with 80% PLA, and 3) 30% HAp with 70% PLA. Following that, the materials were crushed to produce the appropriate shape. The composite samples were formed into the proper forms for tensile test using an injection molding machine.

2.6 Mechanical Testing

Mechanical test of the composite is done to decide the quality of the composite material. As such, tensile test using ASTM D3039 was performed on the samples. Tensile tests are carried out to measure the force required to break a plastic sample and to determine to what extent the samples stretch and elongate to the specific breaking point. The test was repeated five times for each sample ratio and will be finalized by the average value for each calculation. The most common sample for ASTM D3039 is a constant rectangular cross-section, 25 mm wide and 250 mm long as shown in Figure 1.



(a) (b)

Fig. 1. Tensile test sample as per ASTM D3039 standards (a) Sample dimensions (b) Tensile sample

3. Results

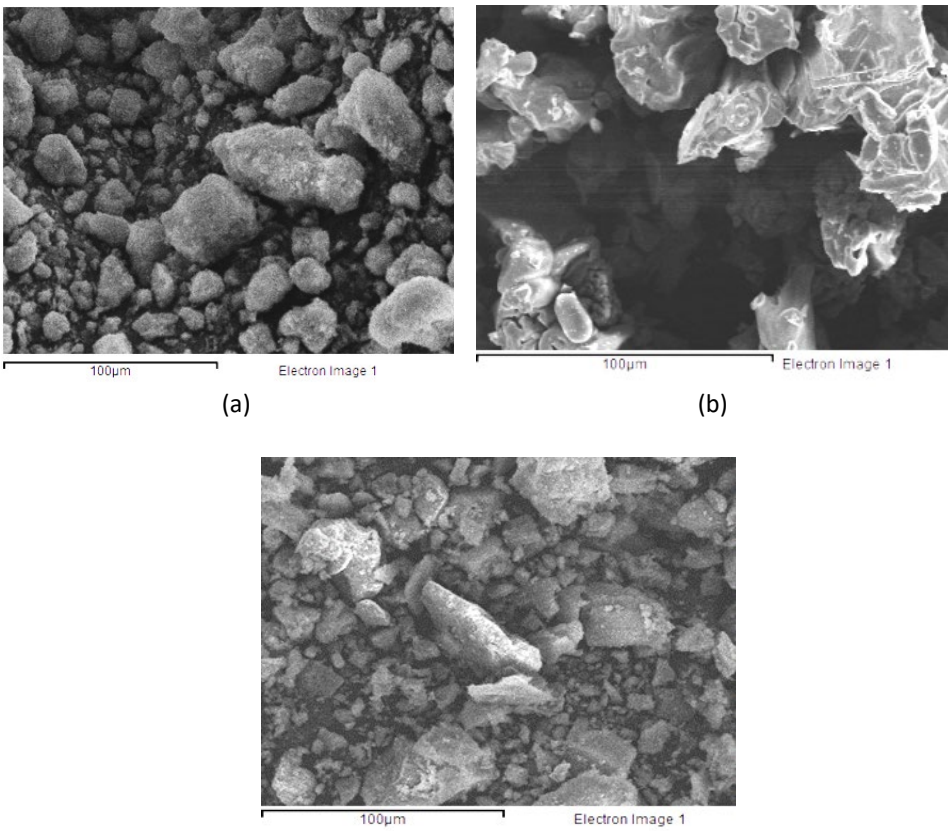
3.1 Micro-Structural Analysis of Sample

Prior to the analysis, samples were coated with platinum or gold to obtain a good quality of SEM images. The image in Figure 2(a) shows the morphology and crystal structure of $CaCO_3$. Figure 2(b) shows the morphology of CaO and Figure 2(c) of HAp. The morphological structure of $CaCO_3$ in Figure 2(a) was defined by a cube-like form, which is typical of calcite [19]. $CaCO_3$ is usually found in three polymorphs in its crystalline form: calcite, aragonite, and vaterite. Different synthesis conditions result in these various morphological changes [19,20]. Calcite and aragonite have distinct crystal development processes and crystal shapes. Analyzing the crystal structure reveals that the nucleation process for calcite is more difficult than that of aragonite. It's also worth noting that rod-shaped crystals are fundamentally less stable than their cube-like cousins. Cube-like crystals were chosen especially for applications involving bone regeneration due to their greater stability. The selection of crystal structure plays a pivotal role in determining the suitability of $CaCO_3$ for specific applications, with cube-like calcite crystals being favored for their advantageous stability in the context of bone regenerative procedures.

