

Effects of phenolic acids on digestion of starch particles

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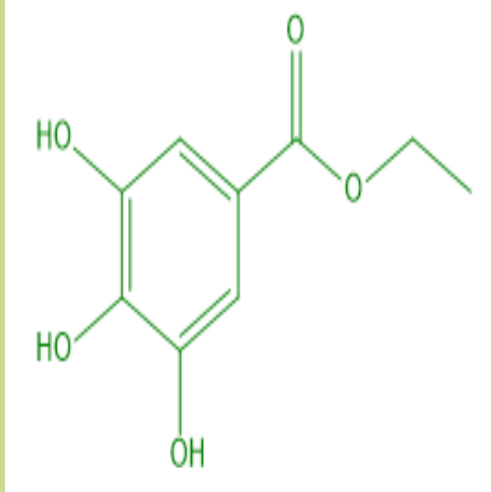
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Introduction

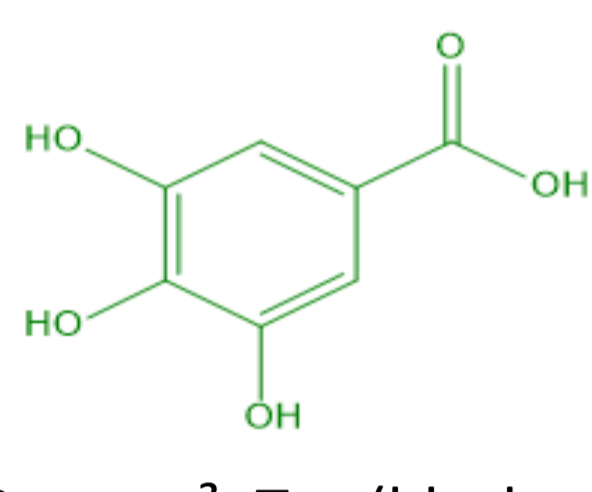
Starch is the principal storage form of energy in plants and is comprised of two polymers: amylose, and amylopectin. Starch is the main dietary source of glycemic carbohydrates and its digestion pattern is directly related to blood glycemic levels and satiety. Controlling starch digestion is therefore key to eliciting a prolonged and steady post-prandial release of glucose into the blood stream in order to control energy levels throughout the day. It is commonly accepted that dietary polyphenolic compounds, plant metabolites that play an important role in the sensory and nutritional properties of food,¹ can slow down or inhibit the starch digestion process; however, the role of the phenolic compounds' structure remains unclear. In this study we hypothesize that: **1-the structure of phenolic acids and their hydrogen bonding capacity and 2- their physical state in the test matrix (incorporated in starch particles or free in solution)** will impact starch digestion kinetics. Starch particles were chosen as a slowly digestible form of starch that could easily be incorporated into products aimed at eliciting a prolonged energy delivery. The phenolic acids used in this study are ones commonly found in the fruit matrix, specifically gallic acid, ethyl gallate and vanillin.

Ethyl Gallate



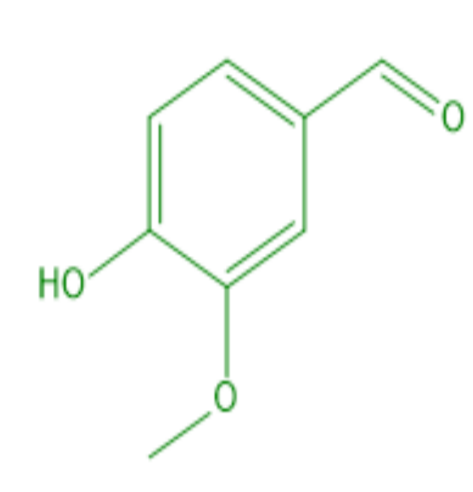
Sources²: wine

Gallic Acid



Sources³: Tea (black, oolong), Nuts (chestnuts, walnuts), Herbs/Spices (cloves, oregano), berries

Vanillin



Sources⁴: primary compound in natural and artificial extract

1. Materials:

Particles:

Corn Starch Ethyl Gallate
Gallic Acid Vanillin

Digestive Solutions:

KH₂PO₄ NaHCO₃
NaCl McCl₂(H₂O)₆
(NH₄)₂CO₃ NaOH
HCl CaCl₂(H₂O)₂
Deionized water

Enzymes:

α -amylase Pepsin
Pancreatin

Amyloglucosidase

*All materials were purchased from Sigma Aldrich (St Louis, MO).

