

Structures of Salivary and Pancreatic Amylase Hydrolysates from Processed Starches

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Abstract

Digestion of starch in humans includes luminal and mucosal steps. Structures from the luminal phase of amylases hydrolysis can impact subsequent steps of digestion at the mucosa of the small intestine. However, structures of the starch digestion products along the gut from the mouth to the small intestines products that impact glucose homeostasis are not well understood. This submission focuses on the luminal step of starch digestion, i.e. impact of salivary and pancreatic amylases on the structure of hydrolysis products obtained from cooked starches from different botanical sources. Starch to water ratios of 1:0.7 (T0.7) or 1:2 (T2) were used to cook normal corn (NCS), wheat (NWS) and potato (NPS) starches. Comparatively, DMSO was used to disperse the starches to remove the effect of granular organisation. Cooked and dispersed starches were then subjected to salivary and pancreatic amylases hydrolysis for 20 min and 120 min. Extent of 20 min hydrolysis was lower at T0.7 compared to T2 and TD for all the starches. The molecular weight profiles of 20 min hydrolysates between the processing treatments were more different for NPS than for the other starches. Oligosaccharide composition of 120 min hydrolysates differed in amounts of DP 2, 3, 5, 6 and 7 between

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processing treatments and also between the starches. These differences, however, did not necessarily follow the intensity of cooking treatment. These differences in structures of hydrolysates, which are the substrate for mucosal hydrolysis in the small intestine, can potentially influence glucose homeostasis.

Keywords: human salivary amylase, starch digestion structures, pancreatic amylase hydrolysates, water content, dextrins

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1 Introduction

Starch is a major component of our diet. It contributes to the energy we use. However, starch digestion in humans may also result in unfavourable high glucose levels in the blood [1]. These levels impact on insulin release into the blood. Thus, long-term eating foods with rapidly digestible starch apparently can negatively affect glucose homeostasis regulatory hormones and places high stress to the regulatory system. This is associated with ill-health such as diabetes, cardiovascular disease and obesity [1]. This needs to be better controlled and managed. To control glucose homeostasis, the many factors that impact on the digestion of starch need to be better understood. Structures of starch digestion hydrolysates produced during the course of digestion appear to impact glucose homeostasis in humans. For instance, it is known that foods from different botanical sources containing similar amounts of starch can yield different postprandial rise in blood glucose and insulin levels [2]. Starch hydrolysis products thus appear to impact on the regulation of insulin release [3]. They also influence the release of incretin hormones, which are in turn involved in insulin release [4]. However it is unclear as to what the structures of starch hydrolysates during the process of digestion are. Are these structures different between starches from different botanical sources? Would different processing or formulations of starch/starchy food yield different hydrolysate structures? How do the hydrolysates affect the rate of glucose production and/or insulin levels? Literature is scant on data about the evolution of molecular structures of cooked starch through the digestion process. This study is the third in a series of studies focusing on these aspects.

Food digestion begins in the mouth and culminates in the small intestine in monogastric in vivo systems. Starch is digested first by luminal (salivary and pancreatic) amylases and finally by sucrase-isomaltase and maltase-glucoamylase at the mucosa in small intestine. Products of luminal enzymes can influence the digestion of starch and production of glucose by gastro-intestine mucosal enzymes [5-7]. For instance, maltase-glucoamylase can be inhibited by luminal α -amylase products such as maltotriose, maltotetraose and maltopentaose [7]. This study focuses on in vitro luminal (oral and duodenal) digestion. Salivary

amylase can yield different types and amounts of oligosaccharides [8, 9]. Moreover, most studies investigated the glycemic index or resistant starch fractions of foods [10-12]. The focus has mostly been placed on the amount of glucose produced at 20 min and 120 min of in vitro digestion. However, the identity and molecular structures of starch hydrolysates from salivary amylase and pancreatic amylase are not well known. It is these structures in the sequential steps during the digestion of starch in the gut that impact (amplify/inhibit) subsequent digestion of starch, and/or possibly influence the rate of glucose release further down in the gut.

