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RESEARCH ARTICLE

IMPACT OF BIOWASTE PORE-FORMERS ON THE PROPERTIES OF MULTI-DOPED CARBONATED HYDROXYAPATITE SCAFFOLDS

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Abstract. Multi-doped carbonated hydroxyapatite, such as MgCoSr-CHA powders (md-CHA), has been developed as a potential alternative material for bone regeneration applications. This is due to their strong affinity for natural bone and good bioactivity, biocompatibility, and osteoconductivity properties. This study investigates the effect of incorporating biowaste as a pore-forming agent (BWPFA) on the structural and mechanical properties of md-CHA scaffolds. BWPFAs used in this study are (1) Empty fruit bunch (EFB) and (2) soybean hulls (SBH). Each BWPFA was milled for 3 hours, sieved to 150 μm, and mixed with md-CHA powders in a fixed ratio of 80:20. The scaffolds were then sintered at 800 °C to solidify the structure and ensure the complete removal of the BWPFA. Various techniques were employed, including X-Ray Diffraction (XRD) analysis, Fourier-Transform Infra-Red spectroscopy (FTIR), Field Emission Scanning Electron Microscopy with Energy-Dispersive X-ray spectroscopy (FESEM/EDX), density and porosity test, and diametral tensile strength (DTS) to characterize the scaffolds. Results confirmed that the scaffolds retained a single-phase CHA with a hexagonal crystal structure, and FTIR analysis verified the presence of B-type CHA. Adding BWPFA in the scaffold effectively creates scaffolds with an open pore structure with an optimal pore size of 200-300 μm, meeting the ideal bone scaffold's requirements. Interestingly, different BWPFAs resulted in distinct pore shapes: elongated pores in EFB scaffolds and subrounded pores in SBH scaffolds, despite the initial particle size of EFB and SBH being similar. Scaffolds with SBH exhibited the highest porosity (15%), followed by EFB (13%), while the control had the lowest (5%). As porosity increased, the DTS value decreased, with the control showing the highest strength (1.86 MPa), followed by EFB (1.09 MPa) and SBH (0.65 MPa).

Keywords: Carbonated hydroxyapatite, biowaste, empty fruit bunch, soybean hull, bone scaffold.

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1. INTRODUCTION

Biomaterials, particularly bioceramics, are engineered substances designed for clinical applications owing to their durability and the ability to integrate with tissues. Bioceramics, particularly those made from calcium phosphate, are widely utilized in bone regeneration and repair. 'Biomaterial' describes any substance or blend of natural or synthetic materials that interacts with living things [1]. Bioceramics are "ceramic materials that are biocompatible and used in medical or dental applications to replace or repair hard tissues, such as bone and teeth" [2].

Bioceramic scaffolds, such as those made from hydroxyapatite and beta-tricalcium phosphate, have been used to mimic bone structure and replicate mechanical strength [3]. Due to their osteoconductivity, biocompatibility, and similarity to bone composition, these materials have been widely employed for bone regeneration. For instance, they have been used as coatings on metal implants to improve biocompatibility and serve as tissue substitutes. Various processes, such as freeze-casting, gas-foaming, sol-gel, gel-casting and CAD/CAM procedures, are employed to fabricate bioceramics, particularly for applications in dentistry and orthopaedics [4].

Carbonated hydroxyapatite (CHA) is a modified form of hydroxyapatite (HA), a mineral predominantly made of calcium apatite. The most common chemical composition of HA is $Ca_5(PO_4)_3(CO_3)(OH)$. This compound is similar to hydroxyapatite but with carbonate ions replacing some phosphate ions. Carbonated hydroxyapatite is more significant as it contains 2-8wt% carbonate, similar to that found in hard tissues such as bones and teeth [4].

Since CHA is biocompatible and promotes cell proliferation, it can be used in bone tissue engineering as bioceramic scaffolds. CHA can be produced from oyster shells and combined with polyethylene oxide and honeycomb which enhances the scaffolds properties. The scaffold's micropores, which are impacted by the concentration of CHA, enhance permeability and promote cell migration, making CHA a potentially useful substance for bone regeneration applications [5].