2. Starch particle formation:

Starch particles were formed from a hot starch paste (20% w/w starch in water) and then the solution was heated (T>75°C) to induce gelatinization. The paste was then deposited on aluminum foil using a syringe to form particles \approx 4-6 mm in diameter. The particles were cooled at 4°C for at least a week to induce retrogradation. Similar particles were made by incorporating the ethyl gallate, gallic acid and vanillin into the solution of starch and water at concentrations of \approx 0,176 M (3g gallic acid/kg starch particles).

3. Moisture and starch content of particles:

Moisture of starch particles was measured using a Mettler Toledo™ HE53 Halogen Moisture Analyzer (Mettler Toledo Mississauga) and given as % of total particle mass. This allowed the total starch content (and therefore glucose content) in the particles to be determined accordingly:
100% mass-% humidity=% total solid content
%total solid content x mass of particles to be digested= mass of solids (-mass of phenolic acids added)

Mass of solids/162 g/mol (molar mass of glucose unit of starch= mols of glucose available for digestion.

4. In-vitro digestion:

Using the protocol described by (Minekus et al., 2014)⁵, the starch particles were subjected to three phase in-vitro digestion process. The protocol was modified to include amyloglucosidase at a concentration of 4 mg/ml. The organization of the samples is described in Figure 1: \approx 5 g of particles were added to each test tube. To the test tubes where the phenolic acids were free in solution, the phenolic acids were added to the salivary digestive solution.

5. Glucose measurements:

To determine the extent to which the starch particles were digested in each tube, a glucometer was used to follow starch digestion throughout the 2-hour intestinal phase. The concentration of glucose within each test tube at the end of the intestinal phase served as the raw data for the final results:
mmol glucose in solution/total mmol glucose units present in starch=% starch digested.

6. Statistical Analysis:

The average final glucose concentration, as well as the standard deviation for each phenolic acid type/location were determined. A pairwise comparison test (Tukey test) was also used to determine if a statistically significant difference was present between the sets of data.
*Aberrant values were discarded due to suspicion of experimental error

