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Optimization of hydroxyapatite synthesis from crab shell waste using response surface methodology (RSM)

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ABSTRACT

Hydroxyapatite synthesis from crab shell waste was optimized using Response Surface Methodology to achieve stoichiometric Ca/P ratio. Central Composite Design investigated calcination temperature ranging from 800 to 1200°C and Na_2HPO_4 concentration ranging from 0.75 to 2.95 M. The quadratic model demonstrated excellent fit with R^2 value of 0.9573 and p-value less than 0.0001, indicating both factors significantly influenced Ca/P ratio. Optimal conditions at calcination temperature of 858.86°C and Na_2HPO_4 concentration of 2.949 M yielded Ca/P ratio of 1.67 with desirability value of 1.0, validated experimentally through Confidence Interval, Tolerance Interval, and Prediction Interval analyses. XRD confirmed phase-pure hexagonal HAp matching JCPDS 09-0432 with high crystallinity as evidenced by sharp, well-defined peaks, conforming to SNI 7949:2013 specifications for biomedical-grade biomaterials. This optimization provides a robust protocol for valorizing seafood waste into standard-compliant hydroxyapatite suitable for clinical applications.

1. Introduction

Indonesia is a maritime country endowed with abundant marine and fishery resources, including crab shells that are often discarded as industrial waste. Crab shells represent a promising natural source of calcium that can be utilized for material synthesis. Hydroxyapatite (HAp), with the chemical formula $Ca_{10}(PO_4)_6(OH)$, is a calcium phosphate compound composed of calcium and phosphate ions, which can be synthesized from both synthetic and natural calcium sources. Synthetic sources such as CaO, $Ca(NO_3)_2$, $Ca(OH)_2$, CaCO, $CaCl_2$ dan $CaCO_3$ are commonly used, while natural sources such as crab shells contain high calcium carbonate content [1]. HAp is classified as a bioceramic material widely applied in the biomedical field due to its similarity to the main mineral component of human bones and teeth. It is frequently employed in bone tissue regeneration, dental implant coatings, and desensitizing toothpaste [2].

Despite its abundance, crab shell waste remains largely underutilized in Indonesia, leading to both environmental and economic concerns. The demand for hydroxyapatite continues to rise owing to its extensive biomedical applications. According to national statistics in 2022, Indonesia imported approximately 1.5 million units of hydroxyapatite per 5 milligrams, highlighting a strong dependency on imported materials [3]. This dependency results in high production costs and underscores the need for local production using natural waste sources. The fishery and seafood processing industries in Indonesia generate around at 56,200 tons of crab shell waste annually [4]. Crab shells typically contain 53.70%–78.40% calcium carbonate (CaCO3), 15.06%–23.90% protein, and 18.70%–32.20% chitin [5]. Through calcination, calcium carbonate can be converted into calcium oxide (CaO), serving as a precursor for HAp synthesis [6]. Utilizing crab shell waste for HAp production not only adds economic value but also contributes to environmental sustainability by reducing marine waste [7].

Noviyanti et al [8] reported that hydroxyapatite is a multifunctional material with high biocompatibility, bioactivity, and osteoconductivity, making it suitable for diverse biomedical and environmental applications. Beyond its use in bone and dental implants, HAp can also act as an adsorbent for heavy metal ions [9], a catalyst or photocatalyst [10], and a precursor in developing advanced materials such as bioceramics and nanocomposites [11]. Romadhona et al. (2023) explained that its physicochemical properties and morphology are influenced by several parameters, including raw material source, calcination temperature, and synthesis method [12]. Among various routes, the precipitation method offers advantages such as simplicity, cost-effectiveness, and better control over particle homogeneity and the Ca/P ratio [13]. Maintaining an optimal Ca/P ratio of 1.67 is essential for producing crystalline, structurally stable hydroxyapatite suitable for high-performance applications.

In modern materials research, computational approaches have become integral to optimizing experimental design and efficiency [14]. Through computational modeling and statistical analysis, researchers can explore complex interactions among process variables and identify optimal synthesis conditions beyond the capabilities of conventional methods [15,16,17] Previous studies have applied RSM to optimize HAp synthesis from various natural calcium sources a such as eggshells and fish bones; however, limited studies have focused specifically on crab shell waste as a calcium precursor. Moreover, variations in calcination temperature and phosphate concentration have

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not been comprehensively correlated with the resulting Ca/P molar ratio.

Therefore, the novelty of this study lies in its application of RSM with a Central Composite Design (CCD) to optimize hydroxyapatite synthesis derived from crab shell waste by varying calcination temperature and disodium hydrogen phosphate (Na_2HPO_4) concentration. This research provides a deeper understanding of how these process parameters influence the physicochemical formation of HAp, aiming to achieve an optimal Ca/P ratio of 1.67 and high crystallinity. The findings are expected to support sustainable utilization of Indonesia's marine-derived resources and contribute to the development of locally sourced, high-quality biomaterials for biomedical applications.

2. Method

2.1. Materials

Crab shell waste samples have been taken from the seafood restaurant seller Kepiting Delta from Siwalanpanji Village (Buduran District, Sidoarjo Regency, East Java Province, Indonesia), Disodium Phosphate (Na_2HPO_4), Hydrochloric Acid (HCl) 37%, Sodium Hydroxide (NaOH), and Aquadest.

