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# STRUCTURAL DETERMINANTS OF STARCH DIGESTIBILITY: A COMPARATIVE ANALYSIS OF α-AMYLASE ACTIVITY IN QUINOA, POTATO, OAT, AND TAPIOCA

Biological Science

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## ABSTRACT

Starch is one of the most important carbohydrate sources in the human diet, but its digestibility can vary significantly depending on its botanical origin. These differences arise from variations in granule size, surface structure, crystallinity, and water absorption properties, which affect how easily digestive enzymes can access and break down the starch. In this study, the early-stage digestibility of quinoa, potato, oat, and tapioca starches was investigated using a standardized three-minute α-amylase assay. Glucose released during hydrolysis was measured as an indicator of starch digestion, and five independent trials were conducted for each starch type to ensure reproducibility. Among the starches tested, quinoa released the highest amount of glucose, followed by potato, oat, and tapioca starches. The results suggest that differences in digestibility are driven largely by granule-level structural characteristics rather than amylose and amylopectin composition alone. This study demonstrates that simple laboratory assays can effectively reveal meaningful relationships between starch structure and enzymatic digestion. The findings further indicate that hydration behavior, granule morphology, and enzyme accessibility play key roles in determining the rate of early starch hydrolysis.

## KEYWORDS

## INTRODUCTION

Starch is the main long-term carbohydrate reserve in plants and remains a fundamental component of the human diet. Although it is often treated as a single class of biomaterial, starches from different botanical sources exhibit substantial variation in their digestibility. These differences arise from variations in granule architecture, crystalline–amorphous organization, hydration behavior, and interactions with minor components such as lipids and dietary fibers.

Starch digestion begins with the action of α-amylase, which hydrolyzes internal α-1,4 glycosidic bonds. This enzyme is most active in hydrated, amorphous regions of the granule, making structural accessibility a key determinant of hydrolysis during the initial stages of digestion [1–4]. Previous studies have shown that starches with smaller granules, porous surfaces, and faster swelling behavior generally exhibit higher rates of glucose release [5–7]. In contrast, larger, more compact granules or those associated with lipids tend to resist enzymatic penetration and therefore digest more slowly [8–10]. While the relative proportions of amylose and amylopectin influence overall digestibility, short-term hydrolysis is more strongly governed by physical features such as hydration rate, granule morphology, and surface characteristics. In this context, the present study examines the first three minutes of enzymatic hydrolysis in four starch sources: quinoa, potato, oat, and tapioca to understand how granule-level structural properties influence initial glucose release.

## LITERATURE REVIEW

Previous studies have shown that starch digestibility is influenced more by structural characteristics than by botanical source alone. Among these features, granule size plays a key role by determining the available surface area for enzymatic attack. Smaller granules provide a higher surface-to-volume ratio, which facilitates faster enzyme binding and hydrolysis. For example, quinoa starch is known to contain some of the smallest granules among common dietary starches (approximately 0.5–2 μm), which contributes to its rapid swelling behavior and relatively high early digestibility [7].

In contrast, potato starch contains much larger granules; however, its surface irregularities and strong swelling capacity can still enhance enzyme accessibility under hydrated conditions [8]. Oat starch typically presents a heterogeneous granule population and is often associated with surface lipids and non-starch polysaccharides such as β-glucans, both of which can restrict enzyme penetration [11]. Tapioca starch, on the other hand, is characterized by smooth, compact granules that hydrate more slowly, thereby limiting early enzymatic access [12].

A range of analytical approaches has been used to evaluate starch digestibility, particularly those that emphasize short-term hydrolysis behavior. Rapid glucose assays, iodine–starch colorimetric methods and standardized in vitro static digestion models have been widely applied in both research and teaching

laboratories [13–15]. Overall, previous work consistently indicates that early-stage starch hydrolysis is governed primarily by structural openness, porosity, crystallinity, and granule integrity rather than by chemical composition alone.

## METHODOLOGY

### MATERIALS

Four powdered starches, quinoa, potato, oat, and tapioca, were used to ensure consistent hydration. Commercial α-amylase acted as the digestive enzyme. Distilled water served as the solvent. Glucose colorimetric strips were used for semi-quantitative glucose detection. Additional materials included reaction cups, digital scales, timers, pipettes, and stirring rods.

