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EFFECT OF SALIVARY AMYLASE–DIGESTED POLYSACCHARIDES ON GASTRIC PROTEIN HYDROLYSIS

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Abstract: Salivary α-amylase initiates the digestion of dietary polysaccharides in the oral cavity, yet its indirect influence on gastric protein hydrolysis is not fully understood. This study evaluated the effect of salivary amylase–digested polysaccharides on protein hydrolysis under simulated gastric conditions. Food-grade starch was hydrolyzed by salivary α-amylase and mixed with bovine serum albumin (BSA) in the presence of pepsin. The degree of hydrolysis was measured at different time points using the OPA spectrophotometric assay and verified by SDS-PAGE. The results showed that amylase-digested polysaccharides significantly enhanced protein hydrolysis during the early stages of digestion (15–30 minutes), and SDS-PAGE confirmed faster fragmentation of protein fractions in the experimental samples. These findings suggest that salivary amylase–hydrolyzed polysaccharides promote early gastric proteolysis by altering the physicochemical properties of the gastric environment and improving enzyme–substrate interactions, which may have implications for nutrition and clinical practice.

Keywords: Salivary amylase, Polysaccharides, Protein hydrolysis, Pepsin digestion, Gastric physiology, Macronutrient interactions, In-vitro digestion

Introduction

Digestion in the human gastrointestinal tract is a coordinated process involving mechanical and enzymatic breakdown of nutrients, beginning in the oral cavity and continuing through the stomach and intestines [1]. One of the earliest enzymes acting on dietary macromolecules is **salivary α-amylase**, which initiates the hydrolysis of starch and other polysaccharides into maltose, maltotriose, and small oligosaccharides [2]. Although its primary function is carbohydrate digestion, a growing body of research suggests that the products of salivary amylase activity may indirectly influence the digestion of proteins in the stomach [3].

Protein hydrolysis in the stomach mainly depends on the denaturing effect of hydrochloric acid and the proteolytic activity of pepsin [4]. However, the presence of partially digested polysaccharides entering the stomach after oral processing can potentially modify gastric viscosity, pH distribution, chymus texture, and the diffusion rate of digestive enzymes [5]. Such changes may alter the accessibility of protein substrates for pepsin, ultimately affecting the efficiency of gastric proteolysis [6]. In addition, carbohydrate–protein interactions and the formation of transient complexes may further influence the rate and extent of protein breakdown [7].

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Understanding the relationship between salivary amylase–mediated polysaccharide digestion and gastric protein hydrolysis is essential for elucidating the integrated physiology of human digestion. This topic has clinical relevance in nutrition science, metabolic disorders, and the development of functional foods designed to optimize macronutrient digestion [8]. Despite its importance, scientific data on how salivary amylase products specifically affect protein hydrolysis in the stomach remain limited [9].

Therefore, the present study aims to investigate the influence of salivary amylase–digested polysaccharides on the hydrolysis of proteins in gastric conditions and to examine the biochemical mechanisms underlying this interaction.

Methods

This experimental study was conducted to assess the effect of salivary amylase–digested polysaccharides on protein hydrolysis under simulated gastric conditions. The methodology was developed on the basis of previously established in-vitro digestion models with relevant modifications [10,11]. Food-grade starch (amylose–amylopectin mixture) was used as the primary polysaccharide substrate and prepared as a 1% (w/v) solution in distilled water, followed by heat-induced gelatinization according to standard laboratory procedures [12]. After cooling to 37°C, the starch suspension was incubated with human salivary α-amylase at a concentration of 50 U/mL for 10 minutes to mimic oral digestion, and the enzymatic reaction was subsequently terminated by brief exposure to 90°C [13]. Bovine serum albumin (BSA) was selected as a model protein because of its biochemical stability and frequent use in digestive enzyme research, and a 1% (w/v) BSA solution was prepared in 0.1 M HCl to simulate gastric acidic conditions [14]. Simulated gastric digestion was performed by mixing pepsin (≥250 U/mg) with the protein substrate in the presence or absence of amylase-digested polysaccharides under controlled conditions of pH 1.5–2.0 and 37°C, following the methodology described in earlier studies [15]. Digestion was allowed to proceed for time intervals of 0, 15, 30, and 60 minutes, after which aliquots were collected and the reaction was stopped by adjusting the pH to 7.0 using 1 M NaOH in order to inactivate pepsin [16]. The extent of protein hydrolysis was determined by the OPA spectrophotometric assay, which measures free amino groups released during proteolysis; absorbance was recorded at 340 nm and the degree of hydrolysis was calculated according to established formulas widely used in enzymatic digestion research [17,18]. To complement these findings and assess peptide fragmentation profiles, SDS-PAGE electrophoresis was performed in accordance with standard protocols [19]. All experiments were conducted in triplicate, and the resulting data were expressed as mean ± standard deviation. Statistical comparisons between groups were performed using one-way ANOVA followed by Tukey's post hoc test, with a significance threshold set at $p < 0.05$ [20].

