Correlation between the in-vitro starch digestibility, predicted glycemic index and in-vivo glycemic index of selected Omani snack foods

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Abstract

In-vitro starch digestibility is a simple experimental method to determine the glycemic response of carbohydrate-rich meals. Five selected Omani snack foods (white bread, chicken sandwich, croissant, doughnut, and samosa) were tested for their starch fractions, in-vitro starch digestibility and predicted glycemic index. The in-vivo glycemic index of these foods was determined in 12 healthy human volunteers. The total starch (TS) content ranged from 32 to 74.36 % being highest in white bread and lowest in chicken sandwich. The resistant starch (RS) content was lowest in white bread (2.0 %), and highest in samosa (4.23%) followed by doughnut (4.17%), croissant (4.07%) and chicken sandwich (3.28%). The starch digestion index (SDI) values ranged from 45.5 to 68.2. The predicted glycemic index (PGI) values were calculated based on the in-vitro starch digestibility and varied from 58.7 to 95.4. The in-vivo glycemic index of these foods measured in human volunteers ranged from 60.4 to 77.8. A regression equation was developed to calculate the estimated in-vivo glycemic index (GI) values based on the in-vitro PGI values. The in-vivo GI values and in-vitro PGI values were found to be well correlated. The result indicated that starch fractions and in-vitro starch hydrolysis can be used to determine the PGI values, which showed a good correlation with in-vivo GI values in this study. The results suggest that in-vitro starch digestibility values can be used to predict the in-vivo glycemic index of foods.

Keywords: in-vitro starch digestibility, predicted glycemic index, in-vivo glycemic index, Omani snack foods

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1. Introduction

Carbohydrates are an important part of everyday meals and are the main source of starch. The starch consists of amylose and amylopectin that contain α-1,4 and α-1,4 and α-1,6 glycosidic bonds respectively and have diverse gastrointestinal and metabolic attributes. Satiety, energy intake and blood sugar levels are influenced by the starch content of food and the rate of digestibility. Starch digestibility is affected by several intrinsic and extrinsic factors and in-vivo studies involving humans may add to further complexities. The nutritional composition of foods (protein, moisture, phosphorous and resistant starch) variably affects the starch hydrolysis and digestibility. Cooking affects the physiological characteristics of starch and its gelatinization with variable access to starch hydrolyzing enzymes. Insufficient heating and moisture content in foods may affect the gelatinization process as the residual starch is not fully gelatinized during the processing[1]. Both the food and starch properties affect the ileal starch digestibility. Starch retrogradation can decrease starch digestibility by increasing the resistant starch as a result of restructuring starch granules through hydrogen bonds. Ingestion of food stimulates the secretion of several gastrointestinal hormones and gastric juices, which affect the gastric motility and subsequently the gastric emptying (GE) of the ingested meal[2-3]. Digestion of foods is vital for maintaining life. It is important to understand the digestive processes and the passage of digested foods as a result of peristaltic contractions. Meal composition, energy content, and subject characteristics variably affect the rate of gastric emptying for solid, semi-solid and liquid meals. The glycemic response of carbohydrate-rich foods is altered by factors such as the amount of processing, particle size, starch structure, type of
fibre, amylose to amylopectin ratio, and genetics. Differences in glycemic index (GI) values are associated with amylose content, resistant starch, and gelatinization characteristics of starch in foods and act as predictors of starch digestion rate[4-5]. However, the underlying mechanisms related to the rate-determining processes, which affect starch digestibility and in-vitro digestion kinetics have not yet been established. The associations among the food properties, digestive activities, and overall health outcomes must therefore be improved for efficient food consumption[6-7].

