Starch Digestion and Glucose Absorption in the Small Intestine
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ABSTRACT
The main objective of this work was to understand phenomena occurring during food digestion by using a dynamic \textit{in vitro} Small Intestine Model (SIM), of particular interest was to study the effect of mixing and food formulation on starch hydrolysis and glucose absorption. The SIM reproduces the characteristic mixing of the human small intestine by expansion and contraction of two cuffs around the external tube. Corn starch hydrolysis was studied in single phase fluids varying the concentration of amylase and the viscosity of digesta by adding guar gum (0.5 %, w/v) under simulated physiological conditions.

Results showed the effect of segmentation motion on nutrient delivery to the intestinal wall as a consequence of changes in the mass transfer coefficient. This is most likely due to the increased mixing in the SIM. The rate of glucose absorption was decreased from 0.75 to 0.48 \( \mu \text{M/s} \) when guar gum (0.5%, w/v) was added to the corn starch solution. As the viscosity of the solution increased from 2 to 200 mPa.s glucose absorption was reduced up to 50%. Experiments of starch digestion in the SIM, with and without the presence of guar, have shown that guar reduces the rate of starch digestion by impairing mixing and reducing diffusion within the fluid.

This research has demonstrated the capability of using an \textit{in vitro} rig to simulate and obtain an engineering understanding of transport phenomena occurring in the small intestine leading to absorption of active components. Current work is aimed at developing a reaction engineering understanding of the gastrointestinal tract.

Keywords: Starch hydrolysis; digestion; absorption; glucose; small intestine.

INTRODUCTION
When refined sugars and rapidly digested carbohydrate are included on a regular basis in our diets, blood glucose and insulin responses are usually enhanced, resulting in dietary related chronic diseases such as diabetes and obesity [1-4]. To avoid this scenario, structured foods with specific health benefits should be designed in conjunction with studies to understand how food formulation impacts on digestion and absorption rates in the gastrointestinal tract (GIT).

Digestion is a very complex process at which the small intestine is a key step responsible for most of the active molecules absorption. To understand and reproduce this process, \textit{in vitro} and \textit{in silico} models have been designed [5-7]. Nevertheless, details of flow dynamics and mixing have not been extensively studied. Mixing of lumens contents is essential in enhancing food digestion and absorption. Intestinal motility allows interactions between food and pancreatic digestive enzymes and biliary salts that enable food digestion.

Intestinal absorption is also influenced by the physical form of the food ingested and the physical properties (viscosity, density, concentration, flow rate) of the lumen content. It has been reported that increasing viscosity of the lumen content, for example, could reduce the nutrient absorption through the epithelium reducing glucose levels in blood [2,3]. Addition of viscous fibers into the food not only increases the viscosity of the lumen but also may protect starch from enzymatic attack. Overall, by changing food formulation, the rate of nutrients absorption could be modified resulting in structured food with a health benefits for the consumer.

The main objective of this work was to reproduce and model starch digestion by studying the effect of mixing and viscosity on glucose absorption rate. Digestion and absorption were studied by using a dynamic small intestine model simulated the physiological conditions of the human small intestine.
MATERIALS & METHODS

All reagents and digestive enzymes were purchased from Sigma-Aldrich (UK). Pancreatic α-amylase from porcine (30 units/mg solid SIGMA-Aldrich, UK) and amylglucosidase from Aspergillus niger (300 units/mL SIGMA-Aldrich, UK) were used for starch digestion. Cornstarch (1.0%, w/v) was previously gelatinized in a boiling water bath during 30 minutes with intermittent mixing. After gelatinization, the starch solution was cooled at room temperature. The enzyme solution (50 ml) was freshly prepared for the digestion analysis by dissolving the pancreatic α-amylase (2.5 and 25 U/ml) and amyloglucosidase (3 U/ml) into a pancreatic mix solution for 10 minutes at room temperature. The Small Intestine Model (SIM) used in this work was designed to represent the characteristic flow and mixing processes in the small intestine by expansion and contraction of two cuffs around the external tube (Fig. 1b). This model functions as a concentrically mass transfer exchanger composed of an inner semi permeable membrane of cellulose (Spectra/Por 7®, MWCO 8000, 3.2 cm of diameter, Medicell International Ltd London, UK) and an outer non-active and impermeable tubing (5 cm of diameter, Flexihose, UK). The reaction was started by pumping the pancreatic solution into the SIM at 0.3 and 3.0 ml/min (Figure 1).

