Investigating Lipid-Modulated Catalysis: The Impact of Lipid Interactions on Pancreatic Alpha-Amylase Activity

Aditi Jakkula, Stephanie Bellas, Dr. Steven Lenhert, Vincent Tocci
Florida State University College of Arts and Sciences

Introduction

Amylase is a key enzyme involved in the hydrolysis of starch into sugars, playing a vital role in carbohydrate metabolism. While amylase is well-studied, the potential impact of lipids on its catalytic function is less explored (Bickel, 2002). Lipids (particularly membrane lipids) can influence enzyme structure and function, affecting activity, substrate specificity, and stability (Gould,1982). This project aims to investigate how interactions with various lipid environments affect amylase activity and catalytic rate/efficiency. The larger widespread of the outcomes of this experiment have potential to enhance drug function, such as insulin related medication.

Methodology

Objective: Measure α-amylase activity via absorbance decrease at 620 nm (starch hydrolysis).

Materials & Reagents

- Phosphate Buffer (200 mM, pH 6.9, 6 mM NaCl): Store cold.
- Iodine Reagent (5 mM I_2 / 5 mM KI): Fresh weekly, amber bottle.
- α-Amylase (0.05 mg/mL): Dissolve in warm buffer (37°C).
- Starch Solution (1% w/v): Dissolve 10 mg rice starch in 1 mL water (heat gently).

Experimental Protocol

1. Spectrophotometer Setup: 620 nm, 37°C.
2. Reaction (In-Cuvette):
   - Mix: 250 µL buffer + 250 µL starch + 100 µL iodine.
   - Insert cuvette, wait 30 sec.
   - Add 250 µL α-amylase, mix.
3. Measurement: Record A620 every 30 sec (5-10 min).

Data Analysis

- Plot A620 vs. time.
- Calculate rate: v=ΔAΔtv = \frac{\Delta A}{\Delta t}v=ΔtΔA.
- Calibration curve for starch quantification.

Controls & Variations

- Control: Omit starch (3x replicates).
- Lipid Effect: Test 10%, 15%, 20% oleic acid.
- Lipid Control: Lipid without enzyme (3x replicates).

Expected Results

- Time-series plots for enzyme kinetics.
- Calibration curve for starch breakdown.
- Effect of surfactants on α-amylase activity.

Discussion

Plant extracts, which are rich in natural antioxidants, can interfere with enzyme activity assays, specifically the starch-iodine method for measuring α-amylase inhibition. In Ononamadu et al., the starch-iodine method showed minimal inhibition for some extracts, whereas the DNSa method indicated a strong inhibition, which suggests inconsistencies in the results.

Extracts AM, EA and QC contained high levels of flavonoids and phenolics which faded the blue-black-starch-iodine complex. This was likely due to their electron-donating properties. It was found that higher extract concentration resulted in greater decolorization, which indicated the observed effect was due to chemical interactions rather than weak enzyme inhibition.

In addition, higher extract concentrations resulted in greater decolorization which showed the observed effect was due to chemical interactions rather than weak enzyme inhibition. In Ononamadu et al., the starch-iodine method produced higher inhibition values when the absorbance was measured immediately after mixing compared to measurements taken after a twenty minute delay.

Interference may have occurred due to the prolonged interaction between the extracts and assay reagents. However, the DNSA method provided more consistent and reliable measurements of the α-amylase inhibition. Modification such as reducing extract concentrations may be used for the starch-iodine assay to improve accuracy. To conclude, these findings show the importance of selecting appropriate analytical methods when evaluating natural plant compounds in order to ensure accurate results.

Results From Ononamadu et al.

Images taken from: Ononamadu, C., Ezeigwe, O., Owolarafe, T., Ihegboro, G., Lawal, T., Salawu, K., ... Aminu, I. (2020a). Starch-iodine assay method underestimates α-amylase inhibitory potential of antioxidative compounds and extracts. BioTechnologia, 101(1), 45–54. doi:10.5114/bta.2020.93103

Table 3. Correlation of α-amylase inhibitory activity (IC_{50}) of the tested extract/fractions and compounds using starch-iodine methods and the DNSA method

	ST-ID method I	ST-ID method II	DNSA method
ST-ID method I	–	$r = 0.901$ [a]	$r = 0.807$ [a]
ST-ID method II	$r = 0.901$ [a]	–	$r = 0.787$
DNSA method	$r = 0.807$ [a]	$r = 0.787$	–

[a] correlation is significant at the 0.05 level; ST-ID I – starch-iodide method (approach I), ST-ID II – starch-iodide method (approach II), DNSA – 3,5-dinitrosalicyclic acid method

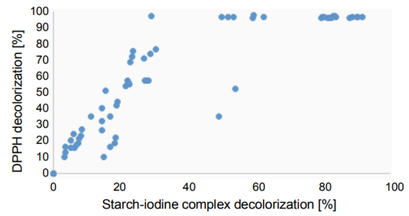
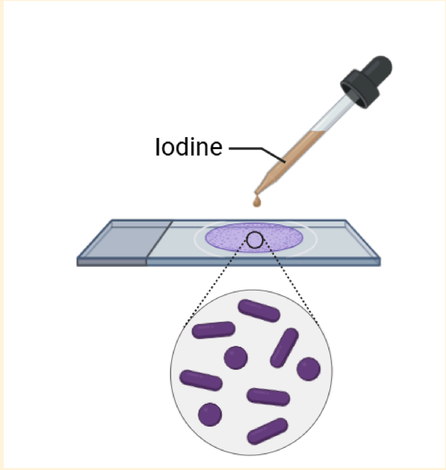


Fig. 3. Correlation of DPPH reduction (%) and starch-iodine complex decolorization by the tested extract, fractions, and compounds (%)



Iodine assay method - image from BioRender

Conclusion

In summary, the inconsistencies observed between the starch-iodine and DNSa methods suggest that the former may not be suitable for screening extracts with high antioxidant potential. The extracts which contain flavonoids and phenolics were significantly decolorized by the starch-iodine complex, due to their electron-donating properties and therefore resulting in misleading data.

The DNSA method proved to be a more reliable method of measuring α-amylase inhibition. Overall, the findings of the study suggested that the starch-iodine method could still be useful for preliminary screening, given only low extract concentrations are used and the absorbance is measured immediately after adding the iodine solution.

References

Gould, R J, et al. "Lipid Effects on the Binding Properties of a Reconstituted Insulin Receptor." Journal of Biological Chemistry, Elsevier, 10 Jan. 1982, www.sciencedirect.com/science/article/pii/S0021925819683894.

Ononamadu, C., Ezeigwe, O., Owolarafe, T., Ihegboro, G., Lawal, T., Salawu, K., ... Aminu, I. (2020a). Starch-iodine assay method underestimates α-amylase inhibitory potential of antioxidative compounds and extracts. BioTechnologia, 101(1), 45–54. doi:10.5114/bta.2020.93103