Determining the in-vivo glycemic index in humans is a time consuming, costly, and difficult task particularly with respect to enrolment of human volunteers for glycemic index testing of foods[8]. In-vitro techniques have been proposed to assess the rate of starch hydrolysis and are believed to be good forecaster of the physiological effects of foods [9-12]. These in-vitro techniques imitate the physiochemical processes involved in carbohydrate digestion in the upper digestive tract of humans. These in-vitro methods estimate the probable rates of carbohydrate digestion and glucose absorption in the small intestine and could be used as an alternative to in-vivo procedure for measuring the GI values of foods[13]. The in-vitro starch hydrolysis is a simple and economical method to estimate the glycemic response of carbohydrate-rich foods without the involvement of humans in in-vivo experimental models[14-16]. Additionally, in vitro methods are non-invasive, and applicable to several food samples and basically measure the hydrolysis of carbohydrates as glucose equivalents. Depending upon the exposure of starch hydrolysis to pancreatic α-amylase, the carbohydrates have been divided into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS)[17-18]. Immediately after ingestion of food, the rapidly digestible starch (RDS) spikes up the blood glucose levels, whereas the SDS is digested slowly in the small intestine. RDS and SDS are regarded as glycemic carbohydrates, whereas resistant starch (RS) that escapes digestion in the small intestine and is fermented by the colonic microorganisms in the large intestine, is regarded as non-glycemic carbohydrate [19-22]. The potential health benefits of RS include prevention of colon cancer, reduction in gall stones formation, hypocholesterolemic and hypoglycemic effects, inhibition of fat accumulation and enhanced absorption of certain minerals [18]. Zhang and Hamaker proposed an in-vivo method to determine the extended glycemic index (EGI) of foods based on slowly digestible starch (SDS) and also to evaluate the metabolic effects and related health consequences of SDS[23]. A universal standardized method has however not yet been adopted from the currently available options[24]. Although some discrepancies have been reported in literature to find out suitable correlations between the starch digestibility, in-vitro and in-vivo glycemic indices[25-26], the data indicates that the in-vitro enzymatic methods for determining the starch digestibility are reliable in predicting the glycemic response of foods and a consistent association can be attained between the starch digestibility, in vitro and in vivo methods for evaluating the GI values[27-28]. The purpose of this research was to evaluate the starch fractions, in-vitro starch digestibility, starch digestion index, predicted glycemic index (PGI), and in-vivo glycemic index as well as to develop the regression equation to estimate the in-vitro PGI values from the in-vitro GI values for some selected snack foods in Oman.

2. Materials and methods

2.1. In-vitro Starch hydrolysis and Predicted Glycemic Index (PGI):

Five selected Omani snack foods (white bread, chicken sandwich, croissant, doughnut, and vegetable samosa) were purchased from the local market and brought to the lab for chemical analysis. For the starch fractions and in-vitro hydrolysis, the samples were ground and stored precisely. Total starch (TS), resistant starch (RS) and in-vitro kinetics of starch digestion were determined according to the method suggested by Goni et al.[10]. The available starch was determined by the difference of TS and RS. For in-vitro starch hydrolysis, 50 mg of ground sample was boiled and incubated with a solution containing 20 mg pepsin at 40°C for 60 min and the volume was made up to 25 ml with a Tris-maleate buffer. This step was carried out to remove protein from the samples. To determine the digestible starch (DS), 5 ml of Tris-maleate solution containing 3.3 IU α-amylase was added and incubated at 37°C in a shaking water bath. One ml aliquot samples were taken from each tube every 30 min from 0 to 180 min and placed in water bath at 100°C and were rigorously shaken for 5 min to deactivate the enzyme. The samples were then kept in a refrigerator till the end of experiment. To hydrolyze the digested starch into glucose 60 μL of amyloglucosidase was added to the samples and were digested at 60°C for 45 min. After that the samples were centrifuged for 15 min, at 4500 g. The glucose content in the supernatant was measured using a glucose oxidase-peroxidase kit and the amount of digestible starch was calculated as mg of glucose × 0.9. The rate of starch digestion was expressed in terms of the glucose released per 100g of sample hydrolyzed at different time intervals (0, 30, 60, 90, 120, and 180 min)[10]. The in-vivo glycemic index of these selected snack foods was determined in 12 healthy human volunteers as described earlier by Ali and colleagues[29]. The study was approved by the Medical Ethics Committee of Sultan Qaboos University and a written consent was obtained from the volunteers. The regression equation was developed to find the correlation between the in-vitro predicted glycemic index (PGI) and the in-vivo glycemic index as determined in human volunteers. The predicted glycemic index (PGI) and starch digestion index (SDI) were calculated using the following formulas:

Predicted Glycemic Index (PGI) = \((39.21 + 0.803*H90)\)

Starch Digestion Index (SDI) = RDS/TS*100, where RDS is equal to rapidly digestible starch at 30 min; and TS = Total starch.

The data was subjected to statistical analysis using one way analysis of variance and the means were compared by student’s t-test at significance level (P <0.05) with the help of SPSS v.16.
3. Results and Discussions

3.1. Starch fractions of selected Omani snack foods:

The data on the starch fraction of 5 Omani selected snacks foods (white bread, chicken sandwich, croissant, doughnut, and samosa) is presented in Table 1. The total starch (TS) content ranged from 32.14 to 74.36%, the highest among all the samples was in white bread. This indicated that the flour used for bread making was highly refined. The more refined is the flour, the higher is the amount of TS is in the product. The total starch content in doughnut, croissant and samosa was 50.56, 44.28 and 47.11%, respectively. The lowest amount of TS was observed in chicken sandwich, which may be because of poultry meat contents that contain more protein and fat and little carbohydrates. RS content is found naturally in foods and can vary due to degree of starch gelatinization, particle size, type of cellular structure, and the presence of other components such as dietary fiber and anti-nutrients[30]. The RS content was lowest in white bread (2.0%), and highest in samosa (4.23%) followed by doughnut (4.17%), croissant (4.07%) and chicken sandwich (3.28%).