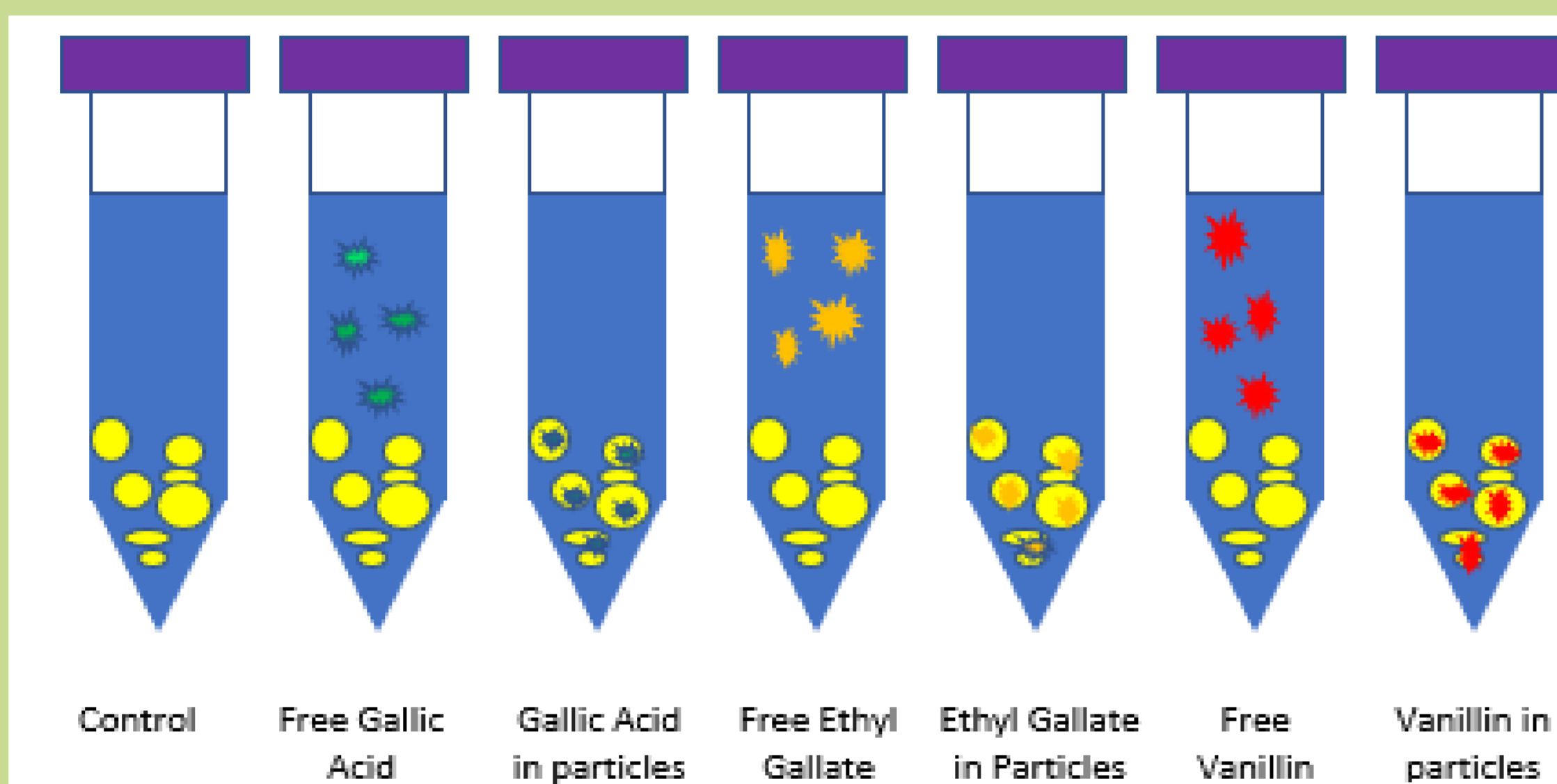
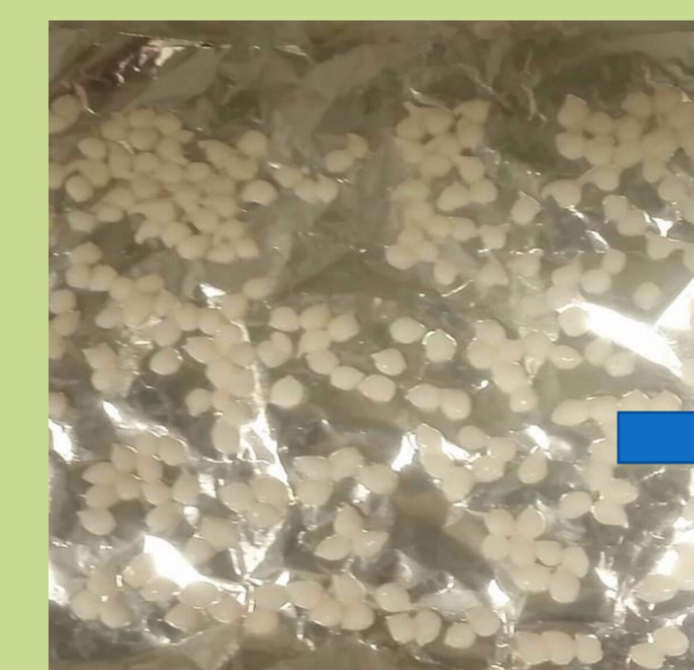


Figure 1. Organization of sample test tubes according to phenolic acid type and location