2.2. Synthesis of hydroxyapatite

Crab shell samples were collected and cleaned by boiling for 1 hour to remove leftover meat, then washed and dried under sunlight for 2 days. The dried shells were crushed and sieved to a 100-mesh size. Calcination was performed by heating 250 grams of shell powder at various temperatures (°C) of 800, 900, 1000, 1100, and 1200 for 5 hours to produce white CaO powder. The calcination results were then analyzed using X-ray Fluorescence (XRF) to determine the calcium content. Next, the precipitation process was carried out by dissolving 19 grams of CaO powder in 3 M HCl solution, then adding Na_2HPO_4 solution with varying concentrations (M) of 0,75; 1,85; 2,95 and stirred using a magnetic stirrer at 90°C for 1 hour. The pH of the mixture was adjusted to 11 using 1 M NaOH. The formed hydroxyapatite precipitate was left for 24 hours, filtered, washed with distilled water, then dried at 110°C for 3 hours and sintering at 900°C for 5 hours to obtain hydroxyapatite crystals. The final product in the form of white hydroxyapatite powder was then analyzed using X-ray Fluorescence (XRF) testing to determine the composition of the sample.

2.3. Experimental design

The effects of calcination temperature and Na_2HPO_4 concentration on the formation of hydroxyapatite (HAp) compounds were investigated using Response Surface Methodology (RSM) with a Central Composite Design (CCD). Each parameter was varied at five levels ($-\alpha$, -1, 0, $+1$, $+\alpha$) as shown in Table 1. The calcination temperature (°C) was varied at 800, 900, 1000, 1100, and 1200, while the Na_2HPO_4 (M) concentration was varied at 0.75, 1.30, 1.85, 2.40, and 2.95. In the CCD experimental design, the number of experiments (Y) was determined using the following equation (1):

$$Y = 2^x + 2x + C_x \quad (1)$$

where Y is the number of trials, x is the number of variables, and Cx is the number of center point repetitions. In this study, the number of variables is 2, and the number of center points.

Table 1. Experimental parameters and their levels

Sample	Parameter	$-\alpha$	-1	0	+1	$+\alpha$
A	Calcination Temperature (°C)	800	900	1000	1100	1200
B	Concentration Na_2HPO_4 (M)	0,75	1,3	1,85	2,4	2,95

The center points and repetitions were 3 and 1, respectively, resulting in a total of 15 experimental runs generated by this design. In Table 1, the alpha (α) value was set to 2 as the star or axial point for the orthogonal CCD design in the case of two independent variables. The parameter values at each variation level were rounded. The mathematical relationship between the independent and response variables was modeled using the following second-degree polynomial equation (2):

$$y = b_0 + \sum_{i=1}^{n} b_i x_i + \sum_{i=1}^{n} b_{ii} x^2_i + \sum_{i=1}^{n} \sum_{j>i}^{n} b_{ij} x_i x_j \quad (2)$$

where b_0 is a constant, b_i is the linear coefficient, and x_1 is the effect of each variable. The effect of the parameters is $x_i x_j$. b_{ij} is the interaction coefficient, and b_{ii} is the coefficient that indicates the effect of the variable $x_i x_i$[18].

The model was then validated through analysis of variance (ANOVA) to evaluate the significance of each factor's effect on the response and to ensure the model's adequacy [19]. The optimization parameters were obtained from the combination of temperature and Na_2HPO_4 concentration that produced a Ca/P ratio closest to 1,67, with a desirability value of 1, indicating an excellent agreement between the model's prediction and the optimum target [20].

3. Result and Discussion

3.1. XRF analysis

Table 2. Ca/P ratio of hydroxyapatite from XRF analysis results

Calcination Temperature (°C)	Concentration Na_2HPO_4 (M)	Ca (%w/w)	P (%w/w)	Ratio Ca/P
800	0,75	28.42	16.91	1,6807
800	1,85	30.8	18.75	1,6427
800	2,95	30.2	18.15	1,6639
900	0,75	28.73	19.42	1,4794
900	1,85	28.71	18.1	1,5862
900	2,95	30.1	18.01	1,6712
1000	0,75	27,12	17,35	1,5632
1000	1,85	31,67	19,07	1,6607
1000	2,95	29,75	16,35	1,8196
1100	0,75	31.67	18.81	1,6837
1100	1,85	63,42	28,80	2,202
1100	2,95	64,91	26,56	2,4432
1200	0,75	62,83	28,14	2,2326
1200	1,85	66.9	27.5	2,4327
1200	2,95	66.8	21.4	3,1215

The XRF results were subsequently analyzed using Response Surface Methodology (RSM) to statistically optimize the synthesis parameters and identify the most influential variables affecting the Ca/P ratio. This analytical approach not only determines the best combination of calcination temperature and Na_2HPO_4 concentration but also provides insight into their interactive effects on the formation of stoichiometric hydroxyapatite. The theoretical Ca/P ratio of 1.67 was used as a benchmark to evaluate the precision of the

optimization model. Through this correlation, RSM enables a quantitative understanding of how thermal and compositional factors govern ionic diffusion, crystallization dynamics, and phase evolution during HAp synthesis.