The surface of the waste cockle shell after calcination producing CaO in Figure 2(b) displayed a significantly smoother texture, characterized by an irregular shape, and featured instances where particles adhered together to create larger aggregates. These observations align with the findings of Sun *et al.,* [21] in 2008, which nearly spherical grain shapes, along with some inter-grain neck growth, were outcomes of the sintering phenomenon occurring during the calcination process.

In Figure 2(c), the SEM analysis of HAp synthesized from cockle shells was observed. The images reveal that the synthesized HAp particles exhibit a nearly uniform shape and size. The natural shell, in contrast, exhibits a characteristic layered structure [22]. As the calcination temperature is increased from 700 to 1,000℃, there is a notable transformation in the microstructure of the natural shell, transitioning from a layered architecture to a porous structure [23]. Hence, the reason why

cockle shells were calcinated at 850℃. Following the calcination process, waste shells displayed irregular shapes, and some of them aggregated or bonded together [24]. This indicates that the calcined shells did not maintain their original form and shape, and instead, they formed irregular conglomerations.



(a) (b)

(c)

Fig. 2. SEM analysis of (a) $CaCO_3$ (b) CaO (c) Synthesized HAp

Figure 3 shows the EDX analyses of $CaCO_3$, CaO and synthesized HAp. The alteration in the material's chemical composition was evident when transitioning from the primary elements of cockle shells, namely calcium (Ca), carbon (C), and oxygen (O), as in Figure 3(a) for $CaCO_3$. This shift was observed in Figure 3(b) with derived CaO, which exhibited Ca and O as its predominant components. In Figure 3(c), for the HAp, the EDX analysis affirmed the presence of calcium (Ca), phosphorus (P) and oxygen (O) in the HAp powder. In terms of elemental analysis, the EDX results highlight the prominence of Ca and P as the primary elements, which are key constituents of HAp, a significant finding in understanding the composition of synthesized HAp.

The characterization of the $CaCO_3$ powder obtained from cockle shells was accomplished through FTIR analysis, as presented in Figure 4. The FTIR spectrum displayed specific peaks indicative of the crystalline phases present. Notably, a peak range spanning from 1460 cm^{-1} to 1473 cm^{-1} was identified, attributing these spectral features to the characteristic vibrational frequencies associated with aragonite. Another distinct peak range between 860.72 cm^{-1} and 860.78 cm^{-1} was observed, firmly establishing the identity of the powder as pure aragonite. Additionally, the FTIR analysis revealed the presence of peaks in the proximity of 718.43 cm^{-1} to 718.81 cm^{-1}, serving as evidence of the coexistence of calcite and vaterite within the powder. This detailed FTIR analysis provided essential insights into the different crystalline phases found in the $CaCO_3$ powder derived from cockle shells, confirming the presence of aragonite as well as the co-occurrence of calcite and vaterite.