The highest content of RS was observed in samosa that may be due to its stuffing materials as it is packed with vegetables like potato, green peas, green chilies, coriander leaves, carrot, spices, and resins. Generally the frozen green peas contain higher quantities of RS[9]. In Oman, the frozen green peas are normally used for samosa filling, which might have altered the RS content of samosa. In addition to this the deep frying of samosa might have increased the RS content of this snack food. Frying, baking, and storage conditions have been reported to increase the RS content in potatoes, and other foods due to the formation of amyllose-lipid complex that might have slowed down the starch hydrolysis[31]. Besides, the thermal processing of foods and degree of gelatinization has also been shown to affect the digestion rate of food starches[32]. Our results partially confirm the data from previously published studies [30-33-35]. Since RS is not digested in the small intestine and is fermented by microbiota in the large intestine producing short chain volatile fatty acids, it has a great impact on the physiological functions. RS has also been shown to have hypocholesterolemic effects and protects the colon cells against the risk of colorectal cancer[36-38].

3.2. In-vitro Starch hydrolysis:

The data on the in-vitro starch hydrolysis of different Omani snack foods is shown in Figure 1. The white bread showed the highest starch hydrolysis rate, whereas the chicken sandwich had the lowest hydrolysis rate. Measuring in-vitro digestion of starch foods is carried out to predict the in-vivo effects of digestion in human system [10-28-39-40]. Englyst and Hudson proposed the utilization of rapidly digestible starch (RDS) for expressing the digestion of foods as eaten[40]. Goni et al. developed a first order equation from the in-vitro kinetics of starch digestion of foods[10]. This model showed a high correlation (r=0.909, P<0.05) with in-vivo glycemic responses in humans and demonstrated good reproducibility and application in other studies. The in-vitro starch hydrolysis approach stimulates the starch digestion in small intestine where the starch is fractionated into slowly digested starch (SDS), rapidly digested starch (RDS) and resistant starch (RS)[41]. The starch degradability in the gut is also influenced by a number of intrinsic and extrinsic factors such as starch structure, composition and association between starch granules and protein and cell wall structure within the foodstuff[33-42]. The other factors which may influence the starch hydrolysis are the extent to which the food is chewed, the amount of available pancreatic amylase, transit time through small intestine, nature of carbohydrate, food form, food processing, fiber, and anti-nutrient contents [40-43]. The starch digestion rate has been shown to correlate well with the glycemic rate, therefore the variability in the physical features in samples may result in different glycemic responses[40-43].

3.3. Predicted glycemic index (PGI):

The data on the in-vivo glycemic index values in human volunteers, predicted glycemic index (PGI) values based on the in-vitro starch hydrolysis and starch digestion index of selected Omani snack foods is presented in Table 2. The in-vivo glycemic index values in human volunteers differed significantly (P <0.05) and ranged from 60.35 to 77.8, whereas the predicted glycemic index values varied from 58.7 to 95.42 in in-vitro conditions. The starch digestion index of these foods also differed significantly (P <0.05) ranged between 45.52 to 68.18, the highest for white bread and the lowest for chicken sandwich and croissant. The highest PGI was recorded in white bread, followed by samosa, doughnut, croissant and chicken sandwich. Our results are in line with the results reported in the literature on similar foods from other parts of the world[12-44-46]. Thermal processing of foods affects the gelatinization and retrogradation of starch granules causing higher availability of starch to enzymatic breakdown. Bread is an open structure with many air holes and its porous structure increases the surface area for enzyme contact during the digestion process. Higher accessibility to starch in the bread by digestive enzymes can contribute to high rate of starch digestibility. The starch digestion in wheat bread has been reported to range from 76.1% to 78.4% after 180[9-47]. A number of factors associated with the preparation and processing of food alter their rate of digestion and glucose absorption ultimately affecting the glycemic index of food[31-48-49]. The impact of starch digesting enzymes is reduced due to combination of compaction and cooking processes that affect the release of glucose from starch granules[1-33-50]. The lowest GI was recorded in chicken sandwich, which may be due to more fat and protein content of it. The protein and fat contents of food affect the glycemic response by delaying the gastric emptying and stimulating the insulin secretion[51]. Protein has been shown to reduce the glucose responses twice more than fat[52]. However, the data is inconclusive about the impact of protein, fat and fiber content of food and meals on glycemic responses. The variability in the glycemic responses with different foods indicated that these foods were digested differently and released glucose in different manner in the blood stream. However, the exact mechanism for the variability in the glycemic responses of foods is not known. It may partly be explained due to variability in the chemical composition, cooking methods, starch gelatinization and retrogradation etc.[53]. The GI values determined in by in-vivo and in-vitro methods were almost similar for croissant, but varied for other snack foods. The GI value of snack foods
observed in our study agree with the values reported earlier by other researchers[29-54-55].