Processing makes food palatable. It gelatinizes starchy foods by disorganising the granular structure of native starch [13]. For instance, cooking native starch in the presence of water, the granule undergoes a series of irreversible physico-chemical changes [14]. These changes are primarily driven by processing temperature and water content [15-16]. Gelatinisation improves the susceptibility of starch to hydrolysis. However, cooling the gelatinized starch causes retrogradation. This involves molecular reorganization and could decrease enzymatic susceptibility of processed starches [17]. Little data can be found on starch digestibility as affected by lower moisture contents during cooking (1:2 starch:water ratio or less) [18-19]. Therefore, this study investigates the digestion of two moisture treatments that represent typical water contents of two different common product categories, such as breads or crackers.

This study shows differences in the composition and type of structures produced by the luminal enzymes (human salivary and pancreatic amylases). Thus, differences in botanical sources of starch and in processing could modulate the type and/or amounts of oligosaccharides, which will potentially be the substrates for subsequent digestion in the gut.

2 Materials and Methods

2.1 Materials

Normal corn (NCS) and normal potato (NPS) starches were gifts from National Starch, Bridgewater, NJ, USA. Normal wheat starch (NWS) was a gift from MGP ingredients, Atchison, Kansas, USA. Human salivary amylase (human saliva form, A-1031, 108 U/mg) and porcine pancreatic amylase (Pancreatin, P1625, from porcine pancreas with activity at least equivalent to $10 \times$ U.S.P specifications) were purchased from Sigma-Aldrich.

2.2 Cooking of starch

Starches were cooked as described in Nantanga et al. [9]. Two separate samples were made for each of the two water concentrations used, i.e. the two treatments were replicated 2 times.

2.3 Dispersed starch preparation

Nongranular starch preparation was carried out as described in Nantanga et al. [9]. Like for the hydrothermal treatments, two separate preparations were made, i.e. the two treatments were replicated 2 times. Dispersed starch sample will be abbreviated as TD.

2.4 Pancreatic amylase activity

To measure the porcine pancreatic amylase activity in pancreatin, normal corn was used as the substrate. Corn starch was solubilized in double distilled water with heating and stirring to form a solution (10 mg/mL). The solution was allowed to return to room temperature with stirring maintained. Pancreatin solution was prepared as described by Englyst et al. [10] with modifications. It was made by using 20 mM sodium phosphate buffer, pH 6.9, containing 6.7 mM sodium chloride. Only pancreatin was used for enzyme preparation. Solutions were pre-incubated at 37°C. Pancreatin solution (0.5 mL) was added to 0.5 mL starch solution and incubated for exactly 1 minute. The reducing sugars content produced by α -amylase of pancreatin was determined by Nelson-Somogyi method [20]. Reducing sugar concentrations were calculated relative to maltose standards. A 1 U is defined as the amount of enzyme that produces 1 μ mol reducing ends (maltose equivalents) per min from a 0.5% starch solution. Based on this method, the pancreatin solution used in this study contained $7.4 \times 10^5 \pm 1.3 \times 10^4$ U/mL.

2.5 Starch hydrolysis

The hydrolysis conditions such as substrate to enzyme ratio, weight to volume of reaction mixture and duration were based on Hoebler et al. [21] and Englyst et al [10] for salivary amylase and porcine pancreatic amylase hydrolysis, respectively. 200 mg of freeze-dried starch was mixed with 800 μ L of sodium phosphate buffer (20 mM, pH 6.9 containing 6.7 mM NaCl) containing 40 U of the enzyme (based on the units given by the supplier). The

starches and the buffer that contained salivary amylase were pre-incubated in a water bath set at 37°C. The reaction mixtures were incubated for exactly 5 min and then immediately a solution of 2950 µL of a pancreatin solution containing about 3.14×10^5 U/mL was added. The reaction mixtures were further incubated for exactly 20 min or 120 min and then immediately placed in ice water and were quickly stored at -80°C to stop the reaction and finally freeze-dried. To inactivate the enzymes, before any further analysis, a sample was dispersed in hot 100% DMSO and heated with gentle stirring for 5 min in a hot (~95°C) water bath. Dispersed starch, starch cooked in 1:0.7 and 1:2 starch to water ratios after being subjected to salivary and pancreatic amylase hydrolysis will be referred to as TD-S&P, T0.7-S&P and T2-S&P, respectively.