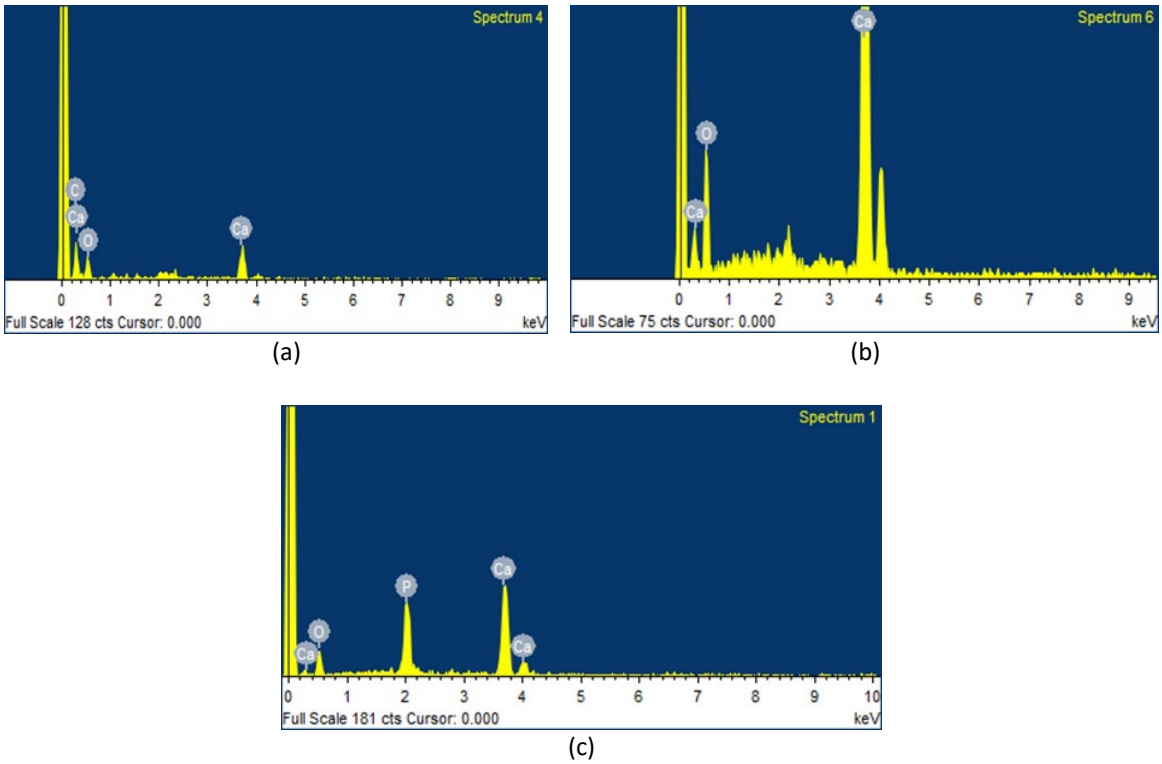


Fig. 3. EDX analysis of (a) $CaCO_3$ (b) CaO (c) synthesized HAp

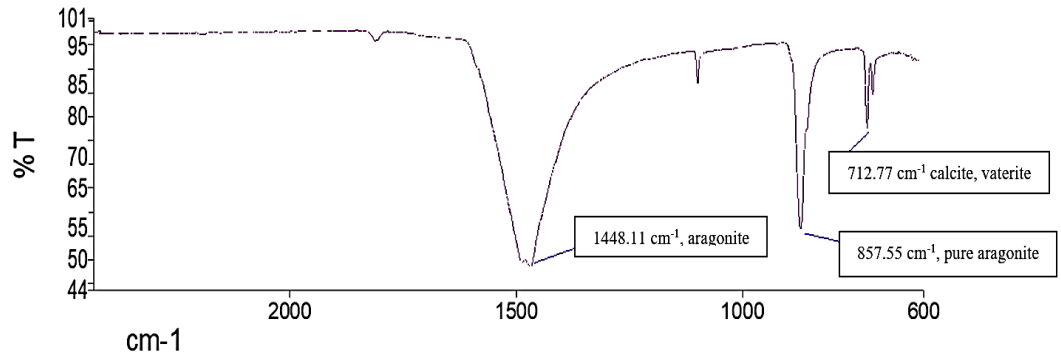


Fig. 4. FTIR analysis spectrum of $CaCO_3$ powder

The phase of CaO powder in Figure 5, which was calcinated at temperatures of 850 ℃ shows a peak range of 1404 cm^{-1} in graphs, indicating aragonite presence in the samples while the sharp band at 711.34 cm^{-1} is related to Ca-O bonds. The absorption peak of calcite at 871.24 cm^{-1} can be observed which indicates the presence of calcite after the calcination process [25].

The FTIR spectroscopy analyses to the HAp of synthesis result on the sintering temperature of 850℃ where specific spectrum patterns for P-O adsorption of HAp can be seen in Figure 6. HAp produced has the C-O band presented at 1418 cm^{-1} and 872 cm^{-1}. Meanwhile, bands 1023 cm^{-1} was corresponded with phosphate band while hydroxyl band can also be seen at 3283 cm^{-1}. The result was compared with commercial medical grade HAp where the C-O band was seen at 1450 cm^{-1} and 873 cm^{-1}. A P-O band was presented at 1020 cm^{-1} indicated the presence of phosphate.