Pore formers (or porogens) are materials used in scaffold engineering to create pores within scaffolds. High porosity scaffolds with interconnected pore networks which are necessary for cell seeding and nutrient diffusion throughout the scaffold, are largely made possible by pore formers. This is essential for promoting tissue integration and cell proliferation [6]. Pore formers aid in modifying scaffolds' mechanical characteristics to better resemble the target tissues. This is crucial for preserving the scaffold's integrity while tissue forms and making sure it can support physiological demands [7]. While pore formers significantly enhance scaffold properties, challenges remain in optimizing their use to balance porosity with mechanical strength and biocompatibility. Selecting appropriate pore formers and fabrication techniques is crucial to meeting the specific needs of various tissue engineering applications. Additionally, ongoing research is focused on developing new materials and methods to improve scaffold performance further and expand their clinical applications [8].

Biowastes have become an essential resource as pore formers in producing porous materials, providing a sustainable and economical alternative to conventional techniques. These materials are increasingly used in various applications, including environmental remediation, energy storage, and adsorption processes [9]. The utilization of biowastes not only reduces environmental impact but also enhances the performance of the resulting porous materials. Collagens, gelatin, and chitosan—biological waste products from the food industry—have been converted into scaffolds for tissue engineering and medication administration. These eco-friendly materials promote tissue regeneration and provide a long-term waste management solution [10].

Empty fruit bunch (EFB) is a major by-product of the palm oil industry, particularly in countries such as Malaysia, one of the leading palm oil producers. By supplying renewable resources for a range of businesses, EFB solves waste management problems and advances sustainability. When producing porous carbons which are necessary for uses like carbon dioxide capture, palm empty fruit bunch (EFB) acts as a sustainable and efficient pore former. In addition to producing porous structures, the pyrolysis

of EFB improves the material's hydrophilic nature through further alterations such hydrogen reduction and phenol treatment [11].

Soybean hulls (SBH) have multiple uses in many industries because of their composition and characteristics. Their main applications include the production of carboxymethyl cellulose, feed ingredients, biotechnological process substrates, and food product ingredients [12]. Soybean hulls, when carbonized and activated, can form a hierarchically porous carbon structure with pores ranging from 1.4 nm to 1000 nm in size. This results in a total pore volume of 0.731 cm^3/g with a high particular surface area of 1455.41 m^2/g, which is advantageous for adsorption and filtration applications [13]. Many techniques, including crusting and three-dimensional printing, have been established for fabricating scaffolds derived from soy. These techniques leverage the self-assembling characteristics of soy biomolecules to construct a three-dimensional matrix conducive to cellular proliferation [14].

Bone tissue engineering requires scaffolds with high bioactivity, biocompatibility, and osteoconductivity, but achieving an optimal balance between porosity and mechanical strength remains a challenge. Conventional pore-forming techniques often rely on synthetic materials, which may lack sustainability and biocompatibility. Biowaste-derived pore-forming agents (BWPFA) offer a promising alternative; however, their impact on scaffold properties, particularly in multi-doped carbonated hydroxyapatite (md-CHA) scaffolds, is not well understood. This study aims to fabricate porous md-CHA scaffolds using empty fruit bunch (EFB) and soybean hulls (SBH) as BWPFA, characterize their structural and chemical composition using XRD, FTIR, and FESEM/EDX, evaluate their porosity and mechanical properties, and analyze the effects of different BWPFA on pore morphology to determine their suitability for bone scaffold applications.

2. MATERIALS AND METHODS

2.1 Preparation of Powders

Multi-doped carbonated hydroxyapatite (md-CHA) was initially synthesized using a nanoemulsion assisted by ultrasonication method synthesized at room temperature. Calcium nitrate tetrahydrate, $Ca(NO_3)_2.4H_2O$ and di-ammonium hydrogen phosphate $(NH_4)_2HPO_4$ acted as the sources of calcium (Ca^{2+}) and phosphate (PO^{3-}_4) ions. $(NH_4).HCO_3$) or more commonly known as ammonium bicarbonate, $Sr(NO_3)_2$ or else known as strontium nitrate, $Mg(NO_3)_2.6H_2O$, which stands for magnesium nitrate hexahydrate and $Co(NO_3)_2.6H_2O$ or else known as cobalt nitrate hexahydrate were used as sources of carbonate (CO_3^{2-}), strontium (Sr^{2+}) ions, magnesium (Mg^{2+}) and cobalt (Co^{2+}). To promote CHA formation and maintain a single-phase HA, sodium hydroxide (NaOH) was used to stabilize the pH at 11 [15]. A nanoemulsion process was then conducted by treating the sample with ultrasonic treatment with a frequency of 360 kHz. An agate mortar and pestle were employed to crush the sample, which is then sieved through a 90 µm sieve for further refinement.