2.6 Molecular size distribution of hydrolysed starches

Gel-permeation chromatography (GPC) was performed as described in Nantanga et al [9]. Analysis of the molecular weight-distributions of fractions was done using a column (1.6 cm × 90 cm) of Sepharose CL 6B (GE Healthcare, Uppsala, Sweden).

2.7 Analysis of oligosaccharides

Samples that were subjected to hydrolysis for 120 min were reduced to hydrolysis products that were analysed by using High Performance Anion Exchange Chromatography (HPAEC). To identify the type of oligosaccharides in those samples, a freeze-dried salivary and pancreatic amylase hydrolysed sample (1 mg) was dissolved in 150 µL of DMSO by gently stirring overnight at room temperature. Then 850 µL of hot (just boiled) HPLC grade water was added to this dispersion. Samples (25 µL) were then analysed by using a HPAEC system (ICS-3000, Dionex, USA) equipped with a Carbo-Pac PA-100 (250 mm × 4 mm) column, a single pump and pulsed amperometric detection (PAD). The column was eluted at 1 mL/min with eluent A (150 mM NaOH) and eluent B (150 mM NaOH containing 500 mM NaOAc) for 2 hours. The elution gradient was as follows: eluent B was increased at 0 to 9 min from 15% to 36%; 9-18 min from 36% to 45%; 18-60 min from 45% to 100%; 60-62 min from 100% to 15% (as it was at the start); and 62-120 min equilibrated at 15%. For quantitative analysis of the major peaks, samples were diluted by a factor of 27 and analysed again with HPAEC. Glucose, isomaltose, maltose, maltopentaose, maltohexaose and maltoheptaose were used as standards.

2.8 Statistical analyses

The effects of water concentrations, starch dispersion by DMSO on the relative amounts of oligosaccharides produced by the hydrolysis of different starches as determined using HPAEC were determined using one-way analysis of variance (ANOVA) and Duncan's least significant difference test (LSD, $p < 0.05$).

3 Results and Discussion

3.1 Molecular size-distribution of hydrolysates

The size-distribution chromatograms of salivary and pancreatic amylase hydrolysates produced within 20 min of hydrolysis are shown in Figures 1-3. The chromatograms were divided into three parts, namely fraction 1 (DP >1500), fraction 2 (DP 1500–60) and fraction 3 (DP < 60). The size-distribution of all control samples was not different between the treatments and the starches. The control samples eluted primarily at the void volume (fraction 1). This indicated co-elution of amylopectin and amylose of large molecular weight. The tail represented portions of the starch polymers that have relatively lower DP. Following hydrolysis, samples yielded variable proportions of dextrans included in fractions 2 and 3 from the hydrolysis and thus decrease of high molecular weight dextrans in fraction 1. Major differences were observed in fractions 1 and 3. For example, T0.7-S&P had dextrans left in fraction 1, whereas T2-S&P and TD-S&P had little if any dextrans remaining after hydrolysis for all the starches. Relative proportions of fraction 3 hydrolysates were in the order of TD-S&P > T2-S&P > T0.7-S&P for NPS (Figure 3). For NCS, the order was TD-S&P = T2-sAA > T0.7-sAA and for NWS it was TD-sAA \geq T2-S&P \geq T0.7-S&P. These results indicate that more gelatinised starch (T2 and TD) led to more conversion of large polymers (fraction 1) to oligosaccharides (fraction 3) when hydrolysed for 20 min. The differences observed between the hydrolysate structures from the different processing treatments may possibly affect the matrix and provide different substrates for mucosal enzymes in the small intestine. The impact of these differences in substrates on glucose production by mucosal enzymes, sucrase-isomaltase and maltase-glucoamylase in the small intestine is underway. Hydrolysis for 120 min chromatograms (data not shown) exhibited size-distributions that were similar to T2-S&P or TD-S&P shown in Figures 1-3 and that of extruded normal corn starch reported by Witt et al. [22]. With no long chains (Fractions 1 in Figures 1-3), these samples were analysed by using the HPAEC-PAD system to determine the oligosaccharide composition.