3.2. Evaluation of RSM analysis

The determination of the Ca/P ratio of HAp under different synthesis conditions was conducted through 15 experimental runs combining calcination temperature and Na_2HPO_4 concentration, as summarized in Table 3. The predicted responses generated using Design Expert version 13 exhibited a strong correlation with the experimental data, confirming that the model accurately represents the interaction between process parameters and the resulting Ca/P ratio [21]. The reliability and significance of the model were further verified through ANOVA, which identified the quadratic model as statistically valid ($p < 0.05$). The high F-value and coefficient of determination ($R^2 = 0.9573$) indicate that the model effectively captures the complex relationship between temperature and phosphate concentration, demonstrating a strong predictive capability. This result suggests that variations in these two factors contribute nonlinearly to the Ca/P ratio due to their combined influence on calcium ion availability and phosphate incorporation during HAp formation.

Table 3. Experimental runs and responses for Ca/P ratio

Run	A Calcination Temperature (°C)	B Concentration Na_2HPO_4 (M)	Ratio Ca/P
1	800	0,75	1,6807
2	800	1,85	1,6427
3	800	2,95	1,6639
4	900	0,75	1,4794
5	900	1,85	1,5862
6	900	2,95	1,6712
7	1000	0,75	1,5632
8	1000	1,85	1,6607
9	1000	2,95	1,8196
10	1100	0,75	1,6837
11	1100	1,85	2,202
12	1100	2,95	2,4432
13	1200	0,75	2,2326
14	1200	1,85	2,4327
15	1200	2,95	3,1215

Table 4. Ca/P ratio of hydroxyapatite from XRF analysis results

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2,90	5	0,5805	63,63	< 0,0001	Significant
A-Calcination temperature (°C)	1,72	1	1,72	188,90	< 0,0001	
B-Concentration Na_2HPO_4 (M)	0,4326	1	0,4326	47,40	< 0,0001	
AB	0,2830	1	0,2830	31,01	0,0003	
A^2	0,4600	1	0,4600	50,41	< 0,0001	
B^2	0,0032	1	0,0032	0,3519	0,5676	
Residual	0,0821	9	0,0091			
Cor Total	2,98	14				

Based on Table 4, the F-value represents the variance ratio of the independent variables, while the p-value indicates the statistical significance of each factor on the observed response. In general, a p-value ≤ 0.05 confirms that a variable exerts a statistically meaningful influence on the response, whereas a p-value ≥ 0.1 suggests an insignificant effect. In this study, both calcination temperature (A) and Na_2HPO_4 concentration (B) exhibited very low p-values (< 0.0001) [22], indicating that these parameters have a pronounced and independent effect on the Ca/P ratio. The significant interaction term between temperature and phosphate concentration (AB, $p = 0.0003$) demonstrates a synergistic relationship, meaning that the combined effect of both variables is greater than their individual contributions.

This synergistic behavior can be explained by the coupled influence of thermal energy and ionic concentration on the reaction kinetics. Higher calcination temperatures enhance CaO reactivity by improving ion diffusion and surface activation, while appropriate phosphate concentration ensures sufficient PO_4^{3-} availability for stable HAp nucleation. When both parameters are optimized simultaneously, the balance between calcium and phosphate ions promotes the formation of hydroxyapatite with a stoichiometric Ca/P ratio and reduced secondary phase formation. The optimization outcomes derived from the RSM–ANOVA analysis are summarized in Table 4.

Table 5. Ca/P ratio of Hydroxyapatite from XRF analysis results

Source	Sequential p-value	Adjusted R^2	Predicted R^2	
Linear	0,0005	0,6762	0,5093	
2R	0,0359	0,7674	0,5216	
Quadratic	**0,0002**	**0,9573**	**0,9257**	**Suggested**
Cubic	0,7556	0,9466	0,7720	Aliased

Based on Table 5, the quadratic model was selected as the most suitable equation for predicting the Ca/P ratio response, as it demonstrates high consistency between the Adjusted R^2 (0.9573) and Predicted R^2 (0.9257) values, with a difference of only 0.0316—well below the acceptable threshold (<0.2) [23]. This high correlation ($R^2 = 0.8–1.0$) indicates that variations in calcination temperature and Na_2HPO_4 concentration strongly influence the Ca/P ratio, confirming that these two parameters are the dominant factors controlling the formation of hydroxyapatite (HAp). The low p-value ($0.0002 < 0.05$) further verifies the model's statistical significance, implying that the relationship between these variables is not random but scientifically meaningful [24].

The close agreement between the statistical parameters demonstrates that the relationship between calcination temperature and Na_2HPO_4 concentration significantly influences the Ca/P ratio, both individually and interactively. Scientifically, this result can be explained by the effect of temperature on the thermal decomposition of calcium carbonate and the subsequent formation of reactive CaO. As the calcination temperature increases, $CaCO_3$ undergoes decarbonation to form CaO, which reacts more effectively with phosphate ions from Na_2HPO_4 to form HAp. Conversely, excessive Na_2HPO_4 concentrations may lead to incomplete precipitation or secondary phase formation (e.g., tricalcium phosphate), thereby lowering the Ca/P ratio. This explains the curvature observed in the response surface plot, where optimal conditions are achieved at moderate Na_2HPO_4 concentrations

and sufficiently high calcination temperatures.