Starch particles

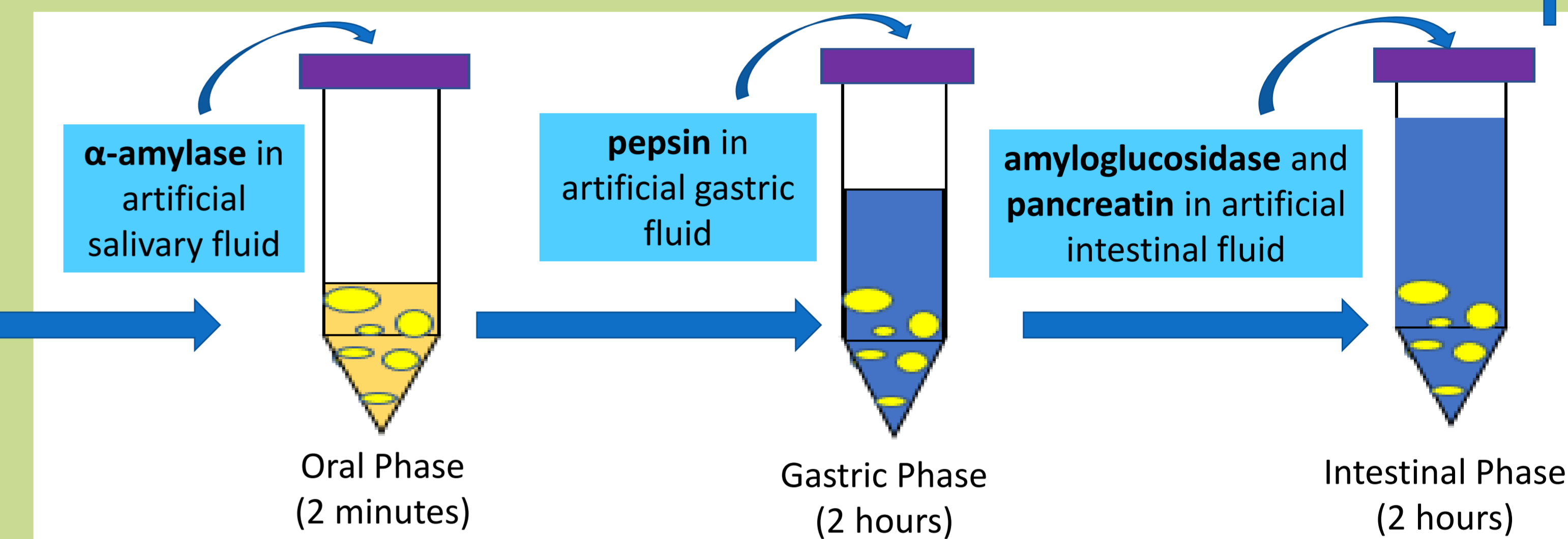


Figure 2. 3-step in-vitro digestion of starch particles. All digestive phases occurred in a water bath at 37°C and were subjected to mechanical shaking to simulate in vivo digestion.

Results and Discussion

Effect of location of phenolic acids on starch digestion kinetics:

While no statistically significant difference was observed between the rate of starch digestion with the phenolic acids within the particles and outside of the particles, a trend nevertheless appeared suggesting that the starch in the tubes containing the polyphenols in solution digested at a slower rate than the starch in the tubes containing the polyphenols in the particles. This may be due to the phenomena that during gelatinization, phenolic acids form hydrogen bonds with starch, which may henceforth have an inhibitory effect on starch retrogradation.⁶ Inhibited retrogradation translates to decreased crystallinity of starch particles and increased accessibility to digestive enzymes.

Effect of phenolic acid structure on starch digestion kinetics:

While no significant difference was found between gallic acid and vanillin on the digestion kinetics of starch, ethyl gallate proved to be significantly less effective at slowing starch digestion when found within the matrix of the starch particles. It is possible that ethyl gallate is less effective at forming hydrogen and conformational bonds with α -amylase, thus blocking the digestive enzymes' action. This may be due to ethyl gallate's ester functional group as compared to the carboxylic acid group found on gallic acid. When compared to vanillin, ethyl gallate is a much larger molecule and potentially has a greater inhibitory effect on starch retrogradation, which may explain why it digested at a much faster rate than vanillin, when found in the particles.

Overall effect of phenolic acids on starch digestion as compared to the control:

When compared to the control, the results, with the exception of ethyl gallate within the particles, suggest that phenolic acids do not have a significant impact on starch digestion kinetics when starch is found in the form of a retrograded matrix, as compared to the impact of the retrogradation process itself. Indeed, after the two hour intestinal phase, \approx 95% of the starch remained undigested (in or out of the presence of phenolic acids) and in an in vivo context, would likely pass through to the colon and therefore be considered dietary fiber. More research is therefore necessary to determine the starch matrix that best lends itself to manipulation, while not entirely inhibiting digestion.

Figure 3. Comparison of total glucose release according phenolic acid type and location

