

Physicochemical and Mechanical Characterization of Hydroxyapatite-Chitosan-PVA Composite Scaffolds Based on Marine Fish Bone Sources

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Abstract

This study aimed to develop a hydroxyapatite (HAp)-based scaffold derived from marine fish bones for bone tissue engineering applications using the freeze-drying method. HAp was extracted from bekre, tongkol, and simba fish bones through a calcination process at 800°C and subsequently characterized using XRD, FTIR, and SEM-EDX to determine the most optimal HAp sample. The characterization results showed that HAp derived from simba fish bone exhibited the best properties, with the highest crystallinity of 90.09%, lattice parameters closest to the JCPDS 09-0432 standard, and the largest crystallite size of 90.6 nm. FTIR analysis confirmed the presence of characteristic hydroxyapatite functional groups, namely PO_4^{3-} and OH^- , while EDX analysis showed a Ca/P ratio of 1.64, which is close to the stoichiometric value of HAp. Furthermore, the HAp was fabricated into an HAp/chitosan/PVA scaffold with a ratio of 3:1:1 using the freeze-drying method. The scaffold characterization results showed a crystallinity of 89.33% with a three-dimensional interconnected porous structure and pore sizes ranging from 180–220 μm . The scaffold exhibited a porosity value of 77.78% and a Ca/P ratio of 1.63. Degradation testing showed a degradation rate of 17–19% over 14 days, while mechanical testing produced an average compressive strength of 21.63 MPa and a Young's modulus of 254.777 MPa. Overall, the HAp/chitosan/PVA scaffold derived from simba fish bone demonstrated good physical, chemical, and mechanical characteristics, indicating its potential as a biomaterial for bone tissue engineering applications.

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Abstrak

Penelitian ini bertujuan mengembangkan scaffold berbasis hidroksiapatit (HAp) yang berasal dari tulang ikan laut untuk aplikasi rekayasa jaringan tulang menggunakan metode freeze-drying. HAp diekstraksi dari tulang ikan bekre, tongkol, dan simba melalui proses kalsinasi pada suhu 800 °C. Selanjutnya, HAp dikarakterisasi menggunakan XRD, FTIR, dan SEM-EDX untuk menentukan sampel HAp yang paling optimal. Hasil karakterisasi menunjukkan bahwa HAp yang berasal dari tulang ikan simba memiliki sifat terbaik, dengan kristalinitas tertinggi sebesar 90,09%, parameter kisi yang paling mendekati standar JCPDS 09-0432, serta ukuran kristalit terbesar, yaitu 90,6 nm. Analisis FTIR mengonfirmasi keberadaan gugus fungsi khas hidroksiapatit, yaitu PO_4^{3-} dan OH^- . Sementara itu, analisis EDX menunjukkan rasio Ca/P sebesar 1,64, yang mendekati nilai stoikiometri HAp. Selanjutnya, HAp difabrikasi menjadi scaffold HAp/kitosan/PVA dengan perbandingan 3:1:1 menggunakan metode freeze-drying. Hasil karakterisasi scaffold menunjukkan kristalinitas sebesar 89,33%, dengan struktur berpori tiga dimensi yang saling terhubung dan ukuran pori berkisar antara 180–220 μm . Scaffold memiliki nilai porositas sebesar 77,78% dan rasio Ca/P sebesar 1,63. Pengujian degradasi menunjukkan laju degradasi sebesar 17–19% selama 14 hari. Sementara itu, pengujian mekanik menghasilkan kuat tekan rata-rata sebesar 21,63 MPa dan modulus Young sebesar 254,777 MPa. Secara keseluruhan, scaffold HAp/kitosan/PVA yang berasal dari tulang ikan simba menunjukkan karakteristik fisik, kimia, dan

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mekanik yang baik, sehingga berpotensi digunakan sebagai biomaterial untuk aplikasi rekayasa jaringan tulang.

1. Introduction

Bone health is essential for body function, as the skeletal system provides structural support, muscle attachment, blood cell formation, protection of vital organs, and mineral metabolism regulation (Rossa, 2021). Bone disorders such as osteoporosis and fractures remain major health issues in Indonesia, particularly among the elderly. Data from the Ministry of Health of the Republic of Indonesia show that 19.7% of women and 7.3% of men over 50 are at risk of osteoporosis, while traffic accidents account for about 5.5% of injuries (Kanis, 2003). These conditions highlight the need for effective therapeutic solutions. Although bone has a natural regenerative ability through osteoblast activity, this is limited in large or critical-size defects, requiring supporting materials. Metallic implants such as titanium and stainless steel provide good mechanical strength but have drawbacks, including elastic modulus mismatch, corrosion risk, inflammatory responses, and high cost due to import dependence (Badan Penelitian dan Pengembangan Kesehatan, 2019). Therefore, scaffold-based biomaterials are a promising alternative for bone tissue engineering. Scaffolds are porous 3D structures designed to support cell adhesion, proliferation, and new bone formation. An ideal scaffold should be biocompatible, biodegradable, sufficiently porous, and mechanically compatible with bone. Hydroxyapatite (HAp), which closely resembles the inorganic mineral phase of bone, is widely used because of its excellent bioactivity and osteoconductivity (Sembiring & Rahmadhany, 2022). Natural sources, particularly marine fish bone waste, provide a cost-effective and environmentally friendly alternative for HAp production (Mahayana et al., 2024). In this study, mackerel tuna (*Euthynnus affinis*), bekre (*Amblygaster sirm*), and simba (*Epinephelus fuscoguttatus*) bones were selected as HAp sources. Differences among fish species may influence bone composition, mineral characteristics, and Ca/P ratio (Aufan et al., 2017), while their abundance in Indonesia provides a potential source of calcium phosphate from fish-processing waste (Rahmatina & Kumala, 2021).