The most appropriate model to describe the effect of calcination temperature and Na_2HPO_4 concentration on the Ca/P ratio was obtained through quadratic regression analysis [21]. Based on the evaluation of several models and ANOVA, it was found that the quadratic polynomial model provided the best fit in explaining the relationship between the two variables and the Ca/P ratio response, as shown in Equation (3), where A represents the calcination temperature (°C) and B represents the Na_2HPO_4 concentration (M).

$$y = 11{,}5026 + (-0{,}0205345)\ A + (-0{,}0205345)\ B + 0{,}0010814\ AB + 1{,}04655e\text{-}05\ A^2 + 0{,}046161B^2 \quad (3)$$

The uniform distribution of residuals indicates that the quadratic model prediction generated by Design Expert 13 software is capable of accurately describing the relationship between calcination temperature (A) and Na_2HPO_4 concentration (B) on the Ca/P ratio. This also demonstrates that the model possesses high accuracy and reliability across the entire range of tested variables.

Figure 1 compares the predicted and actual Ca/P ratio responses, while Figures 2 and 3 show the contour and 3D surface plots describing the relationship between calcination temperature and Na_2HPO_4 concentration on the Ca/P ratio. The contour plot illustrates two-dimensional variations, whereas the 3D plot provides a more comprehensive visualization of the interactive effects between the variables. Both plots clearly demonstrate that the Ca/P ratio is strongly influenced by changes in temperature and phosphate concentration [22]. The red-to-yellow regions in the contour plot correspond to higher Ca/P ratios (up to 3,12), which occur under high calcination temperatures 1200 °C and low phosphate concentrations 0,75 M). This trend can be explained by the thermal decomposition of $CaCO_3$ into CaO at elevated temperatures, increasing calcium ion availability and leading to calcium-rich phases. However, excessive heating may also induce partial volatilization of phosphate species, slightly disturbing stoichiometry. Conversely, the blue regions, corresponding to lower Ca/P ratios (around 1,48), are observed at lower calcination temperatures 800–900 °C and higher Na_2HPO_4 concentrations 2,95 M. Under these conditions, the presence of excess phosphate ions promotes the formation of calcium-deficient apatite or amorphous calcium phosphate, indicating incomplete crystallization [22].

These observations highlight that achieving a stoichiometric Ca/P ratio depends on maintaining an equilibrium between the thermal decomposition rate of calcium precursors and the phosphate availability during nucleation. A calcination temperature of 900 °C with an Na_2HPO_4 concentration near 3 M provides optimal thermal energy for complete $CaCO_3$ decomposition while preserving sufficient phosphate content for stable HAp lattice formation. This synergistic interaction ensures that the resulting hydroxyapatite possesses high structural uniformity and minimal impurities, confirming the reliability of the RSM-optimized parameters for producing high-purity HAp from crab shell waste [18].

This optimization approach provides a deeper understanding of the distribution and trends of the Ca/P ratio across various combinations of variables, enabling more accurate prediction and validation of the hydroxyapatite formation process. In addition, the increased number of data points enhances the reliability of the optimization model by providing a stronger statistical basis for analyzing the effects of calcination temperature and Na_2HPO_4 concentration on the resulting Ca/P ratio.

Based on the XRF analysis results, the Ca/P ratios were obtained from 15 sample points. Subsequently, optimization was carried out using the Response Surface Method (RSM) to generate 10 additional points through the substitution of variable values into Equation (2). In total, 25 data points representing the desired optimum conditions were obtained, as shown in Table 6.

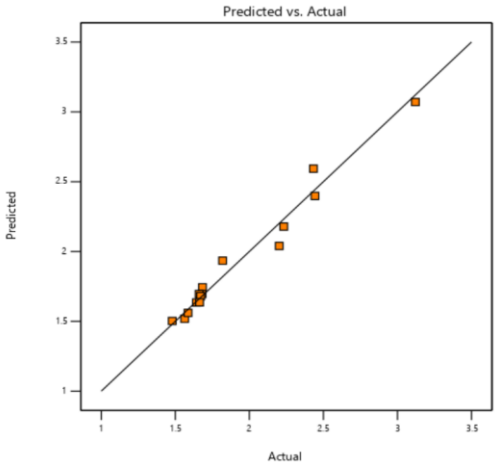

Fig. 1. Comparison graph of Ca/P ratio response in predicted vs. actual

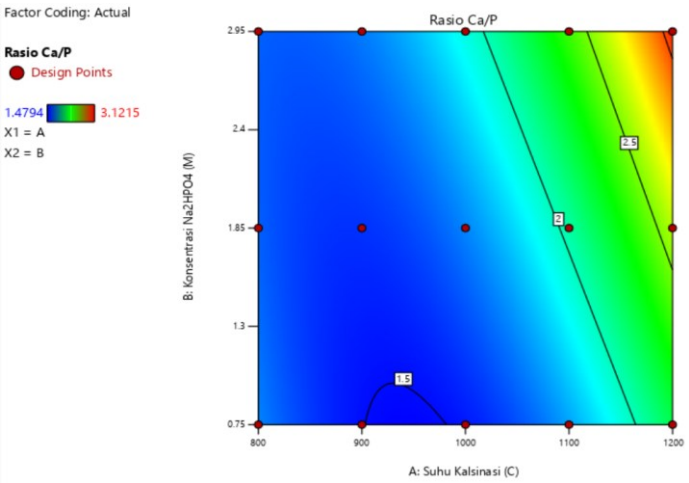

Fig. 2. Contour plot of calcination temperature versus Na_2HPO_4 concentration on the Ca/P ratio