### EXPERIMENTAL DESIGN

In this study, the independent variable was the type of starch (quinoa, potato, oat, and tapioca), while the dependent variable was the glucose concentration measured after three minutes of enzymatic digestion. To ensure fair comparison across samples, key experimental conditions such as enzyme concentration, starch mass, reaction volume, incubation time, and ambient temperature were carefully kept constant.

Each starch type was tested in five independent trials to improve reliability and reduce experimental error. A negative control containing enzyme without starch was included to confirm that no external glucose source contributed to the results. A positive control using a glucose standard ensured that the detection method was functioning properly. In addition, an iodine test was performed to qualitatively verify the presence and extent of starch degradation during the reaction.

### PROCEDURE

For each trial, 0.3 g of α-amylase was first dissolved in 5 mL of distilled water to prepare the enzyme solution. A fixed amount of starch was then added, and timing was started immediately upon mixing. The reaction mixture was gently stirred to ensure uniform dispersion of the starch and enzyme and was allowed to incubate for three minutes at room temperature (22–24 °C).

At the end of the incubation period, glucose test strips were immersed in the reaction mixture and allowed to develop for approximately 30 seconds.

The resulting colour change was compared against the manufacturer's reference chart, and the corresponding glucose concentration was recorded. In parallel, an iodine test was performed by adding a few drops of iodine solution to the reaction mixture. The resulting colour intensity was used as a qualitative indicator of the extent of starch hydrolysis, with a lighter colour reflecting greater breakdown of starch.

### DATA HANDLING

Glucose values from all trials were entered into data tables. Means,

standard deviations, and standard errors were calculated. Trends across starch types were analyzed and compared with known structural properties documented in prior research.

## RESULTS

### QUALITATIVE GLUCOSE STRIP OBSERVATIONS

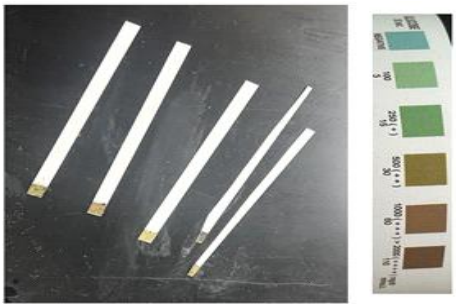

**Figure 1. Hydrolysis of Quinoa Starch by α-Amylase: Glucose strip test indicating a moderate-high glucose production following enzymatic digestion of quinoa starch.**

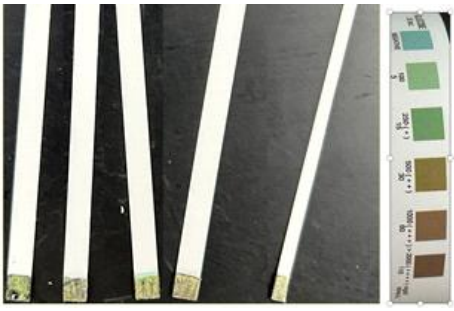

**Figure 2. Hydrolysis of Oat Starch by α-Amylase: Glucose strip test indicating moderate-low glucose production following enzymatic digestion of oat starch.**

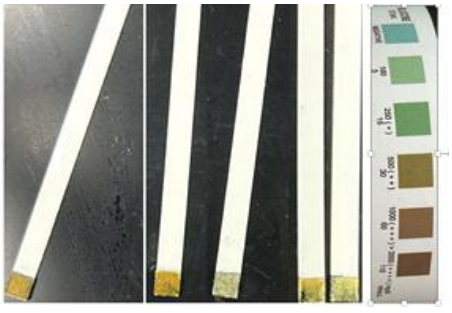

**Figure 3. Hydrolysis of Potato Starch by α-Amylase: Glucose strip test indicating moderate glucose production following enzymatic digestion of potato starch.**

**Figure 4. Hydrolysis of Tapioca Starch by α-Amylase: Glucose strip test indicating a low glucose production following enzymatic digestion of tapioca starch.**

The glucose test strips showed noticeable differences among the starch samples. Quinoa starch produced the darkest colour, suggesting that it released the greatest amount of glucose during the three-minute digestion period. Potato starch also showed a strong response but was lower than quinoa. Oat starch generated a moderate amount of glucose, while tapioca starch produced the lightest colour, indicating the lowest level of starch breakdown. These visual observations closely matched the measured glucose values, providing confidence in the reliability of the results.

