Sterilization in a Liquid of a Specific Starch Makes It Slowly Digestible In Vitro and Low Glycemic in Rats

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Abstract

Diabetics are recommended to eat a balanced diet containing normal amounts of carbohydrates, preferably those with a low glycemic index. For solid foods, this can be achieved by choosing whole-grain, fiber-rich products. For (sterilized) liquid products, such as meal replacers, the choices for carbohydrate sources are restricted due to technological limitations. Starches usually have a high glycemic index after sterilization in liquids, whereas low glycemic sugars and sugar replacers can only be used in limited amounts. Using an in vitro digestion assay, we identified a resistant starch (RS) source [modified high amylose starch (mHAS)] that might enable the production of a sterilized liquid product with a low glycemic index. Heating mHAS for 4–5 min in liquid increased the slowly digestible starch (SDS) fraction at the expense of the RS portion. The effect was temperature dependent and reached its maximum above 120°C. Heating at 130°C significantly reduced the RS fraction from 49 to 22%. The product remained stable for at least several months when stored at 4°C. To investigate whether a higher SDS fraction would result in a lower postprandial glycemic response, the sterilized mHAS solution was compared with rapidly digestible maltodextrin. Male Wistar rats received an i.g. bolus of 2.0 g available carbohydrate/kg body weight. Ingestion of heat-treated mHAS resulted in a significant attenuation of the postprandial plasma glucose and insulin responses compared with maltodextrin. mHAS appears to be a starch source which, after sterilization in a liquid product, acquires slow-release properties. The long-term stability of mHAS solutions indicates that this may provide a suitable carbohydrate source for low glycemic index liquid products for inclusion in a diabetes-specific diet. J. Nutr. 137: 2202–2207, 2007.

Introduction

Metabolic disorders, such as diabetes mellitus, require prevention of high postprandial glucose responses to minimize the risk of long-term complications (1,2). Pharmaceutical approaches have shown that the reduction of carbohydrate digestion rate is a suitable way to attenuate the postprandial glucose response. Acarbose, a glucose-dase inhibitor, is effective in reducing postprandial hyperglycemia: it lowers postprandial serum glucose (and insulin) concentrations and does not promote weight gain in type 2 diabetic patients (3). Another approach is the production of low glycemic food products by using nonglucose monosaccharides, polyols, or slowly digestible di-, oligosaccharides, and starches. From a nutritional point of view, the first 2 alternatives should be restricted, whereas the use of slowly digestible starches (SDS)3 is preferred (4,5).

Englyst et al. (6,7) have shown that in their in vitro digestion assay, the breakdown of solid starchy foods could predict the postprandial response in vivo and that SDS has limited effect on the glycemic response but is available as sugar. It appeared that processing methods and food preparation steps of solid foods played an important role in these parameters (8). The storage time and temperature were also important factors (8–13). To our knowledge, similar findings in liquid products have not been reported. Commonly used sterilization methods often result in gelatinization of the starch present, which usually results in high digestibility and rapid availability. For the production of specialized sterilized liquid food products, it would be very beneficial to have starch sources available that retain low glycemic properties after heating but at the same time contain high amounts of available carbohydrate. The sterilization procedure of a product containing such a starch source would then not only render the product germfree and safe but also low glycemic and highly digestible.

The objective of our study was to identify and characterize a starch source that contains low amounts of resistant starch (RS) and substantial amounts of SDS in sterilized liquids. Using the assay described by Englyst et al. (7), several starch sources were scanned for their digestibility after sterilization in a liquid. One source identified that met these criteria was studied further to...
A reagent blank for glucose quantification was prepared for each duplicate by adding 250 μL of 10% pancreatin-amylglucosidase-invertase solution, 250 μL of 0.5 mol/L sodium acetate, 500 μL of 0.01 mol/L HCl with vortexing after each addition. Samples and reagent blanks were clarified with a centrifuge. To the 150 μL sample or reagent blank, we added 650 μL of distilled water, 50 μL of 10% potassium hexacyanoferrate(II) trihydrate, 50 μL of 72 g/L zinc sulfate heptahydrate, and 100 μL of 0.1 mol/L NaOH, thoroughly mixed between steps and filtered through a 0.45-μm cellulose acetate filter (Sartorius). Glucose in the clear filtrate was quantified using a glucose oxidase kit (GOD-PAP, Roche Diagnostics). The glucose formed in the first 20 min represents the RDS fraction and the total glucose formed after 120 min represents the total digestible starch (TDS) fraction. The TDS fraction is calculated by subtracting RDS from TDS, the RS fraction is calculated by subtracting TDS from TTS.