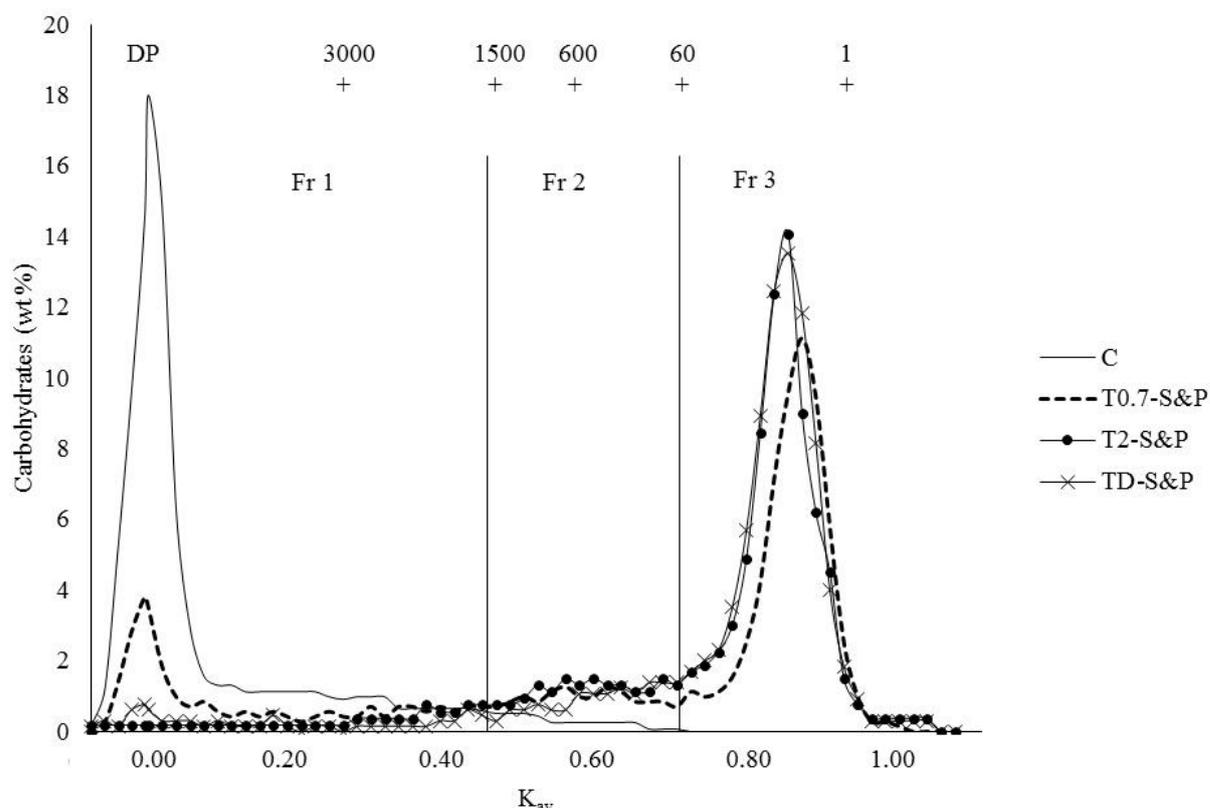


Figure 1: Gel-permeation chromatograms of salivary and pancreatic amylase hydrolysates of normal corn starch digested for 20 min. C = control; T0.7-S&P = starch cooked in 1:0.7 starch: water and subjected to salivary and pancreatic amylases hydrolysis. T2-S&P = starch cooked in 1:2 starch:water and subjected to salivary and pancreatic amylases hydrolysis. TD-S&P = starch dispersed with DMSO and subjected to salivary and pancreatic amylases hydrolysis. DP = degree of polymerization. Fr 1-3 represents divisions of DP fractions; fraction 1 (DP >1500), fraction 2 (DP 1500-60) and fraction 3 (DP < 60). K_{av} = gel partition coefficient.