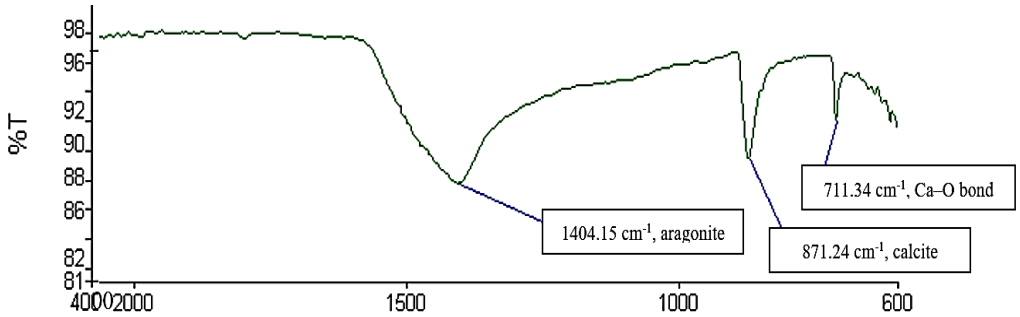


Fig. 5. FTIR analysis spectrum of CaO powder

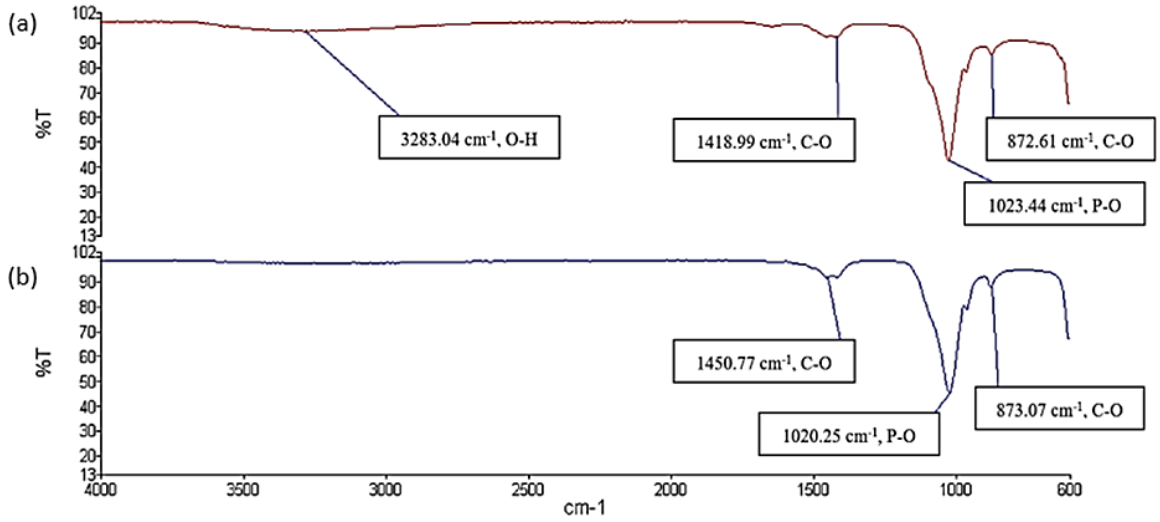


Fig. 6. FTIR analysis spectrum comparison (a) Synthasized HAp (b) Commerci4al HAp powder

3.2 Mechanical Testing

Young's modulus, often referred to as a material's elastic modulus, characterizes its ability to regain its original shape when subjected to external forces. Young's modulus of the composite samples should theoretically increase with higher weight percentages of HAp. In existing literature, a wide range of Young's modulus values for cortical bone (7 to 30 GPa) has been reported. These values exhibit variations based on measurement techniques, parameters, bone sources, and structural differences within bone samples from the same source [26].

In Table 1, the tensile test results display Young's Modulus values corresponding to varying weight percentages of Hydroxyapatite (HAp). Notably, sample S1, composed of 10% HAp and synthesized at a ratio of 850°C, exhibited a lower maximum force compared to S2 and S3, with Young's Modulus value of 7.79 GPa. S2, comprising 20% HAp, generated a maximum force of 317.80 N and had a Young's Modulus of 7.97 GPa, while S3, containing 30% HAp, produced a maximum force of 284.20N and possessed the highest Young's Modulus value of 8.17 GPa. Figure 7 shows the young's modulus and tensile strain results presented in graph. Notably, all the samples, characterized by varying HAp/PLA ratios, consistently maintained Young's Modulus values that fell within the range of Young's Modulus values typical for cortical bone (7 to 30 GPa), affirming their potential suitability for bone-related applications.