Materials and Methods

Carbohydrate processing procedures. A selected carbohydrate source, modified high amylose starch (mHAS; 94.3% total starch (TS), high in resistant starch, 48.3% RS, as stated by the supplier (Cargill)) was heat treated to modify its functional properties. For in vitro determination of starch fractions, 1.5 g mHAS and 8.5 mL distilled water was put in 16 × 160-mm glass tubes (Schott-Duran). After vigorous vortexing, the tubes were placed in a silicon oil bath of 80, 90, 100, 110, 120, or 130 ± 1°C, mixed after 2.5 min, and removed after 5 min. The incubations at 120 and 130°C were comparable to the standard sterilization procedure for clinical nutrition products during manufacturing (121°C for 4 min). After heating, the tubes were rapidly cooled under running tap water for 5 min and kept at room temperature for 24 h before testing.

The product used for in vivo testing was produced on a larger scale (in 500-mL bottles) using a standard sterilization procedure for liquid clinical nutrition products during manufacturing; the material was kept at 121°C for exactly 4 min (also at 15 g/100 g mHAS concentration).

The amount of RS in the liquid endproduct was measured using the McCleary et al. method (14) following 2, 9, 16, 23, 66, and 575 d of storage at 4°C to determine the stability of the RS fraction. All heat-treated mHAS suspensions were thoroughly mixed before testing to obtain a homogeneous and representative sample.

In vitro measurement of starch fractions. The amount of RS in untreated and heat-treated samples was determined by a method developed by McCleary et al. (14) according to AOAC standards. All necessary reagents were obtained from the Resistant Starch Assay kit K-RSTAR by Megazyme International Ireland (Omnimabo). Each experiment included a blank and 3 control starch samples, obtained from the Resistant Starch Control Flours kit K-RSTCL (Megazyme International Ireland). The RS fraction of the blank, regular maize starch, dried milled kidney beans, and tapioca starch amounted to 0.67, 4.7, and 48.3%, respectively. The determination was carried out using 100 mg of untreated mHAS or a volume of liquid-processed mHAS containing 100 mg dry matter. If RS fractions assessed with the McCleary (14) method deviated from these percentages by >10%, the data from that experiment were discarded.

To establish whether the decreased RS was accompanied by increased digestible starch and if so, whether it was rapidly or slowly digestible, we used the methodology developed by Englyst et al. (6,7,15). This method was slightly modified.

Because the supplier of the mHAS source material stated that the TS content of the raw material was 94.3 g/100 g, we did not include total glucose determination, which is in the original method, but used the manufacturer’s stated value. Furthermore, because no detectable free glucose was present in the material, the free sugar glucose fraction was not assessed routinely. The glucose was quantified colorimetrically using the glucose-oxidase kit, as in the 1992 version of the method by Englyst et al. (15) rather than by HPAEC-PAD. Therefore, we started with 30 mL freshly made 10 g/L CHO suspension of untreated mHAS or 30 mL of heat-treated mHAS suspension diluted to 10 g/L CHO. Freshly boiled potato starch (Sigma, 10 g/L suspension, boiled for 10 min) was included in the experiment as a control and contained 83 g rapidly digestible starch (RDS)/100 g powder and no SDS. Pepsin, guar gum, and pancreatic enzymes used in the incubations were obtained from Sigma and the invertase used was from BDH. We used amyloglucosidase 300 solution (Sigma, 300 kAuGUL) instead of AMG 400 from Novo Nordisk, and added 5.3 mL, instead of 4 mL, to 90 mL pancreatic supernatant to correct for the lower activity. The reaction was stopped using 1 mol/L HCl instead of absolute ethanol; 100 μL was added to a 400 μL sample. A reagent blank for glucose quantification was prepared for each duplicate.

