# Effects of Chemical Modification on in Vitro Rate and Extent of Food Starch Digestion: An Attempt To Discover a Slowly Digested Starch

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Differences in glycemic and insulinemic responses to dietary starch are directly related to the rate of starch digestion. Chemical modification of starch may allow for the production of a slowly digested starch that could be used for the treatment of certain medical modalities. An in vitro method was utilized to evaluate the effects of chemical modification on the rate and extent of raw and cooked starch digestion. The extent of starch digestion was significantly reduced by dextrinization, etherification, and oxidation. However, the rate of starch digestion was not significantly affected by chemical modification. For most modified starches, as the degree of modification increased, the extent of digestion decreased, suggesting an increase in the amount of resistant starch. The results of this study suggest that chemically modified starch has a metabolizable energy value of <16.7 kJ/g. Chemically modified starch ingredients may serve as a good source of resistant starch in human and animal diets.

**Keywords:** *Chemically modified starch; in vitro digestion; resistant starch*

## INTRODUCTION

As part of a healthy diet, it is generally recommended that the intake of fat (as a percentage of calories) be decreased with a concomitant increase in the intake of complex carbohydrates (i.e., starch) and dietary fiber to meet caloric requirements. For individuals with diabetes mellitus, however, the carbohydrate must not exacerbate postprandial hyperglycemia and must prevent hypoglycemic events. Differences in glycemic and insulinemic responses to dietary starch are directly related to the rate of starch digestion (O'Dea et al., 1981).

Chemical modification of starch may ultimately affect its rate and extent of digestion in the small intestine. Recently, Flickinger et al. (1998) found that little digestion of a dextrinized corn starch hydrolysate occurred in the small intestine of ileally cannulated dogs. Furthermore, they documented that the rate and extent of in vitro fermentation were lower compared to those of other low molecular weight carbohydrates. These data are supported by Tsuji and Gordon (1998), who estimated that this dextrin has a caloric value of 2.2 kJ/g.

Numerous chemically modified food starches are available as ingredients for processed foods. Chemical reactions currently allowed and used to produce modified starches for food use in the United States include esterification, etherification, acid modification, bleaching, and oxidation (Whistler and BeMiller, 1997). Multiple modifications of starch are a common occurrence for tailor-making starches with specific applications in the food industry. It was postulated that the use of these modifications may allow for the production of a slowly digested starch that could be used for the treatment of certain medical modalities (e.g., glycogen storage disease and diabetes mellitus).

Given the high cost associated with the conduct of in vivo studies, a number of groups have developed in vitro methods to predict the digestion of starch in the small intestine (Englyst et al., 1992; Muir and O'Dea, 1992, 1993; Brighenti et al., 1995). Methods have been adapted to predict the amounts of starch that would be rapidly digested, slowly digested, or not digested (resistant) in the small intestine. These methods provide an inexpensive and rapid means to screen large numbers of starch substrates for their in vivo glycemic response (glycemic index). Utilizing an in vitro starch digestion method, we evaluated the effects of chemical modification on the rate and extent of starch digestion. The purpose of this research was to screen potential starch ingredients that may serve as a source of slowly digested starch in liquid enteral formulas.

## MATERIALS AND METHODS

**Starch Sources.** The extent and rate of in vitro starch digestion were determined on chemically modified starch and their unmodified controls. Various chemical modifications were evaluated to include substitution with propylene oxide (etherification), cross-linking with phosphorous oxychloride (creating phosphate intermolecular bridges between starch molecules), acid modification with heat (dextrinization), and oxidation with sodium hypochlorite. The extent of chemical modification varied among starch ingredients and was within the legal limits allowed by the U.S. Food and Drug Administration. In addition, different genetic varieties were evaluated: common starch (~27% amylose), waxy starch and dull waxy starch (0% amylose), and a high-amylose variety (50% amylose). Several