3.2 Oligosaccharide composition

A representative HPAEC chromatogram of the products from the hydrolysis of processed starches by salivary and pancreatic amylase after 120 min of hydrolysis is shown in Figure 4. The carbohydrates detected corresponded to mostly maltose (DP 2), maltotriose (DP 3), and oligosaccharides corresponding to DP 5, DP 6 and DP 7. In addition, traces of larger oligosaccharides were observed. Peaks corresponding to glucose and isomaltose were also observed in all hydrolysed samples irrespective of the processing treatment. However, a

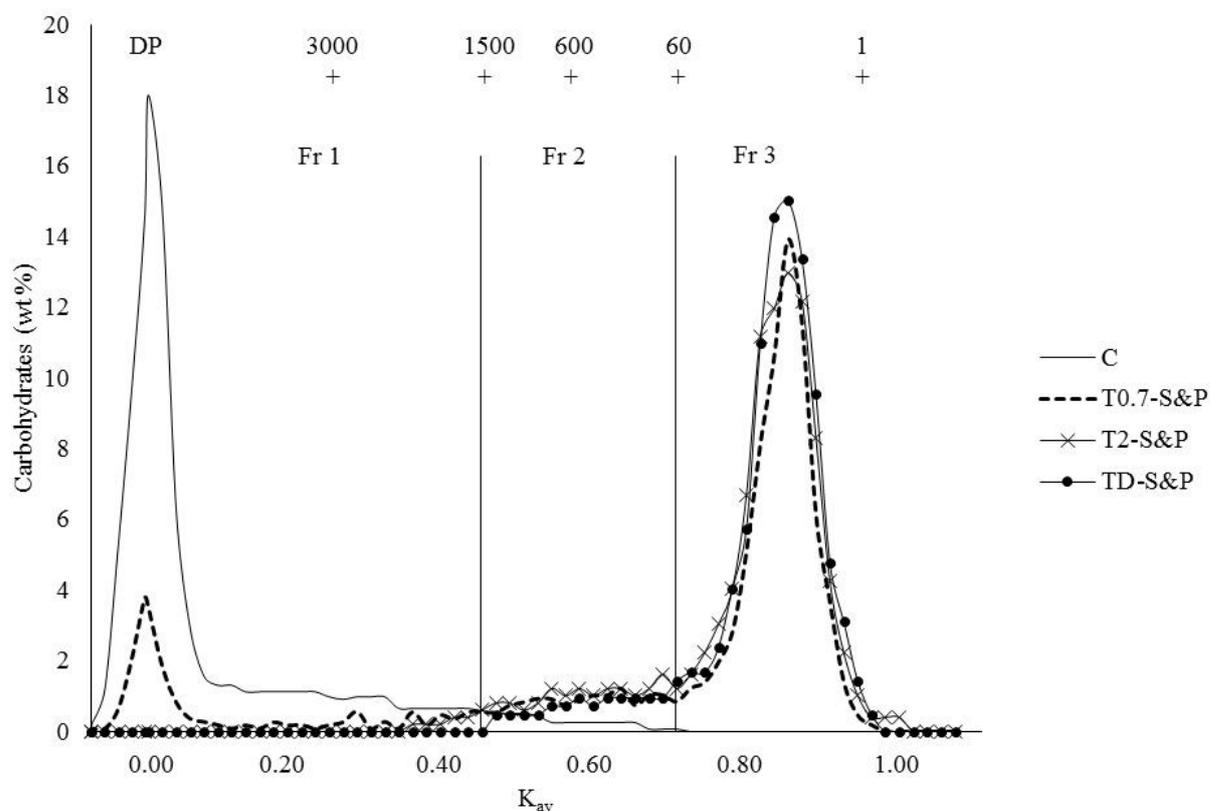


Figure 2: Gel-permeation chromatograms of salivary and pancreatic amylase hydrolysates of normal wheat starch digested for 20 min. C = control; T0.7-S&P = starch cooked in 1:0.7 starch:water and subjected to salivary and pancreatic amylases hydrolysis. T2-S&P = starch cooked in 1:2 starch: water and subjected to salivary and pancreatic amylases hydrolysis. TD-S&P = starch dispersed with DMSO and subjected to salivary and pancreatic amylases hydrolysis. DP = degree of polymerization. Fr 1-3 represents divisions of DP fractions; fraction 1 (DP >1500), fraction 2 (DP 1500-60) and fraction 3 (DP < 60). K_{av} = gel partition coefficient.

pancreatin solution in the buffer with no starch had peaks that eluted at the same positions as the peaks of glucose and isomaltose standards. This compounded our analysis of these two oligosaccharides. Peaks 5, 6 and 7 indicated the presence of branched oligosaccharides with DP 5, DP 6 and DP 7, respectively, because peak 5 eluted between that expected from maltotetraose and maltopentaose standards. Similarly, peak 6 fell at elution time in between those