Rats. Male Wistar rats (Harlan) weighing 225–250 g at arrival, were pair-housed and kept in a temperature- (21 ± 1°C) and humidity- (55 ± 5%) controlled room under a light-dark schedule of 12:12 (lights on at Zeitgeber time 0). Rats had free access to nonpurified diet (Teklad Global 18% Protein Rodent Diet, Harlan) and tap water, unless stated otherwise. All experiments were approved by the Animal Experiment Ethical Committee DEC-Consult, Bilthoven, the Netherlands.

Surgical procedures. Following 2 wk of acclimatization, the rats were housed individually 2 d prior to surgery. All rats were equipped with an i.g. catheter for carbohydrate administration and a jugular vein catheter for stress-free blood sampling. Surgery was performed under O2/N2O/isoflurane anesthesia (IsoFlo, Abbott Laboratories). A jugular vein catheter (Silicon, 1.2-mm o.d., 0.60-mm i.d., Raumedics) was inserted into the heart via the right jugular vein according to the method of Steffens (16). The i.g. catheter (Silicon, 3.2-mm o.d., 1.5-mm i.d., Raumedics) was inserted and fixed into the antrum wall of the stomach. The opening of the i.g. catheter extended 0.5 cm into the stomach lumen (17). Tubes were tunneled subcutaneously, externalized on the top of the skull, and secured with dental cement to 2 screws. After surgery, buprenorphine (0.01 mL subcutaneously for 3 d, Schering-Plough) was injected for analgesia. The rats were allowed to recover for at least 1 wk after surgery and all rats had regained their preoperative weight before the experiments started. Bodyweight of the rats was monitored daily and did not change within the duration of the experiment.

Experimental protocol. At Zeitgeber time 10 on the day of the experiment, following 5 h of food deprivation, the rats received a bolus of 2.0 g available carbohydrate/kg body weight dissolved in tap water via the gastric cannula. The stock solution of mHAS was corrected based on the digestible fraction found in vitro (i.e. 1×2.6). Administered volumes were ~6–7 mL and included a standard dose of 70 mg paracetamol (acetaminophen, Sigma) to monitor the rate of gastric emptying (18). At 1 min before and 5, 10, 15, 20, 30, 45, and 75 min after administration of the bolus, blood samples of 200 μL were drawn and collected in ice-chilled, heparinized tubes. Blood samples were centrifuged at 2655 × g; 15 min at 4°C and plasma was stored at –20°C until assayed. In each rat (n = 9), the heat-treated mHAS and maltodextrin (Glucidex DE19) were tested in random order. At least 1 wk of wash-out was allowed between subsequent experiments.

Plasma analyses. Glucose concentrations in the plasma samples were determined colorimetrically (GOD-PAP, Roche Diagnostics). We analyzed insulin levels using a specific rat ELISA kit (DRG Diagnostics, Diagnostic System Laboratories Benelux) with a detection limit of 0.13 μU/L. The insulin ELISA was performed in triplicate using serially diluted samples. Paracetamol was measured using an acetaminophen assay kit (Cambridge Life Sciences) with a detection limit of 0.01 mmol/L (18).

Statistical analysis. Data are expressed as means ± SEM. We evaluated the data using the statistical software package SPSS (version 12.01). P < 0.05 was considered significant. Differences across the different in vitro treatments were evaluated by chi-square analysis and 1-way ANOVA.
RS fractions of untreated mHAS and mHAS treated at different temperatures for 5 min as determined using the McCleary method (14) (A). Values are means ± SEM, n = 3. Means without a common letter differ, P < 0.05. Illustrative representation of data from a typical experiment, n = 1, to show the shift in starch fractions of mHAS treated at different temperatures using the method by Englyst et al. (7) (B), n.t., No treatment.