Based on prior research findings, it was noted that there is a systematic decline in both material properties as the filler content increases. This phenomenon can be attributed to the substantial agglomeration of HAp particles within the PLA matrix, resulting in a reduction in contact area and the formation of physical defects within the composite samples. This effect was visually confirmed

through SEM observations. The weakened tensile properties observed are thus likely a consequence of this agglomeration-induced structural compromise [27].

Upon analysis, it becomes evident that the increment in weight percentage of HAp exerted an influence on the strength of the materials. Consequently, as the weight percentage of HAp increased, there was a reduction in the strength of the composites. This decline in strength values observed across the various samples indicates a tendency towards greater brittleness and decreased material resilience.

Table 1
Tensile test result

Sample	Max force (N)	Tensile stress, σ (MPa)	Tensile strain, ε (%)	Young's modulus, E (GPa)
S1	317.43	39.68	4.76	7.79
S2	295.45	36.93	4.77	7.97
S3	284.20	35.53	4.48	8.17

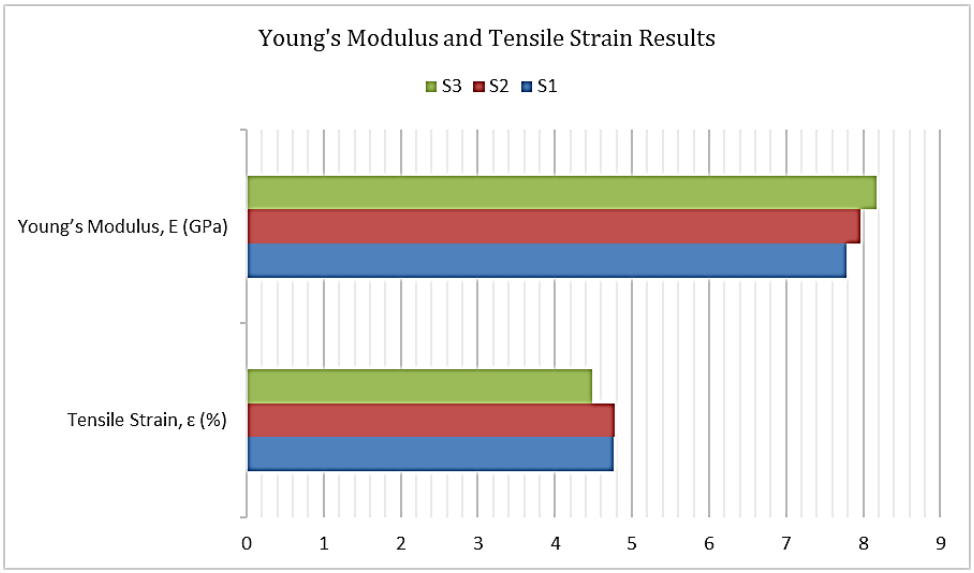


Fig. 7. Young's modulus and tensile strain results

4. Conclusions

In conclusion, this study involves the application of mechanical testing to assess material strength and the efficacy of HAp extracted from $CaCO_3$ as a bioceramic material for promoting the deposition of Ca and P minerals to facilitate new bone formation. In addition to confirming the suitability of the sample's elemental composition for biomedical applications, the study also ensures that the mechanical properties align with the criteria for mimicking the properties of the host bone and facilitating effective load transfer, particularly when subjected to external forces, as indicated by previous research.

FTiR analysis that has been done upon the samples shows that all samples are HAp with the presence of Ca and P. The same peaks appeared as the materials shows that the sample can be used as biomaterials in biomedical fields. The elements contained in the samples such as O, C and Ca which are important elements to be given attention in the medical field. Ca is known to help build strong teeth and bones, help blood vessels to regulate blood flows and contraction of body muscles as proven by Morris [28].

The tensile test was conducted to determine the force required to break a plastic sample specimen and measure its elongation until it reaches the breaking point or maximum force. The

values of Young's modulus for all samples obtained through the tensile test are nearly the same as the value for cortical bone (7 to 30 GPa), indicating their potential suitability for biomedical applications. A significant finding in this study is that the higher the Young's modulus value, the stiffer the material. Among the samples, Sample 3 has the highest Young's modulus value, indicating it is the stiffest; however, it exhibits a lower maximum force compared to Sample 2 and Sample 1. Additionally, the study identified the maximum tensile strength of each sample, providing crucial insights into their mechanical performance under stress.

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