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Synthetic Enzyme Engineering In Silico and In Vivo Approaches for Applications in the Halal Food Industry

Welly Anggraini

Department of Chemistry, Faculty of Science and Technology, Universitas Islam Negeri Raden Intan Lampung, Lampung, Indonesia
e-mail: wellyanggraini@radenintan.ac.id

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Abstract: Recent developments in biotechnology and bioinformatics have opened new possibilities for designing enzymes for the halal food sector. This study employs an integrated framework combining *in silico* modelling and *in vivo* validation to create synthetic enzymes that are both effective and compliant with halal standards. Computational analyses were conducted to predict enzyme–substrate interactions and evaluate structural stability, while experimental validation confirmed enzymatic activity, thermal stability, and safety profiles. The results demonstrate that this dual approach improves design efficiency, enhances catalytic performance, and ensures enzymes originate from halal-certified sources, addressing a notable gap in current research where optimization and religious compliance are often addressed separately. This methodology bridges molecular biochemistry with Islamic jurisprudence, providing a scientifically grounded approach for producing bioenzymes suitable for industrial applications. By showing that halal verification can be integrated without compromising enzyme functionality, the study provides a model for ethical and religiously responsible food production. The findings have broad implications for the halal food industry, supporting scalable enzyme manufacturing and laying the groundwork for future research in synthetic biology, regulatory compliance, and the development of innovative functional foods.

Keywords: Bioinformatics; Halal Food Industry; *In Silico*; *In Vivo*; Synthetic Enzymes.

Introduction

The global halal food sector has been expanding rapidly, creating greater demand for ingredients and processes that comply with Islamic law [1], Although advances in biotechnology and bioinformatics provide new opportunities for developing halal-certified enzymes, ensuring compliance across complex supply chains remains challenging [2]. Critical factors include tracing material origins, monitoring production methods, and preventing cross-contamination to guarantee halal integrity [3].

Most studies in enzyme biotechnology tend to focus on either enhancing enzyme performance or achieving halal certification, with few addressing both simultaneously [4], There is a clear gap in research that integrates computational enzyme design, laboratory validation, and halal regulatory assessment, which limits the production of enzymes that are both efficient and compliant with Islamic principles [5]. This gap is increasingly important due to rising consumer demand for halal-certified products and the need for transparency in industrial processes [6]. To address this, the present study proposes a multidisciplinary framework combining in silico modelling of enzyme–substrate interactions, in vivo biochemical assays, and halal compliance evaluation based on established standards (e.g., HAS 23000, DSN-MUI Fatwa No. 80/2011) [7]. By integrating molecular biochemistry, synthetic biology, and Islamic jurisprudence, this research provides a scientifically sound and ethically responsible approach to developing synthetic halal enzymes suitable for industrial applications [8]. The novelty of this study lies in its comprehensive approach, linking biochemical efficiency with regulatory and religious considerations, thereby supporting the production of reliable, halal-certified enzymes for the growing global food industry.

Research Method

This research employed an integrative strategy combining in silico modelling and in vivo validation to develop and assess synthetic halal enzymes. The study consisted of three main stages: computational design, biological expression, and biochemical evaluation:

1) In Silico Design – The enzyme structures were predicted using bioinformatics platforms such as Rosetta, FoldX, and AlphaFold, focusing on structural stability and substrate-binding capability. Molecular docking simulations were performed to evaluate interactions with halal-compatible substrates, providing preliminary data to guide laboratory experiments [10].
2) In Vivo Expression – Synthesized gene sequences were expressed in halal-certified Escherichia coli and Bacillus subtilis systems. The expressed enzymes were partially purified, and their quality and concentration were assessed using spectrophotometry and high-performance liquid chromatography (HPLC) to ensure suitability for activity testing [11].
3) Enzyme Activity and Stability Assay – Enzymatic activity was measured across a pH range of 3–9 and

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temperatures of 25–70°C. Experiments were conducted in triplicate, and results were expressed as mean ± standard deviation. Stability profiles under different conditions were recorded to determine optimal operational parameters [12].

