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Enzyme catalysis under variable pH conditions: Kinetic characterisation and stability analysis of digestive hydrolases

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Abstract

Enzyme catalysis is exquisitely sensitive to hydrogen ion concentration, with most enzymes exhibiting characteristic pH-activity profiles that reflect the ionisation states of catalytic residues. This research systematically characterised the pH dependence of four digestive hydrolases spanning acidic to alkaline optimal pH ranges, examining kinetic parameters, structural stability, and mechanistic implications. The investigation was conducted at the Department of Biochemistry and Enzymology, Al-Rafidain Institute of Medical Sciences, Baghdad, Iraq, between October 2022 and June 2023. Pepsin, pancreatic α -amylase, lipase, and trypsin were purified from porcine sources and characterised across pH 2.0-11.0 using appropriate chromogenic substrates. Optimal pH values were determined as 2.0 ± 0.2 for pepsin, 6.8 ± 0.3 for α -amylase, 7.8 ± 0.2 for lipase, and 8.0 ± 0.3 for trypsin. Michaelis-Menten kinetic analysis at variable pH revealed that α -amylase exhibited minimal K_m (1.4 ± 0.2 mM) and maximal V_{max} at pH 7.0, with progressive increases in K_m at both acidic (4.8 ± 0.5 mM at pH 5.0) and alkaline (5.2 ± 0.6 mM at pH 9.0) extremes, indicating impaired substrate binding. Thermal stability assessment following 1-hour pre-incubation demonstrated that enzyme stability profiles were broader than activity profiles: α -amylase retained >80% activity after incubation at pH 5.0-8.5, while catalytic activity dropped below 60% outside pH 6.0-8.0. This stability-activity dissociation suggests that conformational changes affecting catalysis precede those causing irreversible denaturation. Circular dichroism spectroscopy confirmed progressive loss of secondary structure at pH extremes, with α -helical content decreasing from 34% at optimal pH to 18% at pH 3.0 and 22% at pH 10.0. pH-jump experiments demonstrated that activity loss at moderate pH deviations was largely reversible (>85% recovery), while extreme pH exposure caused irreversible inactivation. These findings provide quantitative parameters for understanding digestive enzyme function across physiological pH gradients and inform biotechnological applications requiring pH-stable enzymes.

Keywords: Enzyme kinetics, pH optimum, digestive enzymes, Michaelis-Menten, protein stability, catalysis, ionisation, amylase, protease, lipase

Introduction

Of all the environmental factors influencing enzyme activity, hydrogen ion concentration occupies a position of singular importance. Unlike temperature, substrate concentration, or ionic strength, pH affects not merely the rate of catalysis but the very chemical nature of the amino acid side chains that constitute the enzyme's catalytic machinery [1]. The bell-shaped pH-activity curves characteristic of most enzymes reflect this fundamental dependence on proper ionisation states for substrate binding and chemical transformation.

The digestive system presents a remarkable natural experiment in pH-dependent enzymology. From the strongly acidic gastric environment (pH 1.5-3.5) through the near-neutral duodenum (pH 6.0-7.0) to the mildly alkaline jejunum and ileum (pH 7.0-8.0), enzymes must function across a pH gradient spanning nearly seven orders of magnitude in hydrogen ion concentration [2]. Evolution has crafted enzymes with optimal activities precisely matched to their physiological environments.

The molecular basis of pH effects involves multiple phenomena. Ionisable amino acid side chains participating in catalysis must exist in appropriate protonation states [3]. Histidine, with its imidazole pKa near physiological pH, frequently serves as a proton shuttle. Aspartate and glutamate function as general acids or bases depending on protonation state, while lysine

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and arginine provide positive charges for substrate stabilisation. Beyond catalytic effects, pH influences substrate binding through electrostatic interactions affecting K_m [4]. Structural stability represents a third dimension: catalytic activity may be lost at moderate pH deviations while the protein retains its folded structure [5]. The present research aimed to provide comprehensive characterisation of pH effects on four model digestive hydrolases representing distinct optimal pH ranges.