To improve the properties of HAp-based scaffolds, HAp was combined with chitosan and poly(vinyl alcohol) (PVA). Chitosan provides biocompatibility, antimicrobial activity, and cellular support, whereas PVA contributes mechanical strength and flexibility (Davis et al., 2022; Prastyo et al., 2023). Freeze-drying was employed to produce highly porous scaffolds with interconnected structures favorable for tissue regeneration (Kassapidou et al., 2021; Kementerian Kesehatan Republik Indonesia, 2022). Previous studies have demonstrated the potential of fish-bone-derived HAp and HAp-based scaffolds for bone tissue engineering. Comparative studies have shown that fish species can affect the phase composition and physicochemical characteristics of HAp (Nam et al., 2019; Zhu et al., 2017). HAp/chitosan/PVA scaffolds fabricated using freeze-drying have also demonstrated promising properties for bone tissue regeneration (Pineda-Castillo et al., 2018), while PVA/chitosan/HAp scaffolds have shown favorable mechanical and biological properties (Januariyasa et al., 2020). However, these studies generally used a single or chemically synthesized HAp source and did not comparatively investigate HAp derived from different marine fish species. Similarly, a freeze-dried scaffold based on mackerel-bone-derived HAp and PVA has been reported (Rohania et al. 2025), but the study was limited to a single fish species and did not incorporate chitosan. Thus, a comparative investigation of HAp derived from different marine fish species and its application in HAp/chitosan/PVA scaffolds remains limited. Therefore, this study compares HAp derived from *E. affinis*, *A. sirm*, and *E. fuscoguttatus* and incorporates the resulting HAp into chitosan/PVA scaffolds fabricated using freeze-drying. The scaffolds were evaluated in terms of structural, morphological, porosity, degradation, and mechanical properties to assess their potential for bone tissue engineering applications.

2. Research Methods

2.1 Extraction of Fish Bones

Hydroxyapatite (HAp) derived from marine fish bones was obtained through an extraction process consisting of cleaning, defatting, drying, grinding, and calcination steps. The fish bones were cleaned to remove residual soft tissues, boiled at 90-100 °C for 30 min, and subsequently sun-dried for three days. The defatting process was carried out using 95% ethanol to remove lipids and residual organic compounds such as proteins and collagen. The bones were then further dried in an oven at gradually increasing temperatures up to 100 °C, followed by crushing and sieving using a 200-mesh sieve. HAp formation was achieved through calcination in a furnace at 800 °C for 3 h to obtain hydroxyapatite powder.

2.2 Fabrication of Scaffold

The fabrication of HAp/chitosan/PVA scaffolds was performed using the freeze-drying method with a composition ratio of HAp:chitosan:PVA of 3:1:1. The chitosan solution was prepared using 1% (v/v) acetic acid, while PVA was dissolved in distilled water at 80-90 °C until a homogeneous solution was obtained. Both solutions were subsequently mixed, and HAp powder was gradually added under continuous stirring until a homogeneous suspension was formed. The suspension was poured into a well plate, frozen at -20 °C for 12 h, and subsequently freeze-dried at -80 °C for 24 h to produce porous cylindrical scaffolds with a height of 1 cm and a diameter of 2 cm.

2.3 Characterization of Materials and Scaffolds

The hydroxyapatite-based scaffolds were characterized using several methods to evaluate their structural, chemical, morphological, degradation, and mechanical properties. XRD analysis was used to determine crystal phase,

crystallinity, crystallite size, and lattice parameters of HAp by comparing diffraction patterns with the JCPDS No. 09-0432 standard.

The degree of crystallinity was calculated using the following equation:

$$CI(\%) = \frac{I_{300} - I_{112}}{I_{300}} \times 100\% \quad (1)$$

The crystallite size of hydroxyapatite was determined using the Scherrer equation:

$$D = \frac{k\lambda}{\beta \cos \theta} \quad (2)$$

The lattice parameters were calculated using the interplanar spacing equation for a hexagonal crystal system as follows:

$$\frac{1}{d_{hkl}^2} = \frac{4}{3} \frac{(h^2 + hk + k^2)}{a^2} + \frac{l^2}{c^2} \quad (3)$$

FTIR analysis was used to identify functional groups and chemical bonds in HAp and scaffold composites, including PO_4^{3-} and OH^- groups of HAp, as well as amide, C-O, and O-H/N-H groups from chitosan and PVA. SEM was used to analyze scaffold morphology, including pore structure, distribution, interconnectivity, and porosity, while pore size analysis was conducted using ImageJ software. The porosity was calculated using the following equation:

$$\text{porosity} (\%) = \frac{w_s - w_d}{w_s - w_i} \times 100\% \quad (4)$$

In addition, Energy Dispersive X-ray (EDX) analysis was conducted to determine the Ca/P ratio as an indicator of the compositional suitability of hydroxyapatite.