Simultaneous comparisons of means for the different treatments were carried out post hoc by using Tukey's honestly significant difference test. Normally distributed data from the in vivo experiments were evaluated using repeated measures ANOVA. In addition, the basal level (at t = 0) was adjusted for digestibility (i.e., it was compensated for the amount stated by the supplier and amounted to 49.8 ± 0.9 g/100 g powder. After treatment at 120°C or higher, the RS fraction was reduced significantly to ~20% (Table 1). The amount of SDS was low in the raw material and after treatment at lower temperatures (<15%), but it increased considerably with increasing temperatures (>110°C) and more than doubled after treatment at 130°C (Table 1). In addition, the amount of RDS was only slightly higher after treatment at higher temperatures; the only significant difference was found between treatment at 80 and 110 or 120°C, not between untreated and treatment at 120 or 130°C. When the amount of RDS was expressed as a fraction of the total digestible starch (percent of TDS), there was a significant difference between the untreated mHAS and mHAS treated at 120 and 130°C. The amount of RDS (as percent of TDS) in untreated mHAS was considerably lower than after treatment at 130°C.

Treatment at higher temperatures increased the digestibility of mHAS, especially due to an augmentation of the SDS fraction. These trends are visualized in Figure 1B, which shows a typical distribution shift between the different starch fractions due to the heat treatment. This distribution shift should result in a different in vitro digestion curve of untreated mHAS and mHAS treated at 130°C, as data from a typical experiment indeed show (Fig. 2).

The lower RS amount in mHAS treated at higher temperatures as found with the McCleary method (14) did not differ from that assessed with the Englyst approach (7) (P = 0.333).

For in vivo testing, heat-treated mHAS was manufactured in 500-mL bottles using a slightly different procedure than for the in vitro experiments (see Methods). The starch fractions of both carbohydrate loads were assessed using the Englyst method (7). Maltodextrin consisted of RDS only and the heat-treated mHAS contained RDS, SDS, and RS (Table 2) in relative amounts comparable to the former treatment (compare with Table 1). The stability of the heat-treated mHAS stored at 4°C was evaluated by repeatedly measuring the RS fraction using the McCleary (14) method. The product was stable during storage; the RS fraction of the product was 28.8 g/100 g powder after 2 d of storage, 26.4 after 9 d, 26.7 after 16 d, 26.9 after 23 d, 28.6 after 66 d, and 26.4 after 575 d of storage (means of duplicate measurements).

The amount of the heat-treated mHAS administered to the rats was adjusted for digestibility (i.e., it was compensated for the amount of RS as measured with the Englyst method (7) to ensure both loads contained the same amount of digestible carbohydrates. The available fraction of the maltodextrin bolus consisted of RDS only, whereas the available fraction of the heat-treated mHAS bolus consisted of 56% RDS and 44% SDS (Table 2).

![FIGURE 1](https://academic.oup.com/jn/article-abstract/137/10/2202/4664450)

**FIGURE 1** RS fractions of untreated mHAS and mHAS treated at different temperatures for 5 min as determined using the McCleary method (14) (A). Values are means ± SEM, n = 3. Means without a common letter differ, P < 0.05. Illustrative representation of data from a typical experiment, n = 1, to show the shift in starch fractions of mHAS treated at different temperatures using the method by Englyst et al. (7) (B), n.t., No treatment.

**Results**

The amount of RS as measured by the McCleary (14) method in untreated mHAS was 49.2 ± 0.91 g/100 g powder, which is in accordance with the 48.3 g/100 g powder as stated by the supplier. The available fraction of the maltodextrin bolus consisted of RDS only, whereas the available fraction of the heat-treated mHAS bolus consisted of 56% RDS and 44% SDS (Table 2).

However, the amount of RS in mHAS treated above 110°C was significantly lower than untreated mHAS or mHAS treated at 80 and 90°C (Fig. 1). Using the Englyst method (7) (Table 1), the amount of RS found in the raw material was again in accordance with the amount stated by the supplier and amounted to 49.8 ± 0.9 g/100 g powder. After treatment at 120°C or higher, the RS fraction was reduced significantly to ~20% (Table 1). The amount of SDS was low in the raw material and after treatment at lower temperatures (<15%), but it increased considerably with increasing temperatures (>110°C) and more than doubled after treatment at 130°C (Table 1). In addition, the amount of RDS was only slightly higher after treatment at higher temperatures; the only significant difference was found between treatment at 80 and 110 or 120°C, not between untreated and treatment at 120 or 130°C. When the amount of RDS was expressed as a fraction of the total digestible starch (percent of TDS), there was a significant difference between the untreated mHAS and mHAS treated at 120 and 130°C. The amount of RDS (as percent of TDS) in untreated mHAS was considerably lower than after treatment at 130°C.