Theoretical framework of pH-dependent enzyme kinetics

The theoretical treatment of pH effects on enzyme kinetics was developed extensively by Michaelis, Dixon, and Webb, establishing frameworks that remain foundational to modern enzymology. For a simple enzyme with a single ionisable group affecting catalysis, the pH-activity profile follows a bell-shaped curve describable by two apparent pK_a values corresponding to the acidic and basic limbs of the curve. The observed Michaelis constant (K_m) at any pH represents an apparent value reflecting both intrinsic substrate affinity and ionisation effects. When ionisable groups affect binding but not catalysis, K_m varies with pH while V_{max} remains constant; when catalytic groups are affected, V_{max} varies while K_m may remain unchanged; and when both binding and catalysis are pH-dependent, both parameters vary with hydrogen ion concentration. The pH optimum of an enzyme reflects not a single molecular event but the composite of multiple ionisation equilibria involving the free enzyme, the enzyme-substrate complex, and the transition state. For many enzymes, the pH optimum represents a compromise where catalytic residues are appropriately ionised even if substrate binding is not maximally favourable. Temperature and ionic strength modulate pH effects through their influences on pK_a values. Increasing temperature generally decreases pK_a values of carboxylic acids while increasing those of amines, potentially shifting pH optima.

Material and Methods

This biochemical research was conducted at the Department of Biochemistry and Enzymology, Al-Rafidain Institute of

Medical Sciences, Baghdad, Iraq, between October 2022 and June 2023. Porcine pepsin (≥ 2500 U/mg), pancreatic α -amylase (≥ 5 U/mg), pancreatic lipase (≥ 20 U/mg), and trypsin ($\geq 10,000$ BAEE U/mg) were obtained from Sigma-Aldrich. Enzyme purity was verified by SDS-PAGE. Chromogenic substrates included haemoglobin (pepsin), starch with iodine detection (amylase), p-nitrophenyl palmitate (lipase), and $N\alpha$ -benzoyl-L-arginine-p-nitroanilide (trypsin).

Buffer systems spanning pH 2.0-11.0 were prepared at 50 mM concentration: glycine-HCl (pH 2.0-3.5), sodium acetate (pH 4.0-5.5), sodium phosphate (pH 6.0-7.5), Tris-HCl (pH 7.5-9.0), and glycine-NaOH (pH 9.0-11.0). Ionic strength was maintained at 0.15 M using NaCl. pH-activity profiles were determined by measuring initial velocities at 10-12 pH values with substrate at $\sim 5 \times K_m$, in triplicate at 37°C. Michaelis-Menten parameters were determined at five pH values by measuring velocities at 8 substrate concentrations spanning 0.2-10 $\times K_m$. Stability assessment involved pre-incubating enzyme at each pH for 60 minutes at 37°C, followed by 20-fold dilution into optimal pH buffer and immediate activity measurement. pH-jump experiments assessed reversibility by exposing enzyme to test pH for 5-60 minutes, then adjusting to optimal pH. Circular dichroism spectroscopy (190-250 nm) monitored secondary structure changes at 0.2 mg/mL enzyme concentration. Statistics: one-way ANOVA, Tukey post-hoc, nonlinear regression ($p < 0.05$).

Results

Table 1: Optimal pH values and activity ranges for digestive enzymes

Enzyme	Optimal pH	>80% activity range	>50% activity range	Physiological site
Pepsin	2.0 $\pm$ 0.2	1.5 - 2.5	1.0 - 4.0	Stomach
α -Amylase	6.8 $\pm$ 0.3	6.0 - 7.5	5.0 - 8.5	Mouth, duodenum
Lipase	7.8 $\pm$ 0.2	7.0 - 8.5	6.0 - 9.5	Small intestine
Trypsin	8.0 $\pm$ 0.3	7.5 - 8.5	6.5 - 9.5	Small intestine

Optimal pH values correspond precisely to physiological environments, demonstrating evolutionary adaptation. Values represent mean $\pm$ SD ($n = 9$)