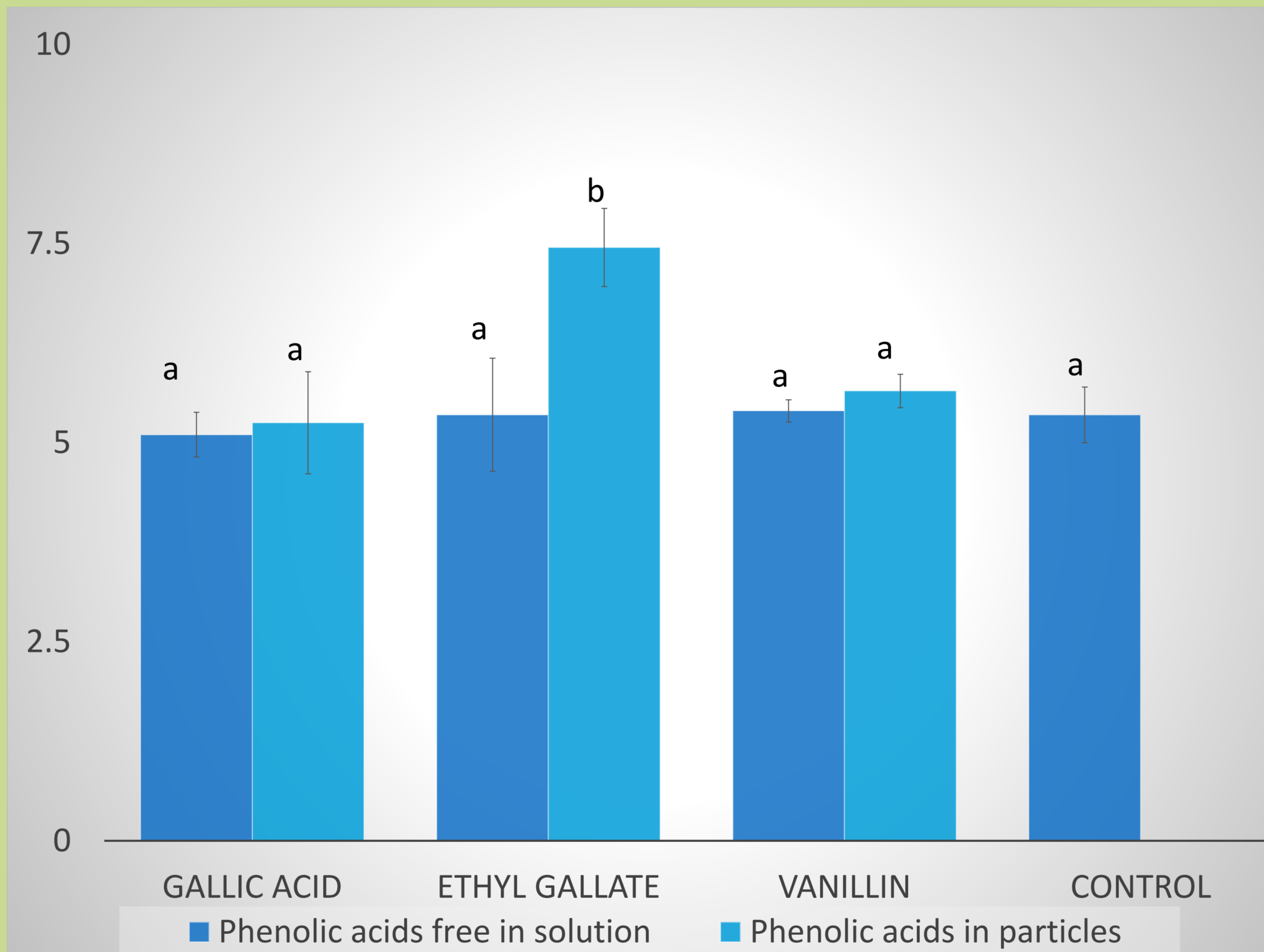


Table 1. Average total final values for glucose release at the end of in vitro digestion, \pm standard deviation

	Gallic Acid	Ethyl Gallate	Vanillin	Control
Phenolic acids free in solution	5.10 \pm 0.28 ^a	5.35 \pm 0.71 ^a	5.40 \pm 0.14 ^a	5.35 \pm 0.35 ^a
Phenolic acids in particles	5.25 \pm 0.64 ^a	7.45 \pm 0.49 ^b	5.65 \pm 0.21 ^a	

Subscripts ^{a/b} indicate significant different differences between data, as determined by a pairwise comparison (95% confidence) using a Tukey test.

Conclusion

The results of this study suggest phenolic acids have the potential to reduce starch retrogradation, thus increasing starch digestion. When the phenolic acids studied are relatively small, their interactions with the digestive enzymes become less effective and as a result, the impact of these phenolic acid-enzyme interactions becomes negligible in comparison to the effects of the retrogradation of starch on its digestion. Indeed, the particles used in this study were very resistant to digestion and in the future it would be preferable to use particles that were slowly digestible rather than mostly resistant.

These insights contribute to a better understanding of interactions among food components and the influence of these interactions on glycaemia, which in turn may lead to dietary solutions enabling athletes to regulate their energy levels, while avoiding spikes in blood sugar throughout a long day of training or competition.

Going forward, more in-depth research is necessary to determine the impacts of various polyphenol functional groups on their interactions with the starch matrix, as well as the digestive enzymes. Studies comparing the effects of smaller phenolic acids to larger tannins on starch digestion would aid in confirming the results obtained in this study.

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