2.2 Preparation of Biowaste Pore Formers

Empty Fruit Bunch (EFB) and soybean hulls (SBH) were first obtained from a palm oil factory in Nibong Tebal, Penang and a tofu production factory in Tanjung Sepat, Selangor. The EFB and SBH were initially washed by stirring them in 1 liter of deionized (DI) water in a beaker for 30 minutes to remove impurities, such as soluble salts or dust, that might interfere with the reaction. An oven was used to dry the EFB at 100 °C while the SBH was dried at 70 °C for 24 hours to eliminate residual moisture after washing. The dried EFB and SBH are subsequently situated within a milling vessel and subjected to milling for 3 hours, 30 minutes and 3 hours, respectively, at a velocity of 280 revolutions per minute. The subsequent sample is then sieved using a 300 µm sieve.

2.3 Preparation of Scaffolds

The md-CHA powder was mixed with each BWPFA separately using a roller mixer at 2 different ratios of 100:0 for control and 80:20 for each of the BWPFAs. The powder blends were subsequently compacted through uniaxial pressing at a pressure of 50 MPa, producing pellets of green bodies. The green bodies were placed in a desiccator for 24 hours to eliminate residual moisture, minimizing the spring-back effect during the sintering process.

2.4 Sintering of Multi-Doped CHA Scaffolds

The sintering process was performed using a chamber furnace (Lenton, United Kingdom) with a two-stages approach. In the first stage, pellets were heat up to 600 °C to burn off most of the BWPFA. Initially, the furnace was regulated to slow heating at a rate of 1 °C/min followed by soaking for 1 hour at 600 °C. For the second stage of the sintering, the pellets were sintered at 800 °C to remove the residual BWPFA further and form the pores at the same time densify the solid parts of the scaffolds. The heating rate was increased to 10 °C/min, followed by 2 hours of soaking and finally cooling at 10°C/min.

2.5 Characterisation Techniques

This study utilized X-ray diffraction (XRD) analysis with a Bruker D8 XRD, which assessed crystal lattice characteristics, phase homogeneity, and the dimensions of the synthesized powders. A reference pattern for Hydroxyapatite (HA), which was attained from the International Centre for Diffraction Data (#09-0432), was used to compare with the resulting data for further evaluation. PANalytical X'pert PRO was used to scan the samples at 0.02° step size within the angular range of 2θ = 20° to 70° using Cu-Kα radiation (λ=1.541Å).

Fourier-transform infrared spectroscopy (FTIR) was conducted to confirm the existence of the functional groups present. About 0.1 g of the md-CHA scaffold is added to 1 g of potassium bromide (KBr), and the mixture was pressed using a hydraulic press at a pressure of 5 MPa with a holding time of 2 minutes. The sample was scanned four time between the wavelength range 400 to 4000 cm^{-1}. Perkin Elmer Spectrum One was employed for the FTIR analysis.

The microstructure of the fabricated md-CHA scaffolds was observed using a Field Emission Scanning Electron Microscope (FESEM; Zeiss Supra™ Gemini 35 VP) with AMETEK® microanalysis system, which comes equipped with an Energy Dispersive X-Ray (EDAX) spectroscope. A gold-palladium layer coats each sample to prevent and reduce surface charging during analysis. The pore size of the sample was then determined using the built-in measurement tools in the open-source FESEM software Image J. Pores were selected manually, and the diameter of the pores were measured. Multiple measurements were taken, and the average was determined.

The porosity of the fabricated scaffolds was determined using Archimedes' Principle (ASTM C 20-00). The dry weight, suspended weight and saturated weight of the scaffolds were measured. The fabricated scaffolds were oven-dried at 90 °C for 2 hours to eliminate any residual moisture. The dry sample's weight was recorded, after which it was immersed in deionized water inside a vacuum desiccator for 2 hours to allow water to fill its pores, resulting in the saturated mass. The scaffolds' suspended and saturated masses were measured using the Sartorius Balance and Bulk Density Apparatus.