The degradation test was carried out by immersing the scaffold samples in phosphate-buffered saline (PBS) solution at 37 °C for 14 days to evaluate material stability under physiological conditions. The degradation percentage was calculated based on the change in scaffold mass before and after immersion using the following equation:

$$\text{Degradation} (\%) = \frac{w_o - w_t}{w_o} \times 100\% \quad (5)$$

The mechanical properties of the scaffolds were analyzed using a Universal Testing Machine (UTM) through a compression test. This test was conducted to determine the compressive strength of the scaffold and evaluate its suitability relative to the mechanical characteristics of natural trabecular bone.

3. Results and Discussion

3.1 Results of Characterization from Fish Bone Sources

The calcination process produced white-colored ash from the three fish bone sources, as shown in Figure 1. The resulting ash was subsequently used for further characterization to evaluate its potential as a hydroxyapatite (HAp) source.



Figure 1. Furnace results of (a) simba fish, (b) mackerel tuna, and (c) bekre fish bones

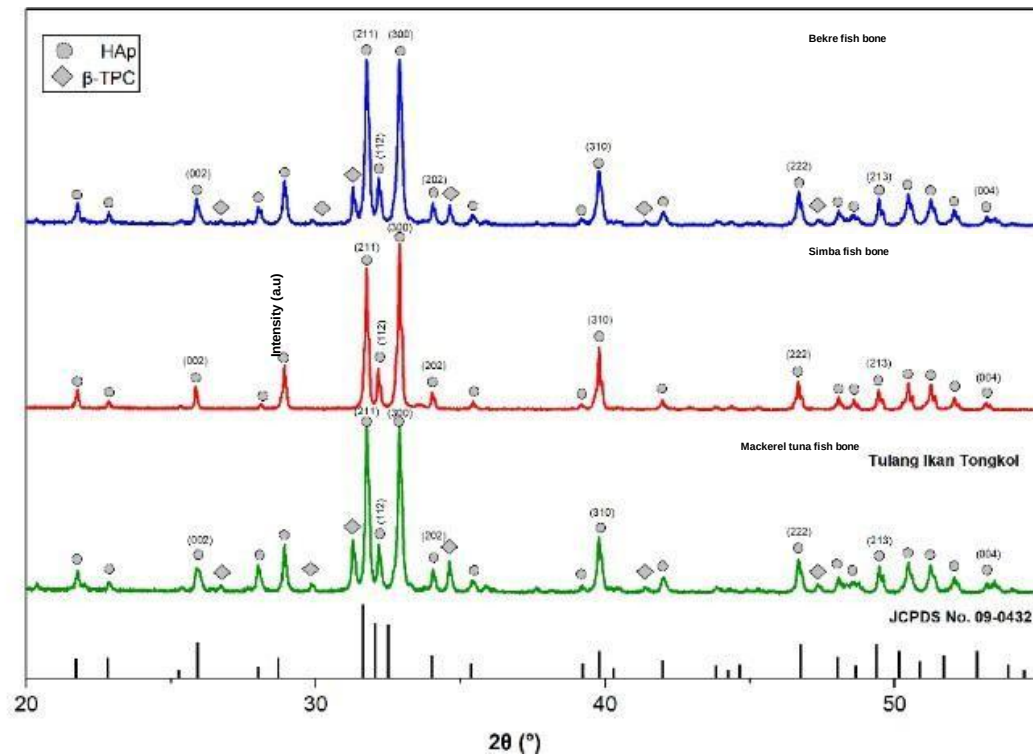
The furnace-treated samples appeared white with a sand-like texture. The extraction effectiveness was quantitatively evaluated by comparing the initial and final weights of each sample after calcination using the following equation:

$$\text{Effectiveness}(\%) = \frac{\text{Final weight}}{\text{Initial weight}} \times 100\% \quad (6)$$

Table 1. Extraction Effectiveness Results

Fish Bone Type	Initial Weight (g)	Final Weight (g)	Effectiveness (%)
Mackerel Tuna	60	20.55	34.25
Simba	60	20.20	33.67
Bekre	60	21.00	35.00

Bekrek fish bones showed the highest extraction yield, but the samples were less dry compared to mackerel tuna and simba fish bones, indicating possible residual organic content or incomplete calcination. In contrast, mackerel tuna and simba samples exhibited better dryness, suggesting more effective removal of organic materials. This shows that extraction yield alone does not determine HAp quality, but also depends on material characteristics after calcination. Characterization using XRD, FTIR, and SEM-EDX was performed to evaluate the suitability of hydroxyapatite (HAp) for scaffold applications.