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**Table 1. Identification and Description of Starch Ingredients[a]**

| ingredient | description[b] | degree of substitution[c] | cross-linking[d] |
|---|---|---|---|
| waxy starch | | | |
| WS | unmodified waxy starch (0% amylose) | | |
| hXL-WS | highly cross-linked, waxy starch | | 0.00093 |
| hPOlXL-WS | highly propylene oxide-substituted, lightly cross-linked, waxy starch | 0.12 | 0.000085 |
| hO-WS | highly oxidized, waxy starch | | |
| mD-WS | moderately converted dextrin, waxy starch | | |
| hD-WS | highly converted dextrin, waxy starch | | |
| dull waxy starch | | | |
| dWS | unmodified dull waxy starch (0% amylose) | | |
| mPOmXL-dWS | moderately propylene oxide-substituted, moderately cross-linked, dull waxy starch | 0.07 | 0.00037 |
| common starch | | | |
| CS | unmodified common starch (27% amylose) | | |
| lD-CS | lightly converted dextrin, common starch | | |
| mD-CS | moderately converted dextrin, common starch | | |
| hD-CS | highly converted dextrin, common starch | | |
| high-amylose starch | | | |
| hAS | unmodified high amylose starch (50% amylose) | | |
| hPO-hAS | highly propylene oxide-substituted, high-amylose starch | 0.15 | |

[a] All chemical modifications were within the legal limits allowed by the U.S. Food and Drug Administration. [b] Degree of dextrinization and oxidation as determined by ingredient manufacturer. [c] Degree of substitution is the average number of hydroxyl groups per glycosyl unit that have been derivatized by etherification. [d] Cross-linking values represent the amount (g/100 g) of phosphorous oxychloride ($POCl_3$) added to the starch during processing.

experiments were conducted to ascertain the effects of chemical modification, variety of starch, and combinations of the above on starch digestion.

Chemically modified starch ingredients and their unmodified controls were obtained from commercial starch suppliers. Modified starch descriptions, including degree of substitution, are presented in Table 1. Details regarding the starch processing parameters are proprietary industry trade secrets. Two laboratory reference materials were used in our experiments: raw potato starch and raw corn starch (Sigma Chemical Co., St. Louis, MO). Potato buds were obtained from a local grocery. Dry matter was determined according to Association of Official Analytical Chemists methods (AOAC, 1984). In general, starch ingredients contained approximately 0.35% protein, 0.55% fat, 0.2% ash, and 98.9% carbohydrate (dry matter basis) as determined by the ingredient supplier.

**In Vitro Digestion.** A modification of the Muir and O'Dea (1992, 1993) in vitro starch digestion technique was used. Approximately 0.1 g of carbohydrate was suspended in 1 mL of pepsin (Sigma) solution (1 g/L; pH was adjusted to 2.0 with HCl) and incubated for 30 min at 37 °C. The solution then was neutralized with 0.5 M NaOH (0.5 mL). Five milliliters of 0.2 M sodium acetate buffer (pH was adjusted to 5.0 with glacial acetic acid) and 1 mL of enzyme solution containing 10 mg of α-amylase (Sigma) and 28 units of amyloglucosidase (Sigma) dissolved in the sodium acetate buffer (pH 5.0) were added, and samples were incubated at 37 °C in a shaking water bath for various times as described below. After the appropriate incubation time, samples were centrifuged at 3000*g* for 10 min and the supernate removed. The pellet was washed three times by resuspending the pellet with 1.5 mL of sodium acetate buffer (pH 5.0) and centrifuging (10 min, 3000*g*). All supernates from washings were pooled with the original supernatant. This fraction allows for the prediction of digestion that would occur in the small intestine (i.e., digestible starch, DS).

The washed pellet was lyophilized; this material was considered the resistant starch (RS) component of the sample. The pellet was resuspended in 5 mL of dimethyl sulfoxide (DMSO) and incubated in a boiling water bath for 5 min. Then 20 mL of 0.15 N sodium acetate buffer (pH adjusted to 4.5 with glacial acetic acid) was added and incubated in a boiling water bath for 20 min. Samples then were autoclaved for 1 h at 15 psi and 121 °C. Samples were allowed to cool to room temperature before addition of 10 mL of amyloglucosidase solution containing 580 units of amyloglucosidase dissolved in water. Samples were incubated for 24 h at 55 °C (with occasional vortexing) and then centrifuged for 10 min at 10000−15000*g*. The supernatant was removed for glucose analysis. After starch digestion (hydrolysis), the released glucose was measured according to a glucose oxidase method (glucose test kit 510-A, Sigma). The final glucose reading was multiplied by 0.9 to convert free glucose back to polysaccharide. All values are reported as means of duplicate analyses, expressed as a percentage of ingredient dry matter. Duplicate samples were reanalyzed if duplicates differed by >5%.