The starch digestion index values observed in this study for these snack foods were lower than the in-vivo GI and predicted glycemic index values. We plotted the in-vivo GI values against the in-vitro PGI values and found a good correlation (Fig. 2). We also developed a regression equation to calculate the in-vivo GI values from the in-vitro PGI values. The regression equation is:

\[ \text{In-vivo GI value (X1)} = 46.3 + 0.319 \times \text{in-vitro PGI value (X2)} \]

In-vivo GI value (X1) is the in-vivo GI value in human volunteers and X2 is the in-vitro PGI value. The results of this study will be useful to assess the predicted glycemic index (PGI) based on in-vitro starch hydrolysis measurements. It is concluded that data on the in-vitro starch hydrolysis can be used to determine the PGI values, which showed a good correlation with the in-vivo GI values in this study.

### Table 1: Starch fractions of commonly consumed Omani snack foods (mg/100g)

<table>
<thead>
<tr>
<th>Food items</th>
<th>Total Starch</th>
<th>Resistant Starch</th>
<th>Digestible Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>White bread</td>
<td>74.36 ± 2.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>72.36 ± 3.24&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chicken Sandwich</td>
<td>32.14 ± 0.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.28 ± 0.05&lt;sup&gt;d&lt;/sup&gt;</td>
<td>28.86 ± 1.74&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Croissant</td>
<td>44.28 ± 0.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.07 ± 0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.73 ± 2.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Doughnut</td>
<td>50.56 ± 4.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.17 ± 0.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.39 ± 1.95&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Samosa vegetables</td>
<td>47.11 ± 0.51&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>4.23 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.88 ± 2.33&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Different alphabets in the same column mean different at (P<0.05)*

![Figure 1](image_url)
Table 2: In-vivo and in-vitro glycemic index of different Omani snack foods

<table>
<thead>
<tr>
<th>Food items</th>
<th>In-vivo GI</th>
<th>In-vitro PGI</th>
<th>SDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>White bread (WS)</td>
<td>77.8 ± 3.7</td>
<td>95.4 ± 3.9</td>
<td>68.2 ± 3.5</td>
</tr>
<tr>
<td>Chicken Sandwich (CS)</td>
<td>65.7 ± 3.4</td>
<td>58.7 ± 3.2</td>
<td>45.9 ± 2.7</td>
</tr>
<tr>
<td>Croissant (CR)</td>
<td>67.5 ± 3.6</td>
<td>67.3 ± 3.3</td>
<td>45.5 ± 2.9</td>
</tr>
<tr>
<td>Doughnut (Donut)</td>
<td>75.5 ± 3.3</td>
<td>67.4 ± 3.5</td>
<td>47.9 ± 3.1</td>
</tr>
<tr>
<td>Samosa vegetables</td>
<td>60.4 ± 3.5</td>
<td>69.2 ± 3.5</td>
<td>61.7 ± 3.1</td>
</tr>
</tbody>
</table>

a, b, c…. different alphabets in the same column means different at (P <0.05)

4. Conclusions

The present study evaluated various starch fractions, in-vitro starch digestibility, starch hydrolysis index, predicted glycemic index, and in-vivo glycemic index of five selected Omani snack foods. The results indicated that measuring the starch fractions and in-vitro starch digestibility is simple, cost effective and reliable way to predict the glycemic index values. The result indicated that starch fractions and in-vitro starch hydrolysis values can be used to determine the PGI values. From a nutritional standpoint, the higher RS and SDS, along with lower RDS and PGI values are acceptable. Based on the regression equation, the predicted glycemic index showed a good correlation with in-vivo glycemic index values measured in human volunteers. The data from the present study is of practical significance for food composition tables, food labelling, and daily meal planning using the concept of glycemic index of foods.

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