**Figure 2.** XRD patterns of bekre, simba, and mackerel tuna fish bones.

The major diffraction peaks of hydroxyapatite (HAp) were observed at 2θ angles of approximately 25.9° , 31.7° , 32.2° , 32.9° , 34.0° , 39.8° , 46.7° , 49.5° , and 53.1° , which are consistent with the JCPDS No. 09-0432 standard. These results confirm the successful formation of the HAp phase in all three fish bone samples. However, additional β -tricalcium phosphate (β -TCP) phases were detected in the bekre and mackerel tuna fish bone samples, indicating partial decomposition of HAp during the calcination process (Rahmiati et al., 2025). In contrast, the absence of the β -TCP phase in the simba fish bone sample suggests better thermal stability and higher HAp phase purity compared to the other samples.

Table 2. Crystallinity, lattice parameters, and crystallite size of bekre, simba, and mackerel tuna fish bone samples.

Sample	Crystallinity (%)	Lattice Parameter		Crystallite Size (nm)
		a (Å)	c (Å)	
Bekre	88.90	9.390	6.8700	32.6
Simba	90.09	9.425	6.8840	90.6
Mackerel Tuna	89.30	9.409	6.8800	42.9

Based on the XRD results, the three fish-bone-derived HAp samples showed differences in crystallinity and lattice parameters. The Simba sample exhibited the highest crystallinity (90.09%), followed by mackerel tuna (89.30%) and Bekre (88.90%), indicating differences in crystal ordering among the samples. All samples exhibited a hexagonal crystal structure with lattice parameters close to the standard values of JCPDS No. 09-0432, confirming the formation of HAp (Purba et al., 2024). The crystallinity values obtained in this study (88.90–90.09%) are comparable to previous reports of calcined fish-bone-derived HAp, which showed crystallinity values of approximately 90–92% (Tekin & Ates, 2024). Thus, the difference between the present results and previous studies is relatively small, with the values in this study being approximately 0–3.1 percentage points lower. This variation may be attributed to differences in fish species, mineral composition, trace-element content, and calcination conditions, which can influence the crystallization behavior of biogenic HAp (Hossain & Ahmed, 2023). Overall, the comparable crystallinity values indicate that the calcination process successfully produced well-crystallized HAp from all three fish-bone sources.

Table 3. Lattice parameter ratio of bekre, simba, and mackerel tuna fish bone samples.

Sample	Lattice Parameter		c/a Ratio
	a (Å)	c (Å)	
Bekre	9.390	6.8700	0.7316
Simba	9.420	6.8840	0.7310
Mackerel Tuna	9.435	6.8800	0.7294

The simba fish bone sample showed lattice parameters and a c/a ratio closest to the JCPDS No. 09-0432 standard. All samples had a hexagonal hydroxyapatite structure with crystallite sizes within the typical range (20–100 nm), with the largest size in the simba sample (90.6 nm), followed by mackerel tuna (42.9 nm) and bekre (32.6 nm), indicating higher crystal order (Yanti & Gandhi, 2020). Overall, the simba sample exhibited the most optimal properties due to its high crystallinity, phase stability, and absence of β -TCP, making it the most promising for bone tissue engineering. FTIR analysis was then conducted to identify HAP functional groups.

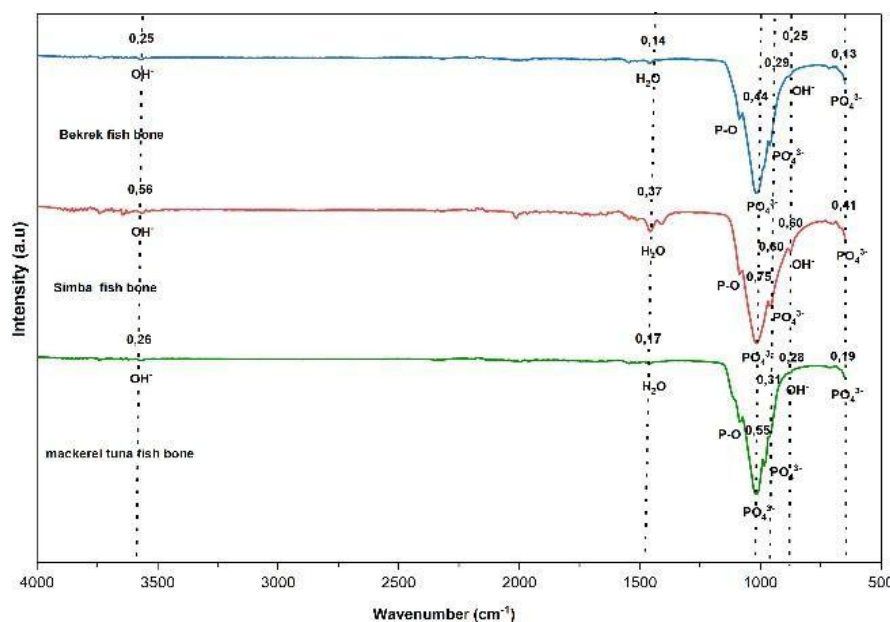


Figure 3. FTIR spectra of bekre, simba, and mackerel tuna fish bones.