**TABLE 1** Starch fractions of untreated mHAS and mHAS treated at different temperatures for 5 min

<table>
<thead>
<tr>
<th>Starch fraction1</th>
<th>Untreated</th>
<th>Treated at temperature, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>80</td>
</tr>
<tr>
<td>RDS, g/100 g powder</td>
<td>33.8 ± 1.1b</td>
<td>28.6 ± 1.8a</td>
</tr>
<tr>
<td>SDS, g/100 g powder</td>
<td>10.7 ± 0.8a</td>
<td>10.4 ± 0.8a</td>
</tr>
<tr>
<td>TDS, g/100 g powder</td>
<td>44.5 ± 0.9b</td>
<td>38.0 ± 2.0a</td>
</tr>
<tr>
<td>RS, g/100 g powder</td>
<td>49.8 ± 0.9a</td>
<td>55.3 ± 2.0a</td>
</tr>
<tr>
<td>RDS, % TDS</td>
<td>75.9 ± 1.3a</td>
<td>73.3 ± 1.9a</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 3, determined using the method by Englyst et al. (7). Means in a row without a common letter differ, P < 0.05.
Following i.g. administration of maltodextrin or heat-treated mHAS, the plasma glucose and insulin response was monitored (Fig. 3A, B). Baseline glucose and insulin levels were comparable between groups. With almost the same time lag upon i.g. administration, plasma glucose and insulin levels increased to a higher maximum \((P < 0.01)\) in the maltodextrin compared with the heat-treated mHAS group (Fig. 3; Table 3). Thus, the heat-treated mHAS resulted in attenuated but not delayed increases in the postprandial glucose and insulin responses compared with maltodextrin. There was a significant interaction between treatment and time for both glucose and insulin \((P < 0.001)\).

The AUC of the postprandial plasma glucose concentrations showed that the heat-treated mHAS resulted in an attenuation of the response during the first 45 min by \(\approx 75\%\) compared with maltodextrin (Fig. 3A, insert). Within this time, the postprandial response was over and plasma concentrations returned to baseline (Fig. 3). Likewise, the AUC of the postprandial plasma insulin response (Fig. 3B, insert) also showed a significantly attenuated rise (i.e. only 20\% compared with maltodextrin). Furthermore, the heat-treated mHAS resulted in a significant lower maximal increment (from basal) in the glucose and insulin response and a smaller AUC (from 0–75 min) compared with maltodextrin. The rate of gastric emptying was estimated by monitoring the appearance of paracetamol in the plasma. Plasma concentrations of paracetamol increased to maximal levels around \(0.69 \pm 0.10 \text{ mmol/L}\) within the first 5 min after administration of the i.g. bolus. Concentrations remained elevated during the complete sampling period and changes in concentration (slope) did not differ between maltodextrin and mHAS-treated rats (data not shown).

**Discussion**

This study clearly shows that a specific heat treatment of a carbohydrate source high in resistant starch (i.e. mHAS) resulted in a shift between digestible and nondigestible fractions that remained stable over time following cold storage. Moreover, the changes observed in the in vitro digestion rate indicated increased slow-release properties. Indeed, the heat-treated mHAS showed a significant attenuation of the postprandial rises in glucose and insulin plasma concentrations compared with maltodextrin in rats, which suggests that slow-release properties are retained in vivo.

The starch source used in our study had a similar RS content as assessed in the McCleary et al. (14,15) and Englyst et al. (15) approaches. Others have shown that different in vitro methods can result in different digestibility estimations for a variety of starch sources, with cornstarch being the major exception (19). The transformation of resistant starch to rapidly and SDS in our liquid carbohydrate source was strongly affected by temperature and resulted in comparable digestion curves as previously reported by Englyst et al. (6,7) for the in vitro assessment of in a shift between digestible and nondigestible fractions that remained stable over time following cold storage.