**Experiment 1.** Raw starch ingredients and cooked starch ingredients were evaluated for their extent of in vitro digestion. A 15 h in vitro incubation was used to determine the amount of digestible starch as this timeframe has been shown to correlate with the amount of starch escaping digestion in the small intestine utilizing this in vitro assay (Muir and O'Dea, 1993). An attempt to quantify resistant starch also was conducted as described above. In addition, raw starch ingredients were evaluated for total starch content according to the method of Thivend et al. (1972). For cooking, raw starch ingredients were made into 5 and 10% (w/v) starch solutions with water. The use of a hot plate (to warm the starch solution) was required to disperse some starch ingredients into a suspension. Samples then were autoclaved for 10 min at 15 psi and 121 °C. The starch solutions were frozen (−20 °C) and lyophilized in a Tri-Philizer MP microprocessor-controlled lyophilizer. Lyophilized samples were ground with a mortar and pestle in preparation for analysis.

**Experiment 2.** The in vitro rate of starch digestion was evaluated by analysis of starch hydrolysis over time. Samples were analyzed at 0, 2.5, 5, 10, and 15 h. Separate tubes containing ∼0.1 g of carbohydrate were used for each time-point. Free glucose (0 h time point) was analyzed for each substrate after pepsin digestion. Starches were processed into 10% (w/v water) solutions and sterilized to mimic their use in a liquid nutritional product. Starch samples that had a negative impact on the solution's viscosity were predigested with α-amylase (Dexlo-S, Genencor International, Inc., Rochester, NY). Starches that did not have a negative impact on the solution's viscosity were cooked into 10% starch solutions at 91 °C for 30 min. Starches that required predigestion were cooked as 10% solutions at 71−77 °C until the starch was hydrated and the solution was translucent in appearance, at which time the temperature was raised to 93−97 °C for 5−10 min to inactivate the α-amylase. An aliquot of 1000*g* of the cooked 10% starch solutions was filled into 1 L ready-to-hang bottles (Ross Products Division, Altavista, VA). The bottles were sealed and sterilized by heating to 122 °C, at which they were held for 12 min, and then allowed to cool to room temperature. The starch solutions were frozen (−20 °C) and

**Table 2. Starch Concentration and in Vitro Digestion of Chemically Modified Corn Starch Ingredients (Experiment 1)**

| | raw starch | | | 5% starch solution[a] | | 10% starch solution[a] | |
|---|---|---|---|---|---|---|---|
| | Thivend[b] | Muir and O'Dea[c] | | Muir and O'Dea[c] | | Muir and O'Dea[c] | |
| ingredient[d] | starch, % | DS, % | RS, % | DS, % | RS, % | DS, % | RS, % |
| waxy starch | | | | | | | |
| WS | 96.8 | 98.0 | 0.0 | 101.9 | 0.9 | 96.1 | 0.0 |
| hXL-WS | 97.8 | 97.6 | 1.5 | 94.2 | 1.7 | 99.1 | 0.0 |
| hPOlXL-WS | 58.9 | 34.4 | 27.1 | 62.0 | 0.8 | 56.7 | 0.2 |
| hO-WS | 99.4 | 92.5 | 1.3 | 91.3 | 1.2 | 84.2 | 0.0 |
| hD-WS | 49.1 | 45.6 | 0.3 | 45.7 | 0.9 | 44.9 | 0.0 |
| dull waxy starch | | | | | | | |
| dWS | 96.3 | 90.6 | 0.9 | 90.6 | 1.6 | 95.2 | 0.0 |
| mPOmXL-dWS | 72.5 | 68.7 | 4.4 | 69.4 | 1.6 | 83.9 | 0.0 |
| common starch | | | | | | | |
| ID-CS | 90.1 | 87.6 | 1.0 | 85.4 | 3.0 | 86.2 | 0.3 |
| mD-CS | 84.2 | 77.8 | 0.2 | 75.1 | 3.2 | 77.2 | 0.0 |
| hD-CS | 63.8 | 63.4 | 0.6 | 64.9 | 1.0 | 62.3 | 0.1 |
| high-amylose starch | | | | | | | |
| hAS | 92.5 | 47.1 | 51.9 | 72.8 | 16.6 | 77.2 | 15.4 |
| hPO-hAS | 58.9 | 53.8 | 7.6 | 59.7 | 2.3 | 58.1 | 0.2 |
| lab reference controls | | | | | | | |
| raw corn starch | 97.9 | 83.9 | 9.0 | NA[e] | NA | NA | NA |
| raw potato starch | 95.0 | 38.3 | 65.7 | NA | NA | NA | NA |