FTIR analysis confirmed the formation of hydroxyapatite (HAp) in all samples through characteristic OH^- , PO_4^{3-} , and H_2O functional groups (Panda et al., 2025). The simba fish bone sample showed more intense phosphate peaks, indicating a more ordered structure and higher crystallinity. Overall, all samples successfully formed HAp with good structural characteristics.

Table 4. Summary of hydroxyapatite functional groups identified by FTIR analysis.

No.	Wavenumber (cm^{-1})	Vibration Type	Functional Group
1	3570	Stretching	OH^- (hydroxyl)
2	1630	Bending	Adsorbed H_2O
3	1030-1090	Asymmetric stretching (ν_3)	PO_4^{3-} (phosphate)
4	960	Symmetric stretching (ν_1)	PO_4^{3-} (phosphate)
5	570-605	Bending (ν_4)	PO_4^{3-} (phosphate)
6	630-635	Librational vibration	OH^- (hydroxyl)

The FTIR results were consistent with XRD analysis, confirming the successful formation of hydroxyapatite (HAp) in all samples according to JCPDS No. 09-0432. Among the samples, the simba fish bone showed more intense phosphate bands, indicating a higher crystal order. This is in agreement with XRD results, where the simba sample had the highest crystallinity, closest lattice parameters to the standard, largest crystallite size, and no β -tricalcium phosphate (β -TCP) phase. Overall, simba fish bone-derived HAp showed the most optimal properties for bone scaffold applications and was selected for further SEM-EDX analysis.

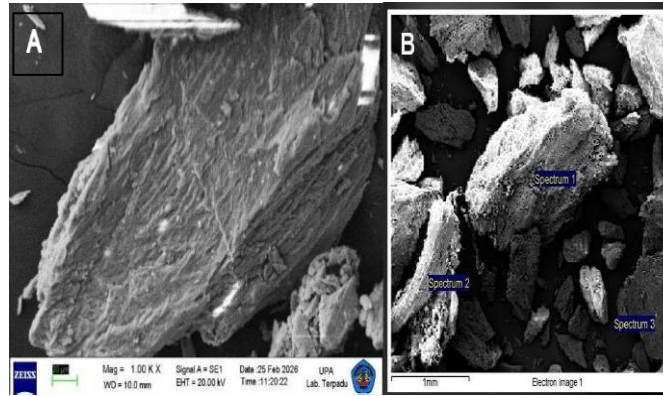


Figure 4. (A) SEM morphology of simba fish bone and (B) reference morphology of natural hydroxyapatite derived from calcined bone

The morphology of simba fish bone-derived hydroxyapatite showed typical characteristics of calcined natural HAp, including irregular particles, rough and layered surfaces, and particle agglomeration, consistent with fish bone-derived HAp morphology (Ghoneum et al., 2025). The rough surface resulted from the removal of organic components during calcination, leaving the mineral phase. These morphological features may improve cell adhesion and biological performance for bone scaffold applications. EDX analysis was also performed to determine elemental composition and Ca/P ratio as indicators of HAp suitability as a biomaterial.

Table 5. EDX results of simba fish bone hydroxyapatite.

Sample	Ca (at.%)	P (at.%)	Ca/P Ratio
Measurement 1	19.63	12.27	1.60
Measurement 2	27.90	17.55	1.59
Measurement 3	20.49	12.27	1.67
Measurement 4	23.70	13.94	1.70
Average			1.64

EDX analysis showed a Ca/P ratio of 1.64, close to the stoichiometric HAp value (1.67), indicating that the material has a chemical composition consistent with hydroxyapatite (Casimiro et al., 2021). This result agrees with XRD and FTIR findings. Finally, the extracted HAp was used to fabricate a chitosan-PVA scaffold using the freeze-drying method.

3.2 Physicochemical, Morphological, Degradation, and Mechanical Characterization of HAp/Chitosan/PVA Scaffold

The fabricated HAp/chitosan/PVA scaffold obtained using the freeze-drying method is shown in Figure 5. The freeze-drying process produced a lightweight and porous scaffold by removing the frozen solvent through sublimation, resulting in a three-dimensional structure suitable for bone tissue engineering.



Figure 5. HAp/chitosan/PVA scaffold fabricated using the freeze-drying method.

The HAp/chitosan/PVA scaffold fabricated via freeze-drying was characterized using XRD, FTIR, SEM-EDX, degradation, and compression tests to evaluate its structural, morphological, compositional, stability, and mechanical properties.