To evaluate the influence of different types BWPFAs on the diametral tensile strength (DTS) of the scaffold, a DTS test was conducted. The Universal Tensile Machine (INSTRON 3367) was employed to measure DTS at a constant strain rate of 0.5 mm/min which is based on the procedure in ASTM C1424-04. Scaffold dimensions were recorded prior to testing (13 mm x 2 mm). A disk-shaped sample was positioned between two plates and subjected to compressive loading until it fractured. 10 samples of each scaffold were tested, and the average was taken.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Analysis

Figure 1 shows the diffraction peaks of each sintered scaffolds compared to the reference pattern of hydroxyapatite (HA) (ICDD: #09-0432). It was observed that a slight shift of the diffraction pattern towards lower angles in the pattern of the multi-doped CHA. The shifting in the pattern suggests successful substitution of the strontium (Sr^{2+}), magnesium (Mg^{2+}) and cobalt (Co^{2+}) ions in the host structure. Nine different distinct peaks were traced between the scanning range of $20^{\circ} \leq (2\theta) \leq 70^{\circ}$, where the overlapping of peaks (211), (112), (300) and (202) = 32-34˚can be observed which is consistent with result obtained with previous studies done by our research group [16]. It can be observed that the substitution of Mg, Co, and Sr into the HA lattice alters the lattice energy, stabilizing the crystalline structure and facilitating larger crystal growth (Table 1). Between the different sintered scaffolds (A, B & C), there are no significant crystallinity differences indicating that BWPFA does not affect scaffold crystallinity during sintering.

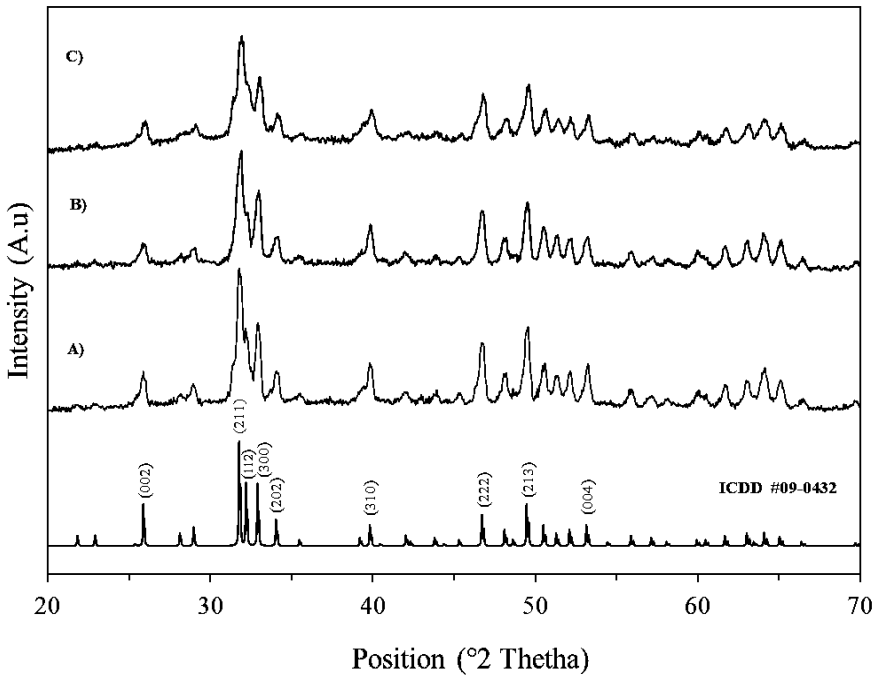


Figure 1: Results of XRD for reference pattern, **(**A) 100:0 ratio md-CHA, (B) 80:20 md-CHA+EFB and (C) md-CHA+SBH scaffolds

Table 1 : Crystallographic parameters of the reference HA and md-CHA doped scaffolds

Samples	**Phase**	**Crystal structure**	**d (Å) (I121)**	**Position (*2θ*) (I121)**	***a=b* (Å)**	***c* (Å)**	***c/a* ratio**	**Crystallite Size (nm)**
HA (09-0432)	HA	Hexagonal	-	-	9.418	6.884	0.731	-
md-CHA (100:0)	CHA	Hexagonal	0.629	32.160	9.352	6.903	0.741	8.00
md-CHA + EFB (80:20)	CHA	Hexagonal	0.627	32.255	9.329	6.910	0.741	13.32
md-+ SBH (80:20)	CHA	Hexagonal	0.803	32.305	9.368	6.923	0.739	10.2