**Figure 1.** Schematic of the experimental SIM

Glucose concentration was monitored in the lumen (inner tube) and recipient side (outer tube) for the control and when the viscosity of the digesta was increased by adding guar gum 0.5% (w/v). The concentration of glucose was calculated following the method of 3,5-dinitrosalicylic acid (DNS) reagent for reducing sugars. All experiments were performed at least in triplicate.

The rate of starch digestion in the lumen side and glucose absorption in the recipient side, were obtained from the slope (mM/s) of each plot by a linear regression analysis. Overall mass transfer coefficients (K) were estimated by Eq. 1.

\[
N_{1} = K (c_{i} - c_{\infty})
\]

where \(N_{1}\) is the molar flux (mol/m²s) including both diffusion and convection and \(c_{i}\) (mol/m³) is the concentration at the interface of the same fluid as the bulk concentration \(c_{\infty}\) (mol/m³).

RESULTS & DISCUSSION

Food digestion was done by using an *in vitro* dynamic small intestine model that simulates representative flow and mixing processes in the small intestine. The effect of mixing and food structure was studied on the glucose absorption phenomena. Figure 2 shows the data from the starch digestion in the lumen side (inner side or membrane) and the glucose absorption after generation in the recipient side (outer side) in the SIM varying the concentration of amylase per minute. An increase of glucose concentration was observed in both sides of the SIM as the concentration of amylase was increased over time. Starch hydrolysis was rapid rate in the first minutes of the enzymatic reaction following by a progressive decreased rate, reaching the steady state after approximately 60 min according to the experimental conditions with maximum glucose concentration up to 40 mM. Glucose absorption was lineal in the recipient side with a maximum of approximately 5 mM, almost ten-fold less than the glucose released from the lumen as a result of the enzymatic starch digestion.
Figure 2. Digestion in the SIM, (a) starch digestion in lumen and (b) glucose absorption in recipient side.

Table 1 shows the rate of digestion of starch and glucose absorption calculated from a linear regression of the data plot in Fig. 2. The Overall Mass Transfer coefficients were estimated from the flux of the lumen to the recipient side with the concentration difference at the maximum concentration reached at the steady state in the lumen.

Table 1. Digestion and absorption rates for the experimental conditions.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Digestion (µM/s)</th>
<th>Absorption (µM/s)</th>
<th>Overall Mass Transfer Coefficient (µm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 U at 0.3 ml/min</td>
<td>6.91 ± 1.23</td>
<td>0.40 ± 0.04</td>
<td>0.33 ± 0.03</td>
</tr>
<tr>
<td>25.0 U at 0.3 ml/min</td>
<td>9.22 ± 2.34</td>
<td>0.49 ± 0.21</td>
<td>0.35 ± 0.09</td>
</tr>
<tr>
<td>2.5 U at 3.0 ml/min</td>
<td>8.43 ± 0.78</td>
<td>0.51 ± 0.01</td>
<td>0.38 ± 0.05</td>
</tr>
<tr>
<td>25.0 U at 3.0 ml/min</td>
<td>54.55 ± 2.11</td>
<td>0.48 ± 0.05</td>
<td>0.30 ± 0.02</td>
</tr>
</tbody>
</table>

The rate of starch hydrolysis increased up to 90% as the concentration of enzyme increased ten-fold in the lumen side, reaching the maximum value at 25.0 U and 3.0 ml/min while the minimum was observed for the lowest concentration of enzyme at 2.5 U and 0.3 ml/min. Results in the lumen also indicated that there was no significant difference between the starch hydrolysis rates when the concentration of enzyme per ml of digesta was identical. This implies that no matter how fast or concentrated the pancreatic solution is feeding into the SIM if the units of enzyme per time per volume are the same, the hydrolysis will take place at similar rates of reaction in well-mixed solutions with viscosities like water.