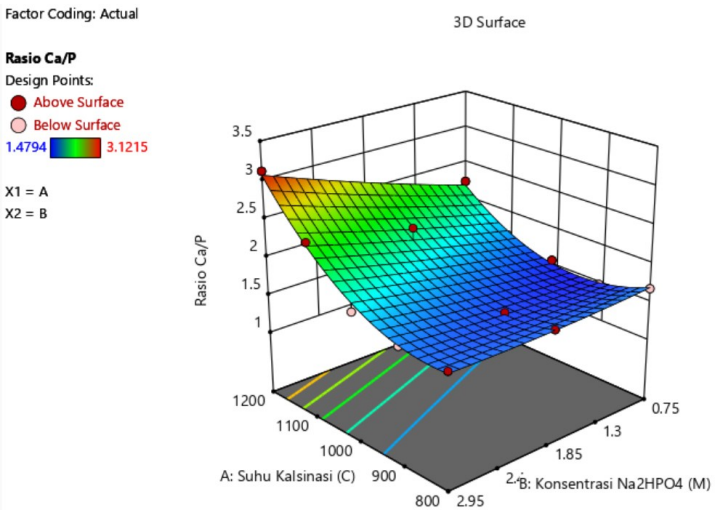

Fig. 3. 3D Surface plot of calcination temperature versus Na_2HPO_4 concentration on the Ca/P ratio

Table 6. Optimization results of Ca/P Ratio of hydroxyapatite at various calcination temperatures and Na2HPO4 concentrations

Run	A Calcination Temperature (°C)	B Concentration Na_2HPO_4 (M)	Ratio Ca/P
1	800	0,75	1,6807
2	800	1,3	1,6575
3	800	1,85	1,6427
4	800	2,4	1,6275
5	800	2,95	1,6639
6	900	0,75	1,4794
7	900	1,3	1,5238
8	900	1,85	1,5862
9	900	2,4	1,6128
10	900	2,95	1,6712
11	1000	0,75	1,5632
12	1000	1,3	1,5993
13	1000	1,85	1,6607
14	1000	2,4	1,8073
15	1000	2,95	1,8196
16	1100	0,75	1,6837
17	1100	1,3	1,8843
18	1100	1,85	2,2020
19	1100	2,4	2,2112
20	1100	2,95	2,4432
21	1200	0,75	2,2326
22	1200	1,3	2,3785
23	1200	1,85	2,4327
24	1200	2,4	2,8243
25	1200	2,95	3,1215

3.3. Optimization results of the Ca/P response using RSM

The optimization process was conducted to determine the combined influence of calcination temperature and Na_2HPO_4 concentration on achieving an ideal Ca/P ratio, which theoretically corresponds to 1.67 for stoichiometric hydroxyapatite (HAp). This optimization was performed using *Design Expert 13* software based on the quadratic model obtained from the previous ANOVA analysis.

Table 7 presents the range limits applied for the optimization process. The selected temperature and phosphate concentration intervals were determined based on preliminary experiments to ensure complete decomposition of calcium carbonate and the effective formation of HAp. The Ca/P ratio target (1.67) represents the theoretical stoichiometric value, indicating perfect hydroxyapatite formation without excess calcium or phosphate phases. After defining these constraints, the software generated several possible combinations of parameters to achieve the target ratio. Among these, the solution with the highest desirability value was selected as the optimum condition.

Table 7. Factor limits and response for optimization

Name	Goal	Lower Limit	Upper Limit
Calcination Temperature	Is in range	800°C	1200°C
Concentration Na_2HPO_4	Is in range	0,75 M	2,95 M
Ratio Ca/P	Is target = 1,67	1,4794	3,1215

Table 8 shows the optimization result generated by *Design Expert 13*. The desirability value of 1.000 indicates perfect agreement between the predicted and experimental results, confirming the accuracy of the response surface model. The close correspondence between the Adjusted R^2 (0,9573) and Predicted R^2 (0,9257) demonstrates that the quadratic model can effectively describe the relationship between the two variables and the Ca/P response. The optimum condition was achieved at a calcination temperature of 858.86 °C and Na_2HPO_4 concentration of 2.949 M, yielding a Ca/P ratio of 1.67.

Table 8. Optimal Ca/P response result

Number	Calcination Temperature (°C)	Concentration Na_2HPO_4 (M)	Ratio Ca/P	Desirability	
1	858,86	0,9466	0,7720	1	**Selected**

It is noteworthy that the optimum condition is located near the mid-range of the investigated variables, which may indicate a potential model bias or a response plateau phenomenon commonly observed in quadratic RSM models. However, the statistical agreement between the Adjusted R^2 and Predicted R^2 values, as well as the high desirability index, indicate that the model's predictions remain reliable.