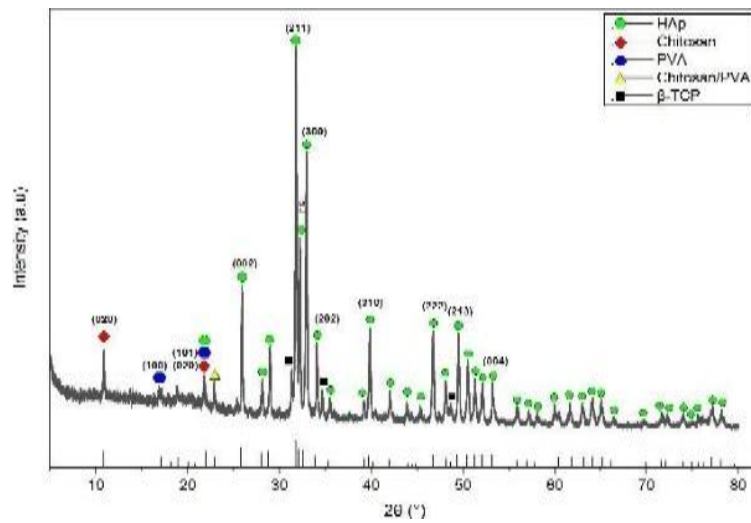


Figure 6. XRD pattern of the HAp/chitosan/PVA scaffold.

The XRD pattern confirmed the presence of hydroxyapatite (HAp) peaks, indicating that the HAp phase was maintained after scaffold fabrication (Hossain & Ahmed, 2023). Peak intensity reduction and broadening were observed due to the amorphous chitosan and PVA matrix, with successful dispersion of HAp in the polymer. A small amount of β -TCP was detected, but it did not significantly affect the main phase. Overall, the scaffold consisted of crystalline HAp and amorphous polymer phases, confirming successful composite formation.

Table 6. Crystallinity, lattice parameters, and crystallite size of the HAp/chitosan/PVA scaffold.

Crystallinity (%)	Lattice Parameter		Crystallite Size (nm)
	a (Å)	b = c (Å)	
89.33	9.398	6.872	40.8

The scaffold exhibited a crystallinity degree of 89.33%, indicating that the crystalline HAp phase was predominantly preserved after incorporation with chitosan and PVA (Wahab et al., 2020). A crystallinity value above 80% indicates a highly crystalline structure with a relatively well-ordered HAp phase, which is important for maintaining the chemical stability, bioactivity, and osteoconductive characteristics of HAp. The lattice parameters remained close to the standard HAp values, confirming that the addition of chitosan and PVA did not substantially alter the HAp crystal structure. The crystallite size was 40.8 nm, which remained within the typical nanoscale range reported for HAp. Compared with the initial HAp samples in this study (88.90 - 90.09%), the scaffold showed a similar crystallinity of 89.33%, indicating that the incorporation of chitosan and PVA caused only a slight change in HAp crystallinity. This value is also comparable to previous studies reporting highly crystalline HAp-based scaffolds, indicating that the HAp crystalline phase was well maintained after scaffold fabrication (Cucuruz et al., 2023; Aminatun, 2024). The slightly lower or comparable crystallinity may be attributed to interactions between HAp and the polymer matrix, which can partially restrict crystal growth. Overall, the high crystallinity of 89.33% confirms that the HAp structure was effectively retained in the HAp/chitosan/PVA scaffold after freeze-drying.

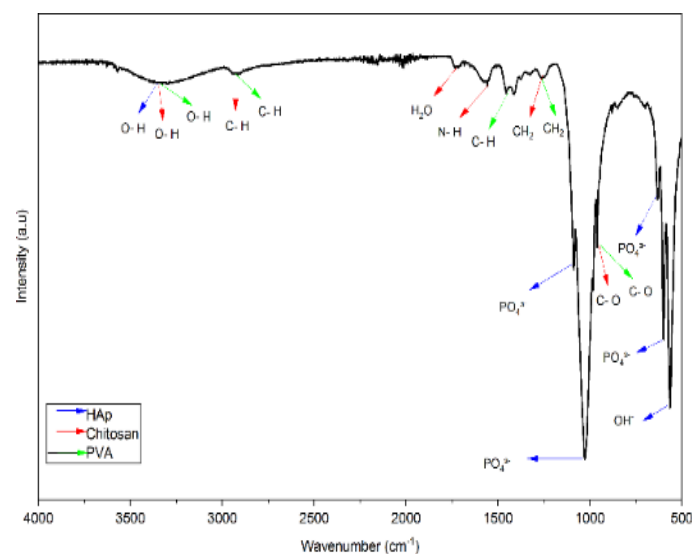


Figure 7. FTIR spectrum of the HAp/chitosan/PVA scaffold.

FTIR analysis confirmed the presence of hydroxyapatite, chitosan, and PVA in the scaffold (Azaman et al., 2022). Characteristic bands of O-H, phosphate (PO_4^{3-}), amide, and polymer chains were observed, indicating hydrogen bonding and good chemical interaction between components. Overall, the results confirmed successful formation of the HAp/chitosan/PVA composite scaffold with good chemical compatibility.

Table 7. Summary of functional groups identified in the HAp/chitosan/PVA scaffold.