[a] For cooking, raw starch ingredients were made into 5 and 10% (w/v) starch solutions with water and then cooked by autoclaving for 10 min at 15 psi and 121°C. [b] Percent starch as determined according to the method of Thivend et al. (1972), expressed as a percent of ingredient dry matter. [c] DS, percent digestible starch, RS, percent resistant starch, as determined according to the method of Muir and O'Dea (1992, 1993), expressed as a percent of ingredient dry matter. All values are means of duplicate samples, and samples were reanalyzed if duplicates differed by >5%. [d] Identification and description of starch ingredients are presented in Table 1. [e] N/A, not applicable.

lyophilized in a Tri-Philizer MP microprocessor-controlled lyophilizer. Lyophilized samples were ground with a mortar and pestle in preparation for analysis.

**Experiment 3.** The initial in vitro rate of starch digestion for a few chemically modified starch ingredients was evaluated by analysis of starch hydrolysis over time. Samples were analyzed at 0, 0.5, 1, 1.5, 2, and 2.5 h. Separate tubes containing ~0.1 g of carbohydrate were used for each time point. Starches were prepared as described in experiment 2.

## RESULTS

**Experiment 1.** The starch concentration and extent of in vitro digestion of chemically modified starches are presented in Table 2. The total starch content of raw starch ingredients as measured according to the method of Muir and O'Dea (1992, 1993; DS + RS) was similar to the values obtained according to the method of Thivend et al. (1972). The unmodified waxy and dull waxy starch ingredients (WS and dWS, respectively) contained high levels of DS (>90%), whereas the unmodified 50% high-amylose starch ingredient (hAS) contained a significant proportion of RS. Total starch was lower in propylene oxide-substituted starches (hPOlXL-WS, mPOmXL-dWS, hPO-hAS) compared to their unmodified controls (WS, dWS, hAS, respectively), suggesting a decrease in their digestibility. A similar phenomenon was noted for the dextrinized starches. As the degree of dextrinization increased, the total amount of digestible starch decreased (lD-CS > mD-CS > hD-CS). Starch modification by cross-linking did not appear to affect starch digestibility (compare WS to hXL-WS), whereas the highly oxidized waxy starch (hO-WS) was less digestible than its unmodified control (WS).

Overall, the amounts of digestible starch and RS were similar between 5 and 10% cooked solutions. Of those raw ingredients that contained a significant amount of RS, this portion became digestible upon cooking. For example, raw propylene oxide-substituted starch ingredients (hPOlXL-WS, mPOmXL-dWS, hPO-hAS) contained more RS compared to their cooked counterparts. In addition, hAS, the raw 50% amylose hybrid (unmodified), had a high concentration of RS (52%) compared to its cooked version (~16%). Digestible starch values were similar for cooked and raw dextrinized starches (lD-CS, mD-CS, hD-CS). The laboratory reference materials, raw corn starch and raw potato starch, contained a high level of total starch; however, a majority of the raw potato starch was quantified as RS.