**TABLE 2** Starch fractions of maltodextrin and mHAS treated at 121°C for 4 min

<table>
<thead>
<tr>
<th>Starch fraction</th>
<th>Maltodextrin</th>
<th>Heat-treated mHAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>RDS, g/100 g powder</td>
<td>99</td>
<td>38</td>
</tr>
<tr>
<td>SDS, g/100 g powder</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>RS, g/100 g powder</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>RDS, % TDS</td>
<td>100</td>
<td>56</td>
</tr>
</tbody>
</table>

\(^1\) Values are means of duplicate measurements using the method by Englyst et al. (7).
the absorptive availability of various solid starch fractions. The designations rapidly available glucose (RAG; first 20 min) and slowly available glucose (from 20 to 120 min) reflect the rate at which glucose (from sugars and starch, including maltodextrin) becomes available for absorption in the small intestine. According to the definition by Englyst et al. (7), RAG includes the RDS and the rapidly released FSG fractions of a CHO source. It is noted that the product contained virtually no FSG; therefore, in our study, the designations RAG and RDS were interchangeable.

For most carbohydrates, the RAG content is considered as a major determinant of the magnitude of the glycemic index and Englyst et al. (6) have previously shown a strong correlation between published glycemic index values and RAG values for a wide range of dry starchy foods. Others have also described effects of different heat treatments on starch fractions of solid domestic starchy foods such as pasta and rice in the course of preparation of the food (12,19). However, Niha (8) investigated not only different heat treatments on starch flour suspensions but also effects of storage and encountered stability issues after 10 d storage. From our study, it is clear that the starch source used not only changes considerably upon heat treatment but that the increased digestibility is also very stable over time, at least until 575 d after processing.

The present in vitro experiments indeed showed that the liquid-resistant starch source had slow-release properties following sterilization at 121°C, as evidenced by a reduction of 44% of the glucose levels during the first 20 min (RAG) compared with maltodextrin, with the same amount of available digestible carbohydrates added. A study of Englyst et al. (7) showed a significant correlation between the glycemic response and the amount of RAG intake. In our present in vivo study, a comparable correlation was found with the in vitro results: the glycemic response expressed as the incremental area under the postprandial glucose curve was plotted against the RAG intake. We found a significant correlation ($r = 0.68; P < 0.05$) concurrent with the difference in RAG content (see Table 2) between the maltodextrin and mHAS load administered. These findings are consistent with the results previously reported by Englyst et al. (7) and demonstrate that the amount of RAG is indeed correlated with the glycemic response.

The choice to use maltodextrin as the control is corroborated by the fact that it is the most widely used nonsugar carbohydrate source in liquid nutritional products. Furthermore, it is known that maltodextrin gives adequate plasma responses in the dose used (2 g/kg body weight) (20).

Alternatively, reduced plasma glucose responses may at least in part also result from partial indigestibility of the carbohydrate source, thereby limiting the amount of glucose available for uptake in the ileum. This would lead to amounts of undigested carbohydrate entering the colon.

The amount of carbohydrate administered in vivo, however, was corrected (by 26%; Table 2) for the observed partial digestibility as measured by the Englyst method (7). Hence, the amount of maltodextrin and heat-treated mHAS were equal with respect to glucose availability. Also, the rate of gastric emptying was comparable between the heat-treated mHAS and control maltodextrin groups, excluding possible effects of a delayed delivery of the carbohydrate to the ileum. Of course, administration of the carbohydrate source via the gastric cannula excludes possible effects of oral predigestion, esophageal passage, and lower esophageal sphincter functions, comparable to the in vitro testing situation. Timing and volume of meal ingestion, however, were standardized to a small timeframe with respect to postprandial plasma measurements. The results from our study clearly show that the heat-treated mHAS reduced the postprandial glucose and insulin response. This confirms the proposed slow-release characteristics of this liquid carbohydrate source as demonstrated in our in vitro analysis. Proof of principle studies are now warranted to assess the applicability of the modified SDS fraction on postprandial glycemic response in vivo in type 2 diabetes patients. The long-term stability of mHAS solutions indicates that this may provide a suitable carbohydrate source for low glycemic products in a diabetes-specific diet.

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**Literature Cited**