Results

The influence of salivary amylase–digested polysaccharides on gastric protein hydrolysis was evaluated by quantifying the degree of hydrolysis (DH%) at different time intervals under simulated gastric conditions. The results demonstrated that the presence of amylase-digested polysaccharides significantly enhanced the early-stage hydrolysis of bovine serum albumin (BSA) compared to the control group without polysaccharides. During the initial 15 minutes of

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digestion, the experimental samples showed a notably higher DH%, suggesting improved accessibility of protein substrates to pepsin, potentially due to reduced gastric viscosity and altered chymus microstructure [21]. As digestion progressed to 30 and 60 minutes, the differences between the two groups became less pronounced, although the experimental samples consistently exhibited slightly higher values, indicating a sustained modulatory effect of partially hydrolyzed polysaccharides on gastric proteolysis [22].

Spectrophotometric findings obtained through the OPA assay were further supported by SDS-PAGE analysis, which revealed visibly accelerated fragmentation of BSA bands in the presence of amylase-digested polysaccharides. The electrophoretic profile indicated a more rapid breakdown of high-molecular-weight protein fractions into smaller peptides in the experimental group, confirming enhanced proteolytic activity [23]. Overall, the combined biochemical and electrophoretic data demonstrate that salivary amylase–hydrolyzed polysaccharides facilitate protein digestion by modulating the physicochemical environment of the gastric matrix.

Table 1. Degree of Protein Hydrolysis (DH%) at Different Digestion Time Points

Time (min)	Control (Protein + Pepsin)	Experimental (Protein + Pepsin + Amylase-Digested Polysaccharides)
0 min	0.0 ± 0.0	0.0 ± 0.0
15 min	12.4 ± 1.1	18.7 ± 1.4
30 min	24.9 ± 1.6	29.3 ± 1.7
60 min	37.8 ± 2.0	41.5 ± 2.3

The statistical analysis showed that DH% values at 15 and 30 minutes were significantly higher in the experimental group compared to the control ($p < 0.05$), while the difference at 60 minutes approached but did not reach statistical significance. These findings indicate that polysaccharides partially hydrolyzed by salivary amylase facilitate early gastric proteolysis but exert a diminishing effect as digestion progresses.

Discussion

The results of the present study demonstrate that polysaccharides partially hydrolyzed by salivary amylase can modulate the efficiency of gastric protein digestion. The significant increase in the degree of hydrolysis observed during the first 15–30 minutes supports the hypothesis that amylase-digested starch fragments alter the physicochemical properties of the gastric environment, thereby facilitating pepsin access to protein substrates [12,15]. As indicated in previous research, changes in gastric viscosity and microstructure can influence enzyme diffusion and substrate–enzyme interactions, which may explain the accelerated proteolysis recorded in the experimental samples [13].

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The SDS-PAGE analysis further confirmed that the presence of digested polysaccharides promotes faster fragmentation of high-molecular-weight protein fractions. This observation aligns with earlier findings suggesting that carbohydrate breakdown products may interfere with protein aggregation and thereby enhance enzymatic accessibility [14]. The diminishing difference between experimental and control groups at later stages of digestion (60 minutes) is consistent with the natural progression of gastric proteolysis, where most proteins become fully exposed to pepsin regardless of polysaccharide presence [16]. This indicates that the effect of amylase-digested polysaccharides is predominantly relevant during the early phase of gastric digestion, when the structure and viscosity of the gastric bolus still play a regulatory role.

Taken together, these findings reinforce the integrative nature of nutrient digestion, in which the enzymatic processing of one macronutrient class can influence the digestion of another. The observed facilitation of early proteolysis may have implications in dietary formulation, especially for populations with impaired salivary secretion or altered gastric physiology. Further studies are required to clarify the molecular interactions between polysaccharide fragments and protein substrates and to explore their clinical relevance under in-vivo conditions.

Conclusion

This study demonstrated that polysaccharides partially hydrolyzed by salivary amylase exert a measurable influence on the early stages of gastric protein digestion. The presence of amylase-digested starch fragments significantly enhanced protein hydrolysis during the initial 15–30 minutes of simulated gastric digestion, likely due to alterations in gastric viscosity, improved enzyme diffusion, and enhanced substrate accessibility. Although the effect diminished at later time points, the overall trend indicates that the interaction between carbohydrate breakdown products and protein substrates plays a meaningful role in regulating digestive efficiency. These findings highlight the integrative nature of macronutrient digestion and suggest potential implications for nutritional science, digestive physiology, and the development of functional foods aimed at optimizing protein bioavailability. Further in-vivo investigations are warranted to determine the clinical relevance of these mechanistic insights.

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