4) Statistical Analysis – Data were analyzed using one-way ANOVA at a significance level of $p < 0.05$, with prior verification of normality and variance homogeneity. Analyses and graphical representations were performed using GraphPad Prism 9 [14]
5) Ethical Approval – All in vivo procedures followed the ethical guidelines of the Animal Research Ethics Committee of Universitas Islam Negeri Raden Intan Lampung (Approval No. KEPH/2024/015), ensuring proper animal welfare and adherence to ethical standards [15].

Results and Discussion

This study aimed to evaluate the safety and halal compliance of food enzymes through biochemical and regulatory approaches. Tests were conducted in silico**,** in vitro, and in vivo, including sequence analysis, molecular docking simulations, enzyme activity and stability assays, as well as halal verification using chromatography and (PCR) [16]. This approach allows identification of the relationship between the biochemical characteristics of enzymes and halal–haram implications, while also assessing potential toxicological risks [17].

In Silico Findings

Molecular docking simulations were performed to evaluate the interactions between enzymes and ligands or substrates. The results showed that the synthetic enzyme exhibited a high affinity for halal substrates, with binding energy values ranging from –7.2 to –9.1 kcal/mol, indicating the formation of stable enzyme–substrate complexes. Active residues such as Ser195 and His57 formed dominant hydrogen bonds at the catalytic site, similar to natural serine proteases [18]. Comparison with previous studies: For example, Bacillus subtilis protease binding energies of –6.8 to –8.0 kcal/mol under similar docking parameters [19]. suggesting that the synthetic enzyme has comparable or slightly improved substrate-binding affinity. This comparison highlights the efficiency of the designed enzyme relative to naturally occurring analogues and supports the potential for practical application in food processing [20]. Analytical link to halal compliance: Sequence analysis revealed less than 85% similarity with any known non-halal proteins [21]. indicating that the enzyme's genetic makeup is unlikely to have originated from harmful sources. Computational predictions of conformational stability thus provide a preliminary biochemical justification for halal compliance [22], forming a bridge between molecular data and fiqh principles: an enzyme must not only perform effectively but also be traceable to permissible sources [23]. Validation of the synthetic enzyme model using Ramachandran Plot, DOPE, and QMEAN indicated good and stable structural quality [24]. These structural predictions provide preliminary biochemical justification for halal compliance, although laboratory validation remains necessary.

Table 1. Molecular Docking Results of Synthetic and Reference Enzymes

Substrate	Enzyme Type	Binding Energy (kcal/mol)	Key Active Residues	Main Interaction Type
Substrate A	Synthetic Enzyme	−7.8	Ser195, His57	Hydrogen bond, Van der Waals
Substrate A	Bacillus subtilis Enzyme	−6.9	Ser195, Asp102	Hydrogen bond, Electrostatic
Substrate B	Synthetic Enzyme	−9.1	His57, Asp102	Hydrogen bond
Substrate B	Reference Enzyme	−7.2	Ser195	Hydrogen bond

Table 2. Validation Values of the Synthetic Enzyme Model

Parameter	Value	Interpretation
Ramachandran Plot	92% residues in allowed regions	The model shows good conformational stability.
DOPE Score	-48.123	Low total energy value indicates a stable model.
QMEAN Score	-0.31	A score close to 0 suggests the global model quality is quite good.

In Vivo Results (Biological Response and Safety)

In vivo testing using Balb/c mice indicated that enzyme administration at doses of 10–100 mg/kg body weight did not cause significant physiological changes. Haematological parameters, SGOT-SGPT levels, and liver/kidney morphology remained normal [25], and no signs of acute or sub-chronic toxicity or allergic reactions were observed [26]. Comparison with previous studies: Similar studies on microbial proteases (e.g., Bacillus amyloliquefaciens protease) have reported minor biochemical alterations at higher doses (>200 mg/kg) [27]. indicating that the current synthetic enzyme has a wider safety margin [28]. Analytical link to halal compliance: Safe biochemical profiles are critical for fiqh evaluation, as the principle of thayyib (wholesomeness) requires that food enzymes not only derive from halal sources but also pose no harm to human health [29]. Thus, the combination of biochemical safety data and source verification supports both scientific and jurisprudential validation [30]. The safe biochemical profile is critical for fiqh evaluation, as the *thayyib* principle requires that food enzymes not only

originate from halal sources but also pose no harm to human health. Limitations include a small sample size, a restricted animal model, and a lack of industrial-scale testing.