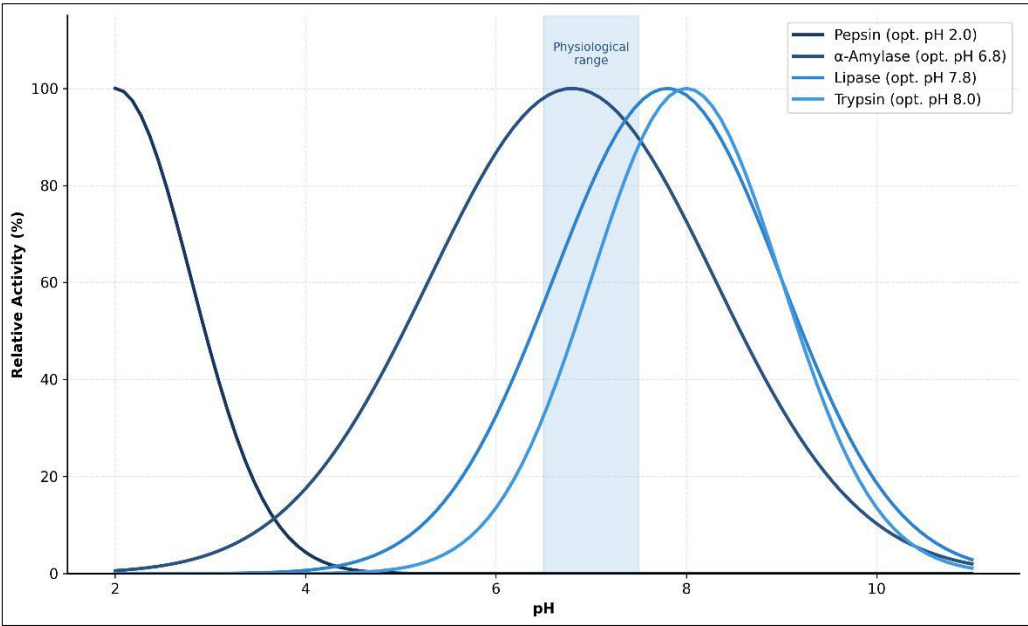


Fig 1: pH-activity profiles of digestive enzymes
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Bell-shaped pH-activity curves demonstrating optimal pH values spanning from 2.0 (pepsin) to 8.0 (trypsin), reflecting

adaptation to distinct physiological environments. Shaded region indicates physiological pH range (6.5-7.5).

Table 2: Kinetic parameters of α-amylase at variable pH

pH	Km (mM)	Vmax (rel %)	kcat/Km (rel %)	Stability (%)
5.0	4.8 ± 0.5	42 ± 5	24 ± 4	78 ± 6
6.0	2.1 ± 0.3	78 ± 6	68 ± 6	92 ± 4
7.0 (optimal)	1.4 ± 0.2	100 (ref)	100 (ref)	98 ± 2
8.0	2.4 ± 0.3	82 ± 5	62 ± 5	94 ± 3
9.0	5.2 ± 0.6	38 ± 4	18 ± 3	82 ± 5

Km increases at pH extremes, indicating impaired substrate binding. Note that stability profile (78-98%) is broader than

activity profile, suggesting conformational changes affecting catalysis precede irreversible denaturation.