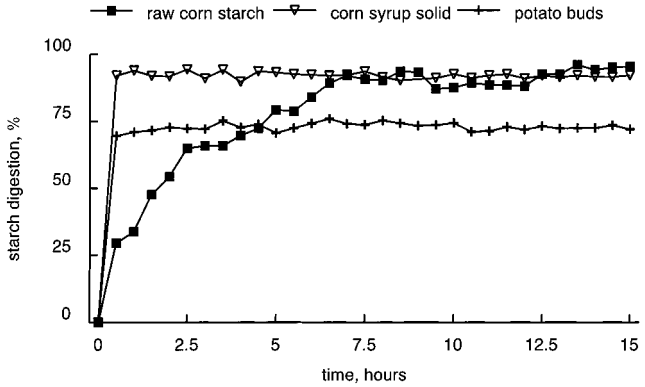


**Figure 1.** Rates of in vitro digestion of raw starches.

Figure 1 shows the rate of digestion of raw corn starch, corn syrup solids (DE = 20), and potato buds. This figure shows that a standard carbohydrate ingredient used in liquid food products, corn syrup solids, is rapidly and completely digested. The digestible portion of potato buds was digested very rapidly, with the remaining portion being resistant to digestion. Raw corn starch was digested more slowly but was also extensively digested over time in this assay (i.e., a slowly digested starch).

**Experiment 2.** Because a 15 h incubation has been shown to correlate with the amount of starch escaping digestion in the small intestine (Muir and O'Dea, 1993), we evaluated the rate of in vitro digestion over this period of time. The rate of in vitro digestion of chemically modified starches is presented in Table 3. In general, these results show that the portion of starch that was digestible was digested within 2.5 h, and the

**Table 3. Rate of in Vitro Digestion of Chemically Modified Corn Starch Ingredients Made into 10% Solutions (w/v) and Processed into I L Ready-to-Hang Bottles (Experiment 2)[a]**

| | time | | | | |
|---|---|---|---|---|---|
| ingredient[b] | 0 h | 2.5 h | 5 h | 10 h | 15 h |
| waxy starch | | | | | |
| WS | 1.1 | 88.5 | 87.6 | 86.3 | 90.6 |
| hXL-WS | 1.0 | 87.5 | 88.0 | 89.1 | 96.3 |
| hPOlXL-WS | 1.0 | 54.5 | 56.0 | 52.9 | 60.2 |
| hO-WS | 1.5 | 84.0 | 82.9 | 86.9 | 89.1 |
| mD-WS | 0.7 | 81.8 | 81.6 | 79.2 | 83.0 |
| hD-WS | 0.9 | 46.1 | 47.5 | 42.2 | 49.6 |
| dull waxy starch | | | | | |
| dWS | 0.7 | 89.7 | 87.1 | 88.5 | 91.2 |
| mPOmXL-dWS | 1.1 | 67.8 | 64.0 | 68.1 | 66.2 |
| common starch | | | | | |
| ID-CS | 0.2 | 86.1 | 86.3 | 82.6 | 90.1 |
| mD-CS | 0.9 | 73.9 | 74.6 | 71.0 | 68.9 |
| hD-CS | 0.1 | 58.0 | 59.2 | 54.1 | 60.6 |
| high amylose starch | | | | | |
| hAS | 0.9 | 77.2 | 77.3 | 76.2 | 83.7 |
| hPO-hAS | 0.3 | 59.3 | 60.0 | 54.3 | 57.5 |
| lab reference controls | | | | | |
| raw corn starch | 0.0 | 67.3 | 69.8 | 78.3 | 86.0 |
| raw potato starch | 0.0 | 32.6 | 29.7 | 38.0 | 37.6 |

[a] Percent digestible starch, expressed as a percent of ingredient dry matter, determined according to the method of Muir and O'Dea (α-amylase and amyloglucosidase enzyme system; 1992); a 15 h in vitro incubation has been shown to correlate with the amount of starch escaping digestion in the small intestine (Muir and O'Dea 1993). Time 0 values represent the percent of free glucose in samples. All values are means of duplicate samples, and samples were reanalyzed if duplicates differed by >5%. [b] Identification and description of starch ingredients are presented in Table 1.

remaining starch was resistant to digestion (i.e., RS). The only starch that demonstrated a "slow" rate of digestion was the laboratory reference material, raw corn starch. Sixty-seven percent of raw corn starch was digested at 2.5 h, and its digestibility rose to 86% by 15 h.

The extent of starch digestion (i.e., 15 h time point) of individual starch ingredients was similar to the values obtained in experiment 1. Thus, the effects of chemical modification on the extent of in vitro digestion were similar between experiments 1 and 2. The extents of digestion for the laboratory reference controls raw corn starch and raw potato starch were 83.9 and 38.3% in experiment 1 and 86.0 and 37.6% in experiment 2, respectively.