### QUANTITATIVE GLUCOSE VALUES

Mean glucose concentrations for each starch followed the pattern: quinoa > potato > oats > tapioca

This trend was consistent across all trials and exhibited low within-group variance.

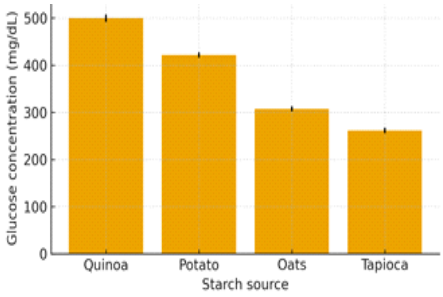


Figure 5. Mean glucose concentration (mg/dL) produced by each starch source after 3 minutes of digestion. Error bars represent standard deviation.

### STRUCTURAL CORRELATIONS

A negative relationship was observed between amylose content and glucose concentration, while amylopectin content showed a positive relationship.

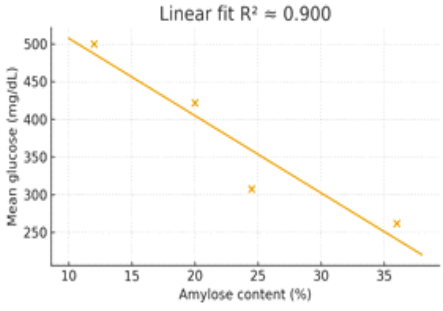


**Figure 6. Relationship between amylose content (%) and mean glucose concentration (mg/dL). A negative linear trend is observed, with an approximate coefficient of determination $R^2 \approx 0.900$, supporting the idea that higher amylose content is associated with lower digestibility in this short-term assay.**

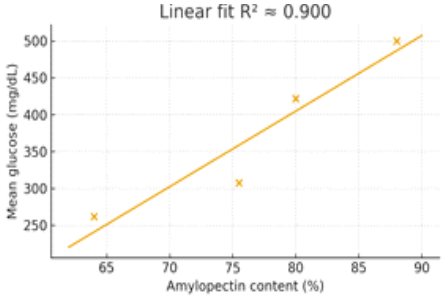


**Figure 7. Relationship between amylopectin content (%) and mean glucose concentration (mg/dL). The positive linear trend ($R^2 \approx 0.900$) indicates that starches richer in amylopectin tend to be more rapidly digested by α-amylase under the experimental conditions used.**

These correlations supported previously documented biochemical expectations.

## DISCUSSION

The results reinforce the idea that early enzymatic digestion is governed by granule accessibility rather than macromolecular composition alone. Quinoa's exceptionally small granules and rapid hydration allow α-amylase to penetrate amorphous regions quickly, resulting in the highest glucose release. Potato starch, although having larger granules, swells rapidly and develops micro fissures that

improve accessibility, explaining its intermediate values. Oat starch displayed moderate hydrolysis, which fits previous reports that β-glucans and surface lipids hinder amylase action by forming protective layers around the granules [11]. Tapioca starch exhibited the lowest outcomes despite its high amylopectin content; its smooth, compact granules restrict early hydration and limit enzyme binding during short digestion intervals.

The observed amylose and amylopectin correlations largely reflect these structural realities. Higher amylose content tends to be associated with greater crystallinity and enzyme resistance, whereas amylopectin's branching architecture generally promotes accessibility. However, during the brief three-minute period used in this investigation, physical properties such as swelling rate, porosity, and granule diameter overshadow compositional influences.

These findings align closely with previous literature and confirm that simple, controlled classroom assays can reveal genuine biological patterns in starch digestion. Future investigations may explore processed starches, cooking effects, or comparison between raw and gelatinized forms.

## CONCLUSIONS

This study demonstrates that the early stages of starch digestion are primarily governed by granule-level structural characteristics. Among the starches evaluated, quinoa showed the highest glucose release during the three-minute assay, followed by potato, oat, and tapioca starches. These differences are largely attributed to variations in hydration rate, granule size, porosity, and surface morphology, which together control enzyme accessibility during initial hydrolysis.

The findings are consistent with previous reports suggesting that structural features play a dominant role in early starch digestion, whereas chemical composition becomes more influential over longer digestion periods. Overall, the results highlight that even simple, short-duration laboratory assays can effectively capture meaningful structure–function relationships in starch systems and provide valuable insights into their enzymatic behavior.

## ACKNOWLEDGEMENTS

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