Figure 2 shows the functional group of the md-CHA scaffolds analysed using transmittance mode. B-type md-CHA formation in the scaffolds was confirmed through analysis of the spectra, which are represented by the bands at 870-875 cm^{-1}, 1410-1430 and 1450-1470 cm^{-1} The FTIR spectra revealed PO_4^{3-} bands at 472, 565, 962, and 970 cm^{-1}, while the characteristic bands of A-type CHA, usually observed at approximately 877–880, 1500, and 1540–1545 cm^{-1}, were absent [17]. Furthermore, signals corresponding to absorbed water and hydroxyl group stretching were identified at 1600–1700 cm^{-1} and 3200–3500 cm^{-1}, respectively. It is observed that no significant differences between the functional groups in different scaffolds which indicates that the addition of the BWPFA does not interfere with the formation of B-type md-CHA.

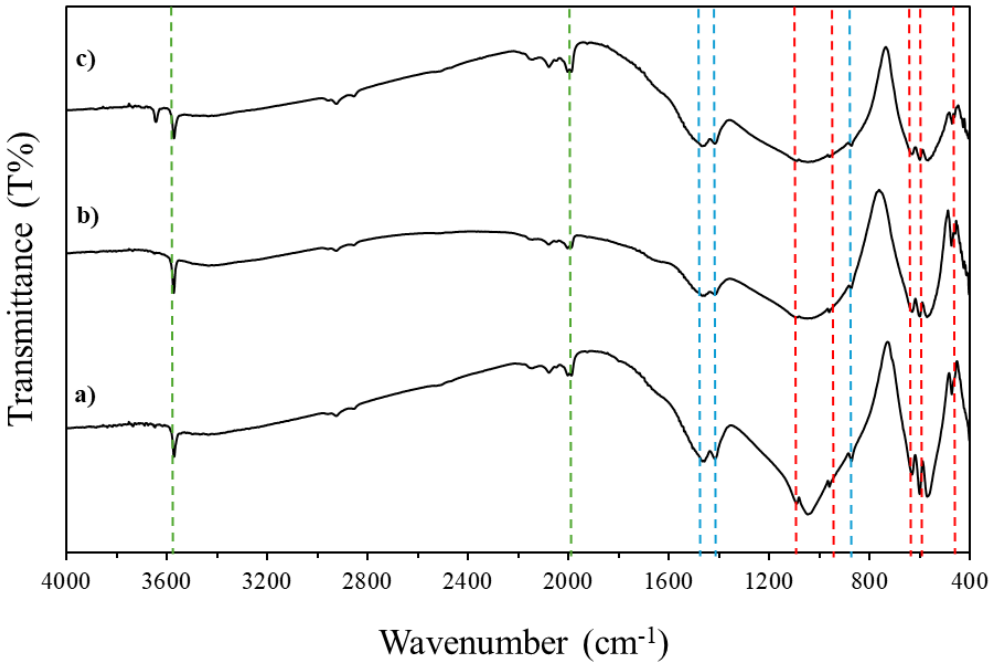


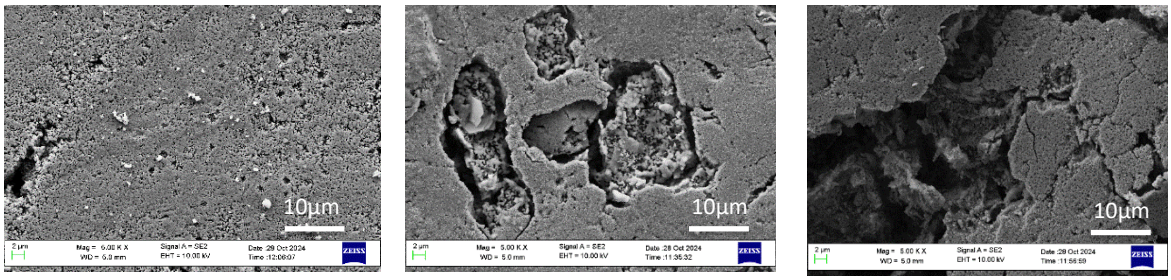
Figure 2: FTIR spectra of md-CHA scaffolds, (a) 100:0 md-CHA (control), (b) 80:20 md-CHA+ EFB and (c) 80:20 md-CHA + SBH. The OH^- group is represented in green, CO_3^{2-} in blue and PO_4^{3-} group in red dotted lines