Similar findings have also been reported by Ebrahimi et al. (2021), who reported that the optimal synthesis temperature of HAp (approximately 850–900 °C) occurred near the mid-range due to the balance between CaO decomposition and phosphate precursor reactions [22]. Similarly, Fitriyana et al. (2024) observed that a phosphate concentration of around 3 M yielded optimal results in HAp synthesis derived from marine waste [7]. Therefore, the present result is in good agreement with previous studies, indicating that the variable range used in this work is sufficiently broad and that the obtained optimum condition is scientifically meaningful rather than a mathematical artifact. Therefore, the optimum point represents a balanced condition between calcium reactivity and phosphate availability, resulting in a Ca/P ratio close to stoichiometric value (1.67) [23].

The validity of the optimization model was further verified using Confidence Interval (CI), Tolerance Interval (TI), and Prediction Interval (PI). The narrow CI and PI ranges indicate that the predicted Ca/P ratio closely matches experimental data with low deviation, confirming the precision of the RSM model. TI analysis also suggests that the variation in experimental runs falls within acceptable limits, reinforcing the reproducibility of the optimized conditions. These statistical validations demonstrate that the developed model is not only statistically significant but also experimentally consistent, ensuring that repeated experiments under similar conditions will yield comparable Ca/P ratios.

3.4. Verification of optimum results

Verification of the optimization results was conducted by comparing the predicted values obtained from the model with the actual experimental Ca/P ratio. The model's accuracy was evaluated using Confidence Interval (CI), Tolerance Interval (TI), and Prediction Interval (PI) analyses at a 95% confidence level. These statistical evaluations aimed to ensure that the developed model accurately represents the interaction between the process variables and can be reliably reproduced under optimum conditions.

Table 9 presents the model verification results based on CI and TI analyses. The CI range of 1.5576–1.7824 indicates a 95% confidence that the population mean lies within this interval, while the TI range of 1.1741–2.1658 encompasses approximately 99% of the total data population.

Table 9. Verification results of confidence interval (C1)

Response	Predicted Mean	Predicted Median	Std Dev	SE Mean	95% CI low	95% CI high	95% TI low	95% TI high
Ratio Ca/P	1,67	1,67	0,0955	0,0496	1,5576	1,7824	1,1741	2,1658

From a scientific perspective, the narrow CI range reflects low experimental fluctuation, indicating that the model exhibits high precision and minimal random error. This stability implies that the optimum condition (858.86 °C and 2.949 M Na_2HPO_4) consistently produces a Ca/P ratio near the stoichiometric value of 1.67. Therefore, the RSM model accurately predicts the relationship between calcination temperature and phosphate concentration in forming homogeneous hydroxyapatite.

Table 10. Verification results of confidence interval (C1)

Response	Predicted Mean	Predicted Median	Std Dev	n	SE Pred	95% PI low	95% PI high
Ratio Ca/P	1,67	1,67	0,0613	1	1,4264	1,4264	1,9135

Table 10 displays the Prediction Interval (PI), which ranges from 1.4264 to 1.9135 at a 95% confidence level. The PI represents the interval in which future experimental data points are expected to fall if additional runs are conducted under the same conditions. The PI range is typically wider than the CI because it accounts for potential variability in future, unobserved samples.

The consistency among CI, TI, and PI results confirms that the RSM model exhibits high accuracy and reliability in predicting the Ca/P ratio response. Scientifically, this finding implies that under the optimum condition of 858.86 °C calcination temperature and 2.949 M Na_2HPO_4 concentration, both the calcination and precipitation processes proceed in a thermodynamically stable manner.

The Ca/P ratio of 1.67 indicates that calcium and phosphate ions are completely integrated into the hydroxyapatite crystal lattice, signifying a well-balanced reaction without the formation of secondary or calcium-deficient phases. The narrow statistical intervals reflect the system's thermochemical equilibrium, supporting the reproducibility and stability of the hydroxyapatite synthesis process.

Thus, CI, TI, and PI analyses not only validate the statistical significance of the RSM model but also provide scientific evidence of the process stability, confirming that the optimized conditions truly represent the equilibrium point for forming hydroxyapatite with high crystallinity and phase uniformity.

The verification results demonstrate that the developed RSM model possesses excellent predictive capability, with strong agreement between the predicted and experimental values. The narrow ranges of CI, TI, and PI confirm that the model is precise, reliable, and reproducible. The combination of 858.86 °C calcination temperature and 2.949 M Na_2HPO_4 concentration is therefore validated as the optimum condition for producing hydroxyapatite with a stoichiometric Ca/P ratio of 1.67. These results are consistent with previous studies by [24], which highlighted that controlled thermal and phosphate parameters significantly improve the crystallinity and phase purity of hydroxyapatite derived from natural calcium sources.