Wavenumber (cm^{-1})	Vibration Type	Functional Group	Compound Source
3200-3500	Stretching	O-H (hydroxyl)	HAp, Chitosan, PVA
2900	Stretching	C-H	Chitosan, PVA
1630	Bending	Adsorbed H_2O	Water (surface adsorption)
1550	Bending (Amide II)	N-H	Chitosan
1400-1450	Bending (deformation)	CH_2	Chitosan, PVA
1200-1300	Stretching	C-O	Chitosan, PVA
1000-1100	Asymmetric stretching (ν_3)	PO_4^{3-}	Hydroxyapatite (HAp)
960	Symmetric stretching (ν_1)	PO_4^{3-}	Hydroxyapatite (HAp)
570-605	Bending (ν_4)	PO_4^{3-}	Hydroxyapatite (HAp)
630	Librational vibration	OH^-	Hydroxyapatite (HAp)

Overall, the FTIR analysis demonstrated that all constituent components of the scaffold, including hydroxyapatite, chitosan, and PVA, were clearly identified through the presence of their characteristic absorption bands. These results confirm that the fabricated scaffold is not merely a physical mixture, but rather a homogeneous composite structure with good chemical integration among its components. Such characteristics are important for enhancing the scaffold's potential in bone tissue engineering applications. SEM-EDX analysis of the scaffold was subsequently performed to evaluate its morphology, pore interconnectivity, pore size, pore distribution, and porosity. The morphology and pore interconnectivity of the scaffold can be observed in the following figure.

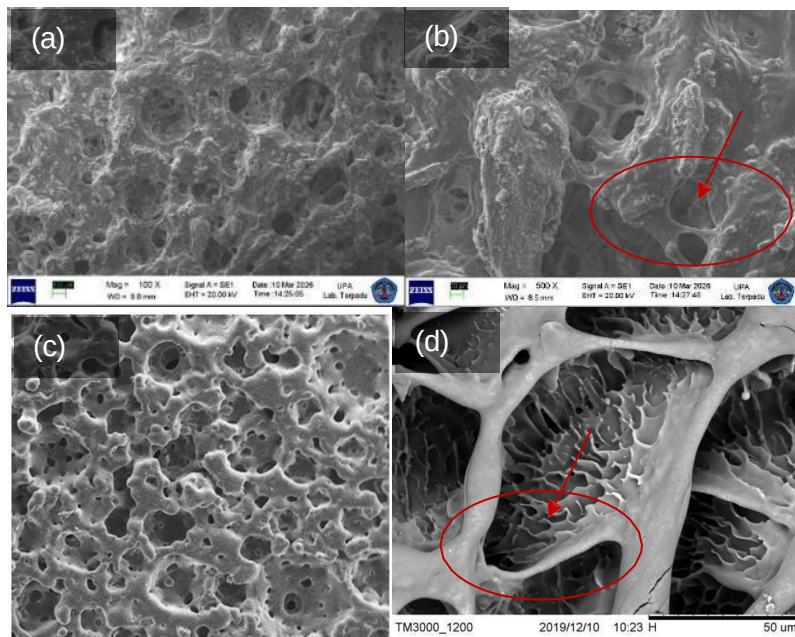


Figure 8. (a) Scaffold morphology, (b) pore interconnectivity, (c) reference scaffold morphology, and (d) reference pore interconnectivity.

Based on the SEM results, the HAp/chitosan/PVA scaffold exhibited a three-dimensional porous structure with irregular pore shapes formed as a result of ice crystal sublimation during the freeze-drying process. At higher magnification, pore interconnectivity was clearly observed, indicating the presence of interconnected pathways that facilitate nutrient and oxygen diffusion, as well as cell migration and proliferation for bone tissue engineering applications. The morphology of the fabricated scaffold showed strong similarity to the scaffold structure reported in the reference biomaterial studies, thereby confirming that the scaffold possessed good porosity and pore interconnectivity characteristics (Devi et al., 2024).

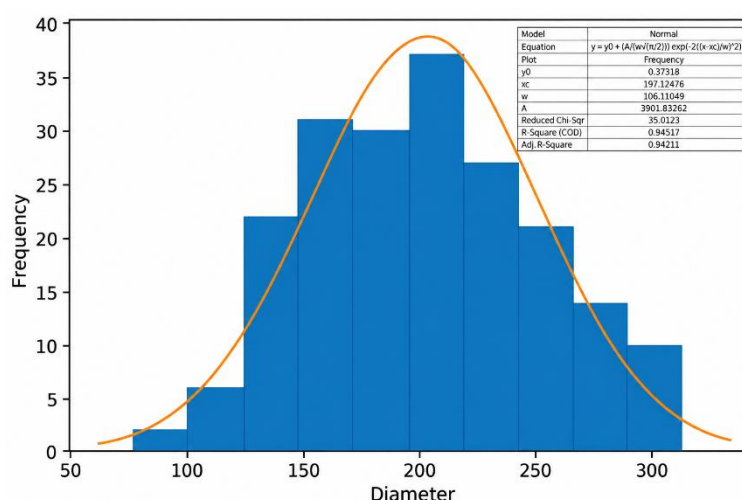


Figure 9. Pore size distribution histogram of the HAp/chitosan/PVA scaffold.