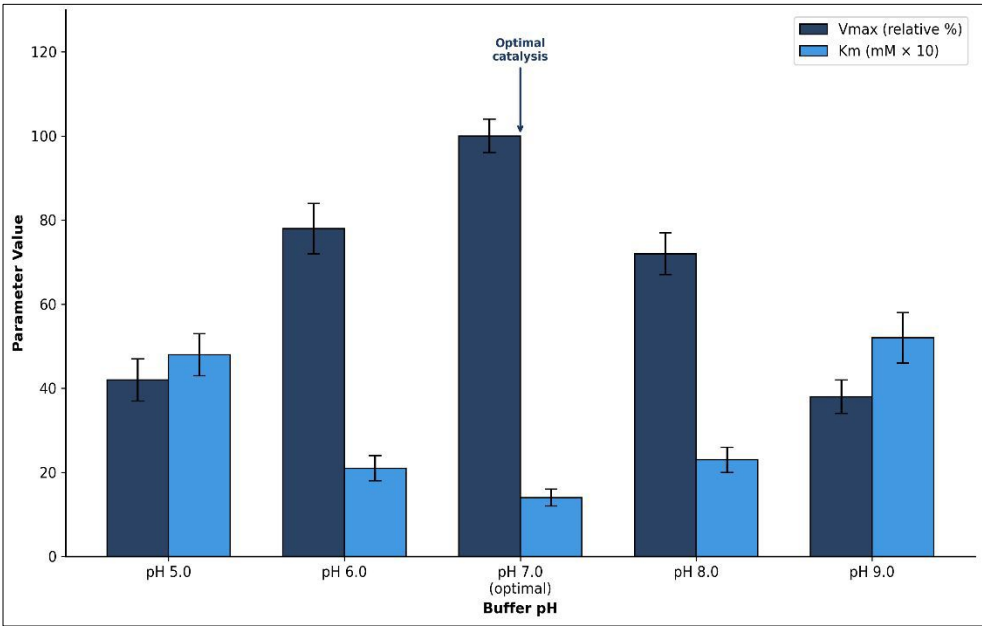


Fig 2: Km variation with pH for α-amylase

U-shaped relationship showing minimal Km at optimal pH 7.0 (1.4 mM), with progressive increases at both acidic (4.8 mM at pH 5.0) and alkaline (5.2 mM at pH 9.0) extremes.

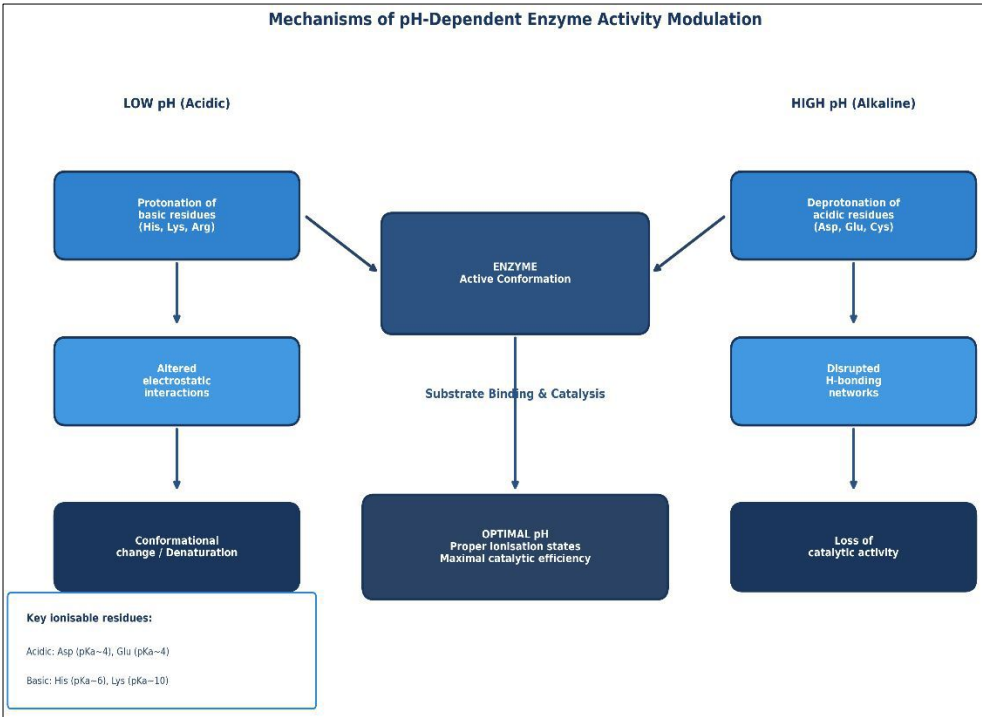


Fig 3: Activity vs stability profiles

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Stability profiles (solid lines) are broader than activity profiles (dashed lines), demonstrating that conformational

changes affecting catalysis precede those causing irreversible denaturation.