**Experiment 3.** Because no slowly digested modified starch ingredients were identified over the 15 h in vitro period, we evaluated the rate of starch digestion over the initial 2.5 h (Table 4). No starch ingredients appeared to be slowly digested compared to our laboratory reference material, raw corn starch. Thirty-five percent of raw corn starch was digested at 0.5 h, and its digestibility rose to 64% by 2.5 h. Utilizing this in vitro procedure, most modified corn starch ingredients chosen for these experiments contained only a small amount of slowly digested starch when processed into a liquid solution.

In vitro digestion values for the 2.5 h time point tended to be higher for the same chemically modified starch ingredients in experiment 3 compared to experiment 2. Potential sources of experiment to experiment variation include variance in water bath temperature (±1 °C), water bath shaker speed (i.e., sample suspension in the buffer), enzyme concentrations, number of samples (i.e., speed at which samples could be processed upon reaching desired incubation time), and the large number of steps for this assay. Thus, appropriate control samples were run with each experiment.

**Table 4. Rate of in Vitro Digestion of Chemically Modified Corn Starch Made into 10% Solutions (w/v) and Processed into 1 L Ready-to-Hang Bottles (Experiment 3)[a]**

| | time | | | | | |
|---|---|---|---|---|---|---|
| ingredient[b] | 0 h | 0.5 h | 1 h | 1.5 h | 2 h | 2.5 h |
| waxy starch | | | | | | |
| hXL-WS | 0.8 | 90.9 | 92.8 | 97.9 | 97.9 | 99.9 |
| hPO1XL-WS | 0.9 | 56.1 | 52.5 | 58.1 | 57.0 | 57.6 |
| hO-WS | 1.6 | 87.0 | 87.7 | 94.0 | 94.6 | 96.1 |
| mD-WS | 0.7 | 82.3 | 81.5 | 89.5 | 90.2 | 90.4 |
| common starch | | | | | | |
| 1D-CS | 0.2 | 83.0 | 92.7 | 95.3 | 94.7 | 95.8 |
| mD-CS | 0.9 | 76.7 | 76.3 | 79.3 | 77.7 | 78.4 |
| hD-CS | 0.1 | 60.5 | 60.4 | 59.9 | 61.2 | 62.4 |
| high-amylose starch | | | | | | |
| hPO-hAS | 0.3 | 62.4 | 63.4 | 64.1 | 63.6 | 63.8 |
| lab reference controls | | | | | | |
| raw corn starch | 0.0 | 35.4 | 46.1 | 50.9 | 62.2 | 63.5 |
| raw potato starch | 0.0 | 29.5 | 27.4 | 28.9 | 22.6 | 25.3 |

[a] Percent digestible starch, expressed as a percent of ingredient dry matter, determined according to the method of Muir and O'Dea (α-amylase and amyloglucosidase enzyme system; 1992); a 15 h in vitro incubation has been shown to correlate with the amount of starch escaping digestion in the small intestine (Muir and O'Dea 1993). Time 0 values represent the percent of free glucose in samples. All values are means of duplicate samples, and samples were reanalyzed if duplicates differed by >5%. [b] Identification and description of starch ingredients are presented in Table 1.

## DISCUSSION

Worldwide, starch and its products constitute most of the digestible carbohydrate in the human diet. The rate and extent of starch digestion in the small intestine are dependent upon several intrinsic and extrinsic factors [reviewed by Englyst et al. (1992)]. It was once assumed that all starch is hydrolyzed and absorbed in the small intestine due to the fact that pancreatic α-amylase can be produced in ample amounts. However, it is now known that a substantial amount of starch escapes digestion in the small intestine and enters the colon (Englyst and Cummings, 1985; Berry, 1986). In an attempt to nutritionally categorize starch, Englyst et al. (1992) developed an in vitro method to classify starch into three categories: rapidly digested starch (RDS), slowly digested starch (SDS), and resistant starch (RS).