3.5. Error bar analysis and standard deviation interpretation

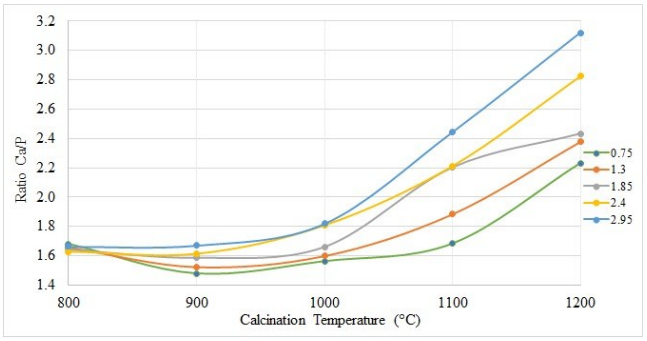

Fig. 4. The Effect of Calcination Temperature on Hydroxyapatite Synthesis on the Ca/P Ratio

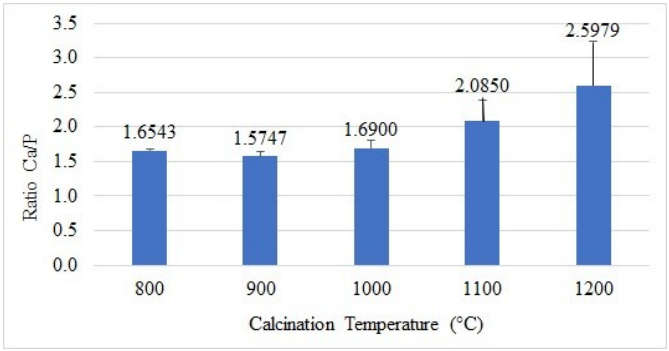

Fig. 5. The effect of calcination temperature on the Ca/P ratio with standard deviation variation

The error bars in the graph represent the standard deviation of experimental replicates, indicating data reliability. The shortest error bar at 800°C (0.0202) indicates optimal reproducibility with stable and controlled synthesis conditions, while the longest error bar at 1200°C (0.6336) demonstrates high variability due to unstable synthesis conditions. This pattern of increasing standard deviation is consistent with the findings of [25], who reported that the variability of HAp synthesis results increases significantly at high calcination temperatures due to thermal instability. Standard deviation as an indicator of reproducibility has been validated in various bioceramic synthesis studies [26], where standard deviation <0.1 indicates excellent process control, while values > 0.5 indicate inconsistent synthesis conditions requiring further optimization.

The progressive increase in standard deviation from 800°C to 1200°C confirms a positive correlation between calcination temperature and result variability. The low standard deviation at 800-900°C (≤0.0955) demonstrates that HAp phase transformation occurs under thermodynamically stable conditions, consistent with literature stating that the formation of stoichiometric HAp crystal structure (Ca/P ratio = 1.67) is optimal in the temperature range of 800-1000°C, where thermal energy is sufficient to remove carbonate groups and crystal water without causing decomposition of the apatite structure. The significant increase in standard deviation at temperatures ≥1100°C (0.2998-0.6336) is attributed to: (1) partial decomposition of HAp into α-TCP/β-TCP with different Ca/P ratios, (2) phosphorus volatilization at high temperatures causing stoichiometric imbalance and (3) microstructural heterogeneity due to non-uniform sintering.

Recent studies by [26] confirm that temperatures >1000°C reduce HAp phase purity and synthesis reproducibility.

Optimum conditions were achieved at 800-900°C (standard deviation ≤0.0955), producing HAp with Ca/P ratios approaching stoichiometric values (1.65-1.69) and high consistency for biomedical applications.

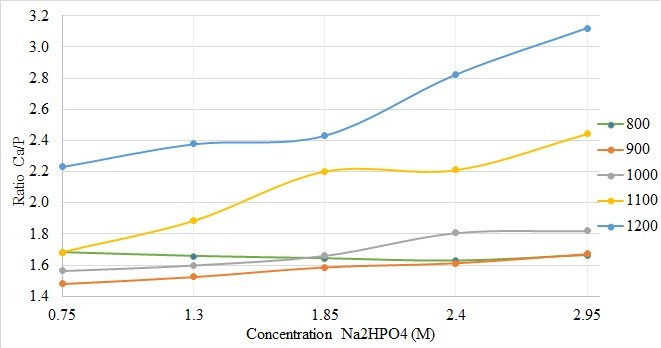

Fig. 6. The effect of concentration Na_2HPO_4 on hydroxyapatite synthesis on the Ca/P ratio

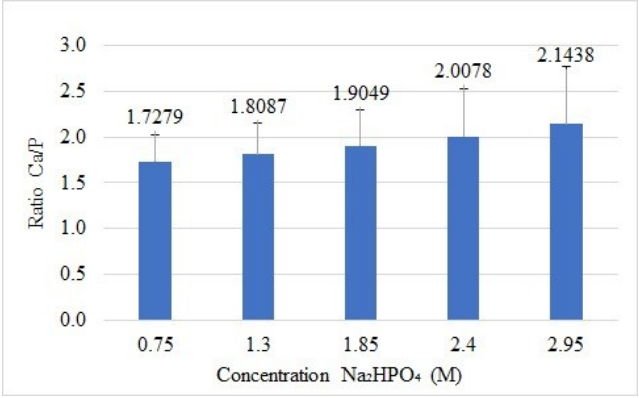

Fig. 7. The effect of concentration Na_2HPO_4 on the Ca/P ratio with standard deviation variation

The error bars in the graph represent the standard deviations of experimental replicates, reflecting the reliability and reproducibility of the Ca/P ratio data obtained from hydroxyapatite (HAp) synthesis. The standard deviation progressively increased from 0.2949 at 0.75 M Na_2HPO_4 to 0.6336 at 2.95 M, indicating greater variability with increasing phosphate precursor concentration. The smallest error bar at 0.75 M (SD = 0.2949) suggests that the precipitation process occurred under stable conditions, where low supersaturation facilitated controlled crystal growth and produced consistent Ca/P ratios across replicates. This observation aligns with precipitation kinetics theory [27], which states that moderate supersaturation promotes uniform nucleation and growth, leading to structurally homogeneous crystals.