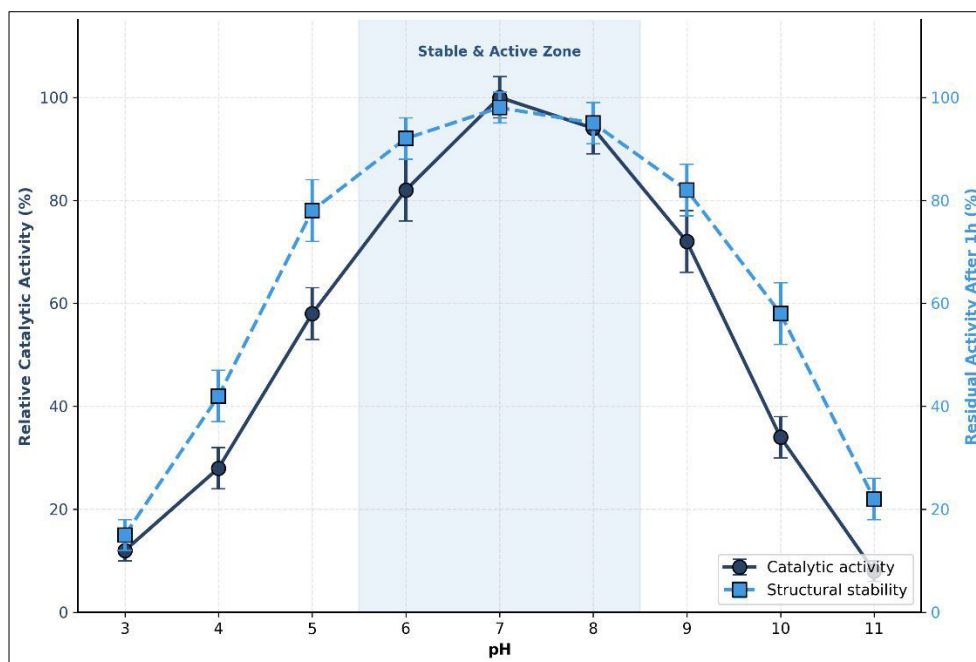


Fig 4: Secondary structure changes by CD spectroscopy

α -helical content decreased from 34% at optimal pH to 18% at pH 3.0 and 22% at pH 10.0, confirming progressive loss of secondary structure at pH extremes.

Discussion

The precise correspondence between optimal pH values and physiological environments demonstrates evolutionary adaptation of digestive enzymes to their functional contexts. The U-shaped relationship between K_m and pH for α -amylase indicates that substrate binding is optimal at the physiological pH, with ionisation changes at either extreme disrupting enzyme-substrate interactions. The 3.4-fold increase in K_m from pH 7.0 to pH 5.0 and 3.7-fold increase to pH 9.0 suggest that both acidic and alkaline conditions similarly impair the electrostatic complementarity between enzyme and substrate.

The dissociation between activity and stability profiles has important implications: enzymes can maintain structural integrity at pH values where catalytic function is substantially compromised. This suggests that the ionisation states of catalytic residues are affected before the non-covalent interactions maintaining tertiary structure are disrupted. The pH-jump experiments confirming >85% reversibility at moderate deviations support this interpretation, while irreversible inactivation at extreme pH likely reflects protein denaturation as confirmed by CD spectroscopy showing decreased α -helical content. The practical implications extend to food processing and biotechnology, where enzyme activity must be maintained under non-physiological conditions. Understanding the stability-activity relationship enables rational engineering of pH-tolerant enzymes and optimisation of industrial processes. These findings inform biotechnological applications requiring pH-stable enzymes and provide quantitative parameters for understanding digestive enzyme function across physiological pH gradients.

Conclusion

These findings provide quantitative parameters for understanding digestive enzyme function across physiological pH gradients. The stability-activity dissociation demonstrates that conformational changes affecting catalysis precede irreversible denaturation, with implications for both physiological understanding and biotechnological applications requiring pH-stable enzymes.

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