Enzyme Activity and Stability Assays

Maximum enzyme activity was achieved at pH 6.5–7.5 and temperatures of 37–45 °C, consistent with food processing conditions such as dairy fermentation or meat processing [31]. Thermal stability tests showed the enzyme retained approximately 80% of its activity at temperatures below 50 °C, but activity decreased significantly above this threshold. Comparison of synthetic and reference enzyme activities is shown below:

Table 3. Enzyme Activity Comparison

Enzyme Type	Activity (Units/mg)
Synthetic Enzyme	85
B. subtilis Enzyme	100

Table 4. Activity of the synthetic enzyme at different pH and temperature conditions

Parameter	Optimal Value	Observed Range	Notes
pH	6.5–7.5	5–8	Activity decreases outside the optimal pH range
Temperature	37–45 °C	25–70 °C	Activity declines sharply above 50 °C

HPLC, SDS-PAGE, and Protein Purity

HPLC analysis showed a dominant peak at ~4.5 minutes with purity >95% [32]. SDS-PAGE and Western Blot confirmed expression of the target protein (~38 kDa) with a relative intensity of 95% compared to the positive control [33]. These results indicate effective removal of contaminants and compliance with halal standards.

Table 5. Chromatogram Results of Enzyme Samples

Component	Retention Time (minutes)	Peak Area	Interpretation
Substrate	2.35	135,200	The signal was reduced in the active enzyme sample due to conversion
Product	4.12	492,600	The signal sharply increased, showing reaction efficiency

Table 6. SDS-PAGE and Densitometry

Lane	Sample	Description	Relative Intensity (%)
M	Protein marker	10–100 kDa	–
1	Negative control	Without plasmid	0
2	4-hour induction	Target expression	80
3	6-hour induction	Maximum target expression	95
4	Enzyme purification	Single 38 kDa band → pure	95

Halal Analysis: Regulatory and Fiqh Perspectives

Based on interviews, certification documents, and laboratory analysis, the enzyme originates from halal microorganisms **(***Bacillus subtilis*), fermentation media are free of haram animal materials, and downstream processes do not involve haram solvents or najis substances [34] [35] [36]. Molecular analyses (PCR, SDS-PAGE) detected no DNA or protein from haram sources, indicating that the enzyme can be categorized as halal and thayyib [37]. This integration of biochemical and fiqh findings establishes a scientific–legal bridge for halal validation, though harmonization of standards across industries remains a challenge

Study Limitations and Recommendations for Future Research

1) In vivo testing limited to small animals and short duration; human or industrial-scale trials are needed
2) Enzyme activity assessed under laboratory conditions; pilot-scale studies recommended
3) Focused on a single enzyme type; broader diversification could improve generalizability
4) Biochemical–halal integration could expand via cross-industry comparison and international certification studies
5) Harmonization of halal standards should be prioritized in future research

Based on comprehensive interviews, certification records, and laboratory tests, the tested food enzyme can be classified as halal and *thayyib* according to both scientific and Islamic jurisprudence standards [38].

Conclusion

This study demonstrates the successful integration of in silico and in vivo approaches in designing synthetic enzymes that are biochemically active, safe, and compliant with halal principles. The primary contribution lies in establishing a unified validation model linking biochemical analysis, bioinformatics, and halal regulation. Practically, this research supports the production of halal-certified bioenzymes with potential applications in the global food industry. Future work should focus on upscaling production, implementing industrial fermentation systems, and integrating artificial intelligence (AI) to enhance halal certification and traceability mechanisms.

Author Contributions

This research was a collaborative effort among all authors. The first author was responsible for study design, conducting in silico simulations, enzyme structure modelling, and bioinformatics analysis for halal assessment. The second author conducted in vivo experiments, including genetic transformation, synthetic enzyme production, and biochemical activity characterization. The third author conducted regulatory analysis, interpreted compliance with halal food industry standards, and formulated recommendations for industrial applications. All authors collectively prepared, critically revised, and approved the final manuscript for publication.

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