ImageJ analysis showed that the scaffold had an average pore size of 180-220 μm , within the optimal range for bone tissue engineering (100–300 μm), and a uniform pore distribution ($R^2 = 0.9728$). The porosity was 77.78%, comparable to natural trabecular bone (75-90%) (Rohmadi et al., 2021), indicating a highly porous structure suitable for tissue regeneration, although slight pore shrinkage may have occurred during freeze-drying. EDX analysis was then performed to determine elemental composition and Ca/P ratio.

Table 8. Ca/P ratio of the HAp/chitosan/PVA scaffold.

Measurement	Ca (at.%)	P (at.%)	Ca/P Ratio
1	28.72	17.20	1.67
2	30.02	19.00	1.58
Average			1.63

EDX analysis showed Ca/P ratios of 1.67 and 1.58 (average 1.63), close to stoichiometric hydroxyapatite (1.67), indicating that HAp remained the dominant phase with good chemical stability and similarity to natural bone mineral, despite slight variations due to non-uniform particle distribution (Yazdanpanah et al., 2023). A degradation test was then conducted to assess scaffold behavior in a biological environment for bone tissue regeneration.

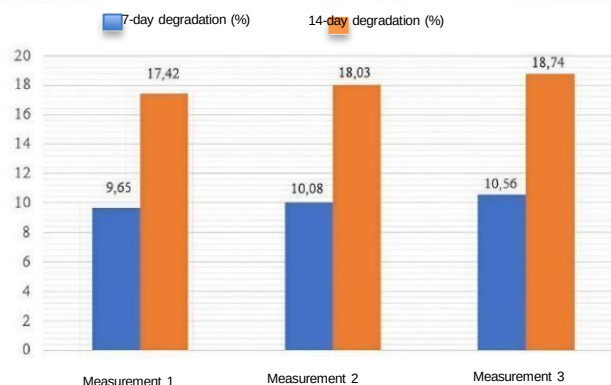


Figure 10. Degradation test results of the HAp/chitosan/PVA scaffold

The scaffolds showed gradual degradation, increasing from 9-11% on day 7 to 17-19% on day 14, which is within the ideal range for early trabecular bone regeneration (5-20% in two weeks) (Nawafi et al., 2022). Differences in degradation behavior were influenced by pore size, porosity, and interconnectivity, where higher porosity led to faster degradation due to easier medium diffusion. Overall, the scaffold demonstrated controlled biodegradability suitable for bone tissue engineering. Compression testing using UTM was then performed to evaluate the scaffold's mechanical strength under load.

Table 9. Compression test results of the HAp/chitosan/PVA scaffold using UTM.

Measurement	Compressive Strength (MPa)	Young's Modulus (MPa)
1	27.71	341.283
2	14.77	171.071
3	22.42	251.978
Average	21.63	254.777

The scaffold exhibited a high crystallinity of 89.33%, together with an average compressive strength of 21.63 MPa and Young's modulus of 254.777 MPa. The high crystallinity indicates that the HAp phase was well preserved after scaffold fabrication and may contribute to the mechanical rigidity of the inorganic phase. However, the mechanical properties of the scaffold cannot be attributed to crystallinity alone. The relatively high porosity of 77.78% can reduce load-bearing capacity, while the interconnected pore structure, HAp-chitosan/PVA interactions, and material homogeneity also influence the compressive strength and Young's modulus. Therefore, the high crystallinity in this study supports the structural stability of the HAp phase, whereas the resulting mechanical performance is determined by the combined effects of crystallinity, porosity, pore architecture, and polymer-HAp interactions. The compressive strength of 21.63 MPa was higher than the reported range for trabecular bone (2-12 MPa), while the Young's modulus of 254.777 MPa was within the reported physiological range (50-500 MPa) (Chung et al., 2021). These results indicate that the scaffold possesses favorable mechanical characteristics for potential bone tissue engineering applications.

4. Conclusion

Hydroxyapatite (HAp) was successfully extracted from bekre, tongkol, and simba fish bones using the calcination method. XRD, FTIR, and SEM-EDX characterization results confirmed that all samples formed the HAp phase; however, simba fish bone exhibited the most optimal characteristics, including the highest crystallinity (90.09%), lattice parameters closest to the JCPDS 09-0432 standard, the largest crystallite size (90.6 nm), absence of the β -TCP phase, and a Ca/P ratio close to stoichiometric HAp (1.64). The HAp/chitosan/PVA scaffold was successfully fabricated using the freeze-drying method, resulting in a three-dimensional porous structure with good pore interconnectivity. The scaffold exhibited a pore size of 180–220 μm , porosity of 77.78%, a Ca/P ratio of 1.63, and a degradation rate of 17-19% over 14 days, which is still suitable for bone regeneration applications. In addition, the scaffold showed a compressive strength of 21.63 MPa and a Young's modulus of 254.777 MPa, indicating favorable mechanical properties for bone tissue engineering applications.

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