For the purposes of this research, we have defined SDS as starch that is likely to be completely digested in the small intestine but at a slower rate, as described by Englyst et al. (1992). Considering this, there are several conditions for which the nutritional use of SDS would be of physiological benefit. For example, individuals with type 2 diabetes may benefit from a foodstuff that contains SDS to improve the postprandial glycemic response (i.e., prevention of hyperglycemia and hypoglycemia). In addition, SDS may prolong satiety and could be incorporated into foodstuffs marketed for weight-loss programs. Finally, SDS may be beneficial in products that are utilized by athletes as SDS would provide a longer, more consistent source of systemic glucose.

Type I glycogen storage disease is associated with the absence or deficiency of glucose-6-phosphatase (glucose-6-phosphate → glucose), which results in hypoglycemia during fasting. Raw (uncooked) corn starch has been shown to serve as an effective oral therapy for the

prevention of nighttime hypoglycemic episodes in these patients (Wolfsdorf and Crigler, 1997). Chen et al. (1984) made similar observations; however, ingestion of cooked corn starch resulted in a sharp rise in blood glucose levels followed by a rapid fall to hypoglycemic levels. These results suggest that raw corn starch is an SDS and that cooking disrupts the corn starch granules, making them more rapidly digestible. Considering these data, we chose to include raw corn starch in our in vitro experiments as a positive SDS control.

In the present study, total starch content of raw starch ingredients as measured by the method of Muir and O'Dea (DS + RS; 1992, 1993) was similar to the values obtained by using the method of Thivend et al. (1972). Compared to their unmodified controls, propylene oxide-substituted and dextrinized starches had lower total starch values (Table 2). This may be due to incomplete hydrolysis of the modified starch ingredients by the enzymes used in these in vitro methods. Both of these in vitro methods are based upon starch hydrolysis by α-amylase and/or amyloglucosidase followed by glucose determination (via glucose oxidase) to quantitate starch. Alternate sources of enzymes (e.g., Megazyme International Ireland Ltd.; Wicklow, Ireland) were evaluated to determine if improved quantification could be obtained. No differences were found in the ability of alternate enzyme sources to improve starch quantification (data not shown). It may be theorized that a substituted starch polymer, although completely hydrolyzed, would not be quantified because glucose oxidase would not react with a substituted glucose molecule. However, the decrease in total starch quantification found in the present study is much greater than the absolute level of substitution. For example, the theoretical reduction in the extent of digestion for hPOlXL-WS (degree of substitution = 0.12) would be 12%; however, an ~40% reduction in digestion was found (Table 2). Thivend et al. (1972) noted that some glucose polymers obtained from certain starch treatments (e.g., dextrinization) were not hydrolyzed by glucoamylase (i.e., amyloglucosidase). They suggested that total hexose content be determined in conjunction with a hydrolysis method (e.g., anthrone method) for these types of ingredients. We attempted to combine the anthrone method with our in vitro procedure but were unable to obtain meaningful results (data not shown).

In the present study, the RS pellet was resuspended in DMSO to gelatinize this material. The limited digestibility of high-amylose starch is related to its gelatinization. This is clearly demonstrated in the hAS ingredient (compare raw sample to 5 and 10% cooked solutions, Table 2). However, solvent (i.e., DMSO) treatment of chemically modified starches did not improve the quantification of RS in these ingredients. We postulated that some of the RS was not quantified because it could not be hydrolyzed by the enzymes used in our in vitro system. As with total starch quantification, the use of alternate enzyme sources did not improve RS quantification (data not shown). We also postulated that the polymer size of the RS was small enough that it was not pelleted during the centrifugation and, therefore, was not quantified in the RS separation. An attempt was made to improve the Muir and O'Dea (1992, 1993) procedure by increasing the force of centrifugation from 1200*g* to 3000*g*; however, it was not successful. This method for measuring RS works only on insoluble forms of RS, and we conclude that this procedure is not appropriate for the quantification of RS in modified starch ingredients as much of the RS was soluble (especially for dextrinized starches). In addition, we attempted to utilize the in vitro method described by Englyst et al. (1992); however, we were unable to replicate the method and obtain meaningful results.

Gelatinization refers to the disruption of molecular order within starch granules as they are heated in the presence of excess water. The incorporation of chemically modified starch ingredients into 5 and 10% solutions with heating significantly increased the digestible component of the starch (Table 2, compare to raw starch values), especially for those ingredients made from high-amylose starch (hAS). Thus, our data support the analysis of digestible starch in the final product as prepared for consumption, because cooking may alter the proportion of digestible starch in the foodstuff.