3.2 Morphological and Elemental Analyses

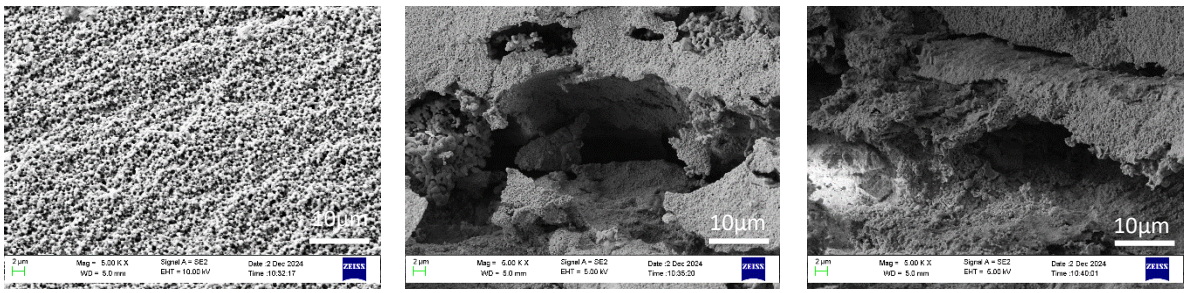
Figure 3 demonstrates that incorporating BWPFA into the scaffolds successfully created an interconnected pore structure with pores ranging from 200 to 300 µm in size, meeting the requirements for ideal bone scaffolds [18].

The cross-sectional view reveals that scaffolds with added EFB exhibit elongated pores, while those with SBH display subrounded pore shapes. In contrast, scaffolds without added BWPFA show no visible pores in their cross-sections. The interconnected pore network and specific pore shapes introduced by BWPFA could also influence the compressive strength and elastic modulus of the scaffolds, balancing porosity with mechanical stability to meet the demands of load-bearing applications. Elongated pores in scaffolds with added EFB may enhance directional cell growth and tissue ingrowth along specific axes, potentially mimicking natural bone anisotropy. Conversely, the subrounded pores in SBH-added scaffolds provide a more isotropic environment, which could uniformly distribute mechanical loads and support multidirectional tissue regeneration [19].

Surface Morphology With BWPFA (80:20 Ratio)
Without BWPFA EFB (5.0 kX) SBH (5.0 kX)
(a)



Cross Section With BWPFA (80:20 Ratio)
Without BWPFA (5.00 kX) EFB (5.0 kX) SBH (5.0 kX)
(b)



Cross Section
Without BWPFA (300 X) EFB (300 X) SBH (300 X)
(c)

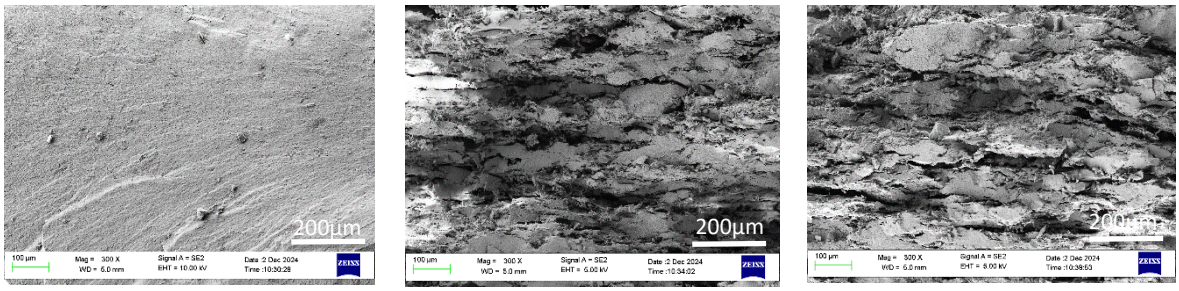


Figure 3: FESEM images of scaffolds (a) surface morphology, (b) and (c) cross section with different ratios of BWPFAs with magnification of 5kX and 300X

Table 2 shows that the scaffold incorporated with SBH exhibited (15%), followed by the scaffold with empty fruit bunch (EFB) at 13%, and the control scaffold without biowaste pore-formers at 5% [20]. This trend confirms that biowaste pore-forming agents significantly enhance porosity, which is critical for bone tissue engineering as it promotes cell infiltration and nutrient diffusion. The porosity achieved on the scaffolds is closely mimics the porosity of cancellous bones which provides a biomimetic structure suitable for tissue engineering applications [17].