The increase of enzyme concentration per ml of digesta markedly increased the hydrolysis of starch but had little effect on glucose absorption (Table 1). The glucose profiles in the recipient side appeared to be linear. No appreciable amounts of glucose were detected at the beginning of the experiment. From the highest enzyme concentration, the change in glucose was detectable at 30 min into the recipient. However, there was a “delay” in glucose absorption (from 30 to 60 min) when the rate of starch digestion decreased. Once glucose was generated and released from the starch structure in the lumen side, absorption took place. Interestingly, this could be correlated to the point where viscosity of the starch solution falls in the lumen.

Effect of food formulation

In Figure 3 glucose absorption data are shown for products having different viscosities in the lumen side. Absorption appeared to be linear (R^2 of 0.95) resulting in a gradient of 7.45 x 10^-3 mM/s. The flux, N, was calculated to be 1.55 x 10^-3 mol/m^2/s. By dividing the molar flux by the concentration difference in mmol/m^3 an overall mass transfer coefficient, K, was obtained as 4.83 x 10^-7 m/s for the aqueous starch solution and 3.10 x 10^-7 m/s for the starch solution added with guar both under segmentation conditions.
Glucose absorption levels in the side of the SIM, with (0.5%, w/v) and without guar gum for two food models (a) corn starch and (b) white bread.

The effect of the presence of guar was shown by 36% reduction in the mass transfer coefficient when mixing was applied (Table 2). The data not only suggest that the rate of enzymatic reactions was reduced when guar was added but also that viscous solutions can delay the access of actives to the absorptive epithelium due to decrease of both propulsion and mixing [2]. Similarly, this tendency was observed when bread digestion was mimicked in an aqueous solution (control) and in the presence of guar. When viscosity of the lumen content was increased from 2 to 200 mPa.s, glucose absorption was reduced almost 15% after one hour, however, no significant difference was observed at the first minutes of digestion (Fig 1b). While the overall mass transfer coefficient from bread with guar gum was reduce 20%. These results are in agreement to those reported for Tharakan et al. [7].

Table 2. Rates of absorption and mass transfer coefficients for two food models.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Absorption (µM/s)</th>
<th>Overall Mass Transfer Coefficient (µm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Starch solution</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.75 ± 0.05</td>
<td>0.48 ± 0.05</td>
</tr>
<tr>
<td>Guar gum (0.5%, w/v)</td>
<td>0.48 ± 0.02</td>
<td>0.31 ± 0.08</td>
</tr>
<tr>
<td><strong>2. Bread</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.67 ± 0.01</td>
<td>0.43 ± 0.04</td>
</tr>
<tr>
<td>Guar gum (0.5%, w/v)</td>
<td>0.50 ± 0.02</td>
<td>0.32 ± 0.02</td>
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</table>

Overall mass transfer coefficient was reduced when guar gum (0.5%, w/v) was added to the solution of starch and bread. That could be explained by inhibition of propulsion and mixing in the membrane making interactions between the starch and amylase also difficult. Thus, starch digestion was lower in the presence of guar. Results suggested that molecular delivery is largely influenced by the fluid dynamics of the lumen side. Viscous polymers create a physical resistance for actives release and delivery that could modified the rate of nutrients absorption for the design of healthy diets with specific targets.

From the starch digestion experiment it was possible to show the effect that segmentation motion and food formulation had on nutrient delivery to the intestinal wall as a consequence of changes in the mass transfer coefficient. On the other hand, the rate of food digestion might be important in assessing the extension at which they raise the blood glucose levels in normal and diabetic people [8]. These insights will help to develop structured foods with advanced properties for preventing dietary related disorders around the world and implement realistic in vitro and in silica models for simulating food digestion processes.
ACKNOWLEDGMENTS

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CONCLUSION

This research has demonstrated the capability of using an in vitro rig to simulate and obtain an engineering understanding of transport phenomena occurring in the small intestine leading to absorption of active components. Current work is aimed at developing a reaction engineering understanding of the GIT. Using an in-vitro system it was demonstrated that increasing the viscosity of food digesta results in a reduced the concentration of nutrients available for absorption.

REFERENCES