In contrast, the largest error bar observed at 2.95 M (SD = 0.6336) indicates substantial variation between replicates, corresponding to an unstable system under high supersaturation. Such conditions accelerate precipitation, resulting in heterogeneous particle size distribution, agglomeration, and potential formation of Amorphous Calcium Phosphate (ACP) or other metastable phases. According to [28], excessive supersaturation enhances phase heterogeneity and decreases reproducibility, explaining the increased variability observed here. These findings reveal that statistical variation correlates directly with the structural uniformity and phase purity of HAp. Therefore, the optimum synthesis range for obtaining reproducible HAp with a Ca/P ratio near the stoichiometric value (1.67) lies between 0.75–1.30 M Na_2HPO_4, where the standard deviation remains ≤0.3458. Prior to performing ANOVA, data normality and homogeneity of variance were verified, ensuring the validity and reliability of the statistical interpretation.

3.6. XRD Analysis

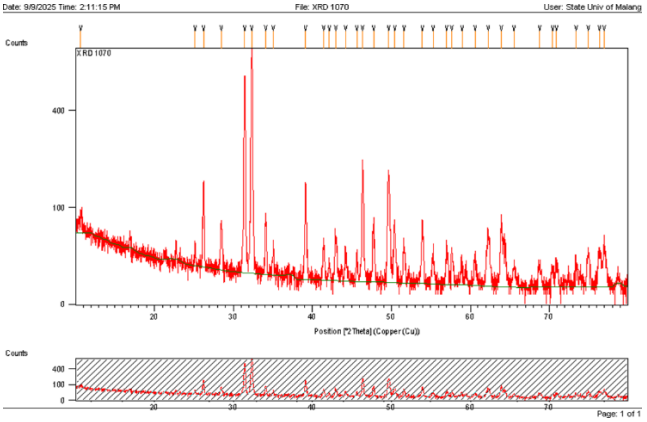

Fig. 8. XRD (X-Ray diffraction) test results on hydroxyapatite product 900°C 2.95M

XRD analysis confirms the formation of phase-pure hydroxyapatite with characteristic diffraction peaks at 2θ values of 25.9°, 31.8°, 32.9°, and 46.7°, corresponding to the (002), (211), (300), and (222) crystallographic planes, respectively. These peaks match JCPDS card No. 09-0432 for hexagonal HAp with space group P63/m. The sharp, well-defined peaks indicate high crystallinity, while the absence of secondary phases such as α-TCP at 2θ = 30.7° or β-TCP at 2θ = 27.8° validates phase purity.

The correlation between Ca/P ratio and crystalline quality is evident from peak characteristics. At optimal synthesis conditions of 0.75-1.3 M Na_2HPO_4 and calcination temperature of 800-900°C, near-stoichiometric Ca/P ratios ranging from 1.66 to 1.70 correspond to narrow peak widths with FWHM approximately 0.3-0.4° and high intensity, indicating well-ordered crystal lattices with minimal defects. These values closely approach the theoretical stoichiometric ratio of 1.67 for pure hydroxyapatite. Crystallite sizes calculated via the Scherrer equation range from 35 to 42 nm, consistent with thermodynamically stable crystallization under controlled supersaturation.

At suboptimal conditions including Na_2HPO_4 concentrations of 1.85 M and above or calcination temperatures exceeding 1100°C, elevated Ca/P ratios beyond 1.90 coincide with peak broadening characterized by FWHM values greater than 0.6°, emergence of shoulder peaks near 2θ = 30.7°, and increased baseline noise. These features indicate partial HAp decomposition to TCP phases and the presence of residual amorphous calcium phosphate. The structural deterioration directly explains the increased standard deviation in Ca/P measurements, as phase heterogeneity introduces compositional variability. Peak broadening reflects both reduced crystallite size below 25 nm and lattice strain from non-uniform ion incorporation during rapid precipitation, consistent with findings [29] on nucleation kinetics under high supersaturation.The XRD data thus validates that the optimal Ca/P range of 1.66-1.70 represents not merely compositional control but structural integrity—yielding phase-pure, highly

crystalline HAp with near-perfect stoichiometry essential for biomedical applications.

4. Conclusion

This study successfully optimized the synthesis of hydroxyapatite (HAp) from crab shell waste using the precipitation method combined with Response Surface Methodology (RSM). The optimum condition was achieved at a calcination temperature of 858.86 °C and Na_2HPO_4 concentration of 2.949 M, yielding a Ca/P ratio of 1.67 with a desirability value of 1.0. Statistical validation through CI, TI, and PI confirmed the model's high accuracy and reproducibility. XRD analysis showed characteristic diffraction peaks at 2θ = 25.9°, 31.8°, 32.9°, and 46.7°, corresponding to the (002), (211), (300), and (222) planes in accordance with JCPDS No. 09-0432, confirming the formation of a single-phase, highly crystalline HAp. These results demonstrate that crab shell waste is a promising, sustainable calcium source for producing high-purity hydroxyapatite suitable for biomedical applications.

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