Table 2: Apparent density and apparent porosity for the md-CHA scaffolds

Scaffold	Relative Density (%)	Apparent Porosity (%)	Diametral Tensile Strength (MPa)
md-CHA (100:0)	95	5	1.86
md-CHA + EFB (80:20)	87	13	1.09
md-CHA + SBH (80:20)	85	15	0.65

However, the increase in porosity comes at the expense of mechanical strength: diametral tensile strength decreases from 1.86 MPa (pure) to 1.09 MPa (EFB) and 0.65 MPa (SBH). This trade-off underscores the need to balance porosity and strength when designing scaffolds for load-bearing applications, suggesting that SBH-based scaffolds may be more suitable for non-load-bearing bone regeneration, while EFB offers a moderate compromise between porosity and strength [17].

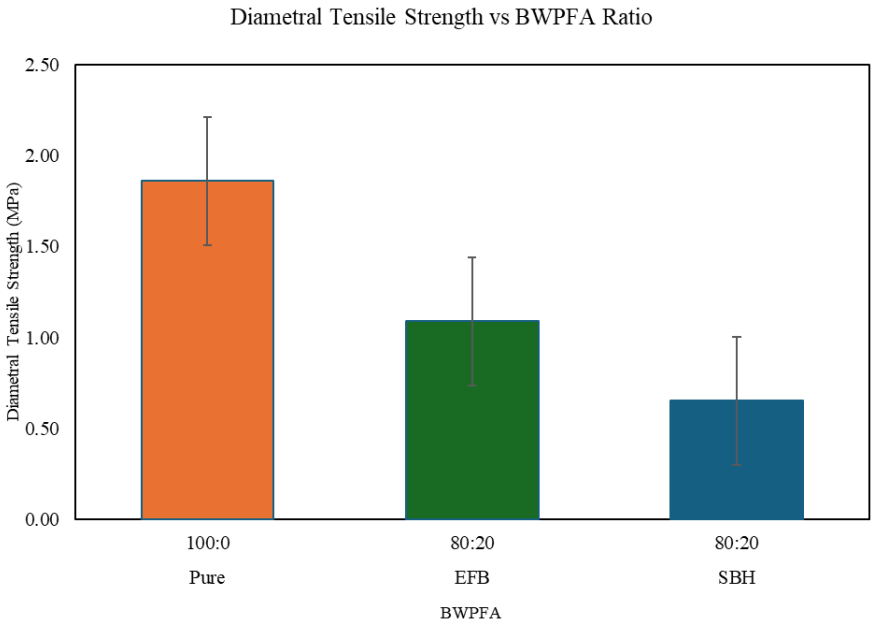


Figure 4: Diametral tensile strength of md-CHA scaffolds with different BWPFAs

4. CONCLUSIONS

In conclusion, this study demonstrates that incorporation of biowaste pore formers, in this case empty fruit bunch (EFB) and soybean husks (SBH), into multi-doped carbonated hydroxyapatite (md-CHA) matrices substantially facilitates the creation of biomimetic scaffolds characterised by porosity levels and pore sizes suitable for osseous tissue engineering purposes. The disparities in pore morphology between EFB and SBH frameworks underscore the potential for customizing scaffold characteristics to fulfil biomedical requirements, facilitating sustainable and efficacious solutions in regenerative medicine. However, some limitations remain. The mechanical strength of the scaffolds decreases with increasing porosity, which may limit their applicability in load-bearing bone repair. Additionally, the study was limited to two types of biowaste pore formers, and further exploration is needed to assess the performance of other sustainable materials. Future research should focus on optimizing the mechanical properties of these scaffolds, exploring hybrid pore formers, and evaluating

their long-term biocompatibility and *in vivo* performance to enhance their potential for clinical applications.

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Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare.

Compliance with Ethical Standards

The work is compliant with ethical standards.

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