The present study shows that the level of digestible starch is decreased by several chemical modifications. Propylene oxide substitution (etherification) significantly reduced the in vitro digestibility of starch. It is proposed that the propylene oxide substitution interferes with the binding of α-amylase and/or amyloglucosidase, thus decreasing starch digestion. Likewise, a high degree of oxidation decreased the extent of starch digestion; however, cross-linking with phosphorous oxychloride did not alter the in vitro digestibility of starch in our experiment. The legal limit of starch cross-linking may be too low to significantly alter starch digestion.

Partial hydrolysis of starch using acid and heat (i.e., dextrinization) results in molecular rearrangement of the starch molecule such that α and *β*-(1,2) and -(1,3) linkages are formed in addition to reconfiguration of existing α-(1,4) and -(1,6) bonds into *β* bonds (Bryce and Greenwood, 1963). Our data document that dextrinization decreases the in vitro digestibility of starch. The data generated by this in vitro assay are supported by the in vivo work of Flickinger et al. (1998) and Tsuji and Gordon (1998). Data presented in Table 2 show that as the degree of dextrinization increases, in vitro digestibility decreases.

Most modified starches contained only RDS and RS. The only ingredient from the present study that contained a significant amount of SDS was raw corn starch. The nature of the ingredients tested (i.e., isolated in pure form) and the fact that they have been "cooked" may explain these results. The use of raw corn starch as a method to prevent nighttime hypoglycemic episodes is fairly common. It appears that the structural nature of raw corn starch retards the ability of α-amylase to hydrolyze the starch molecule, thus slowing its rate of digestion. In the case of the modified starches used in this experiment, that component of the starch which was digestible was hydrolyzed rapidly, with the remaining component being completely resistant to α-amylase hydrolysis.

According to U.S. food regulations, "[n]on-digestible dietary fiber will be determined by the method 'Total Dietary Fiber in Foods, Enzymatic Gravimetric Method, First Action, in the *Journal of the Association of Official Analytical Chemists* (JAOAC) 68: 399, 1985, as amended in JAOAC 69: 370, 1986.' [21 CFR 101.9(c)(3)]". The dietary fiber that is recovered with this gravimetric procedure includes celluloses, hemicelluloses, pectins, and some other nonstarch polysaccharides, lignins, and a portion of RS. Not detected by this method are some nondigestible polysaccharides that are soluble in 78−

80% ethanol, such as inulin and polydextrose. In addition, this method would not quantify all of the RS fractions of the chemically modified starches evaluated in this study as this RS would remain soluble in 78−80% ethanol. Thus, this material would be removed during the filtration process in the analysis for dietary fiber. Given the fact that a significant amount of chemically modified starch is neither digested nor quantified as RS, alternative methods of its determination/quantification must be sought to improve and allow meaningful nutritional labeling (energy values) of food products that contain a significant amount of chemically modified starch. The use of these ingredients (modified food starch, dextrin) may result in a higher intake of "dietary fiber" by the U.S. population.

Our results verify the slowly digestible properties of raw corn starch. The level of SDS in cooked chemically modified starches was small; however, these modifications resulted in an increase in the levels of RS. For most modified starches, as the degree of modification increased, the level of digestible starch decreased, suggesting an increase in the amount of RS. The present in vitro procedure was not appropriate for the quantification of RS in chemically modified starch ingredients. The use of chemically modified starches allows for the fortification of many foods with a dietary fiber-like material. However, alternative methods of RS quantification should be sought to allow appropriate nutritional labeling for foods containing these ingredients. Because of their increased RS composition, the use of chemically modified starch ingredients should attenuate the glycemic response and decrease the caloric density of foods containing them. Caution must be used in the interpretation of starch values for foodstuffs as most enzymatic based methods are probably underestimating the content of RS, and some may be overestimating the availability of starch digestion in the small intestine. Appropriate clinical studies are necessary to validate these hypotheses. This study highlights the need for methodologies to accurately and reproducibly quantify chemically modified starches that are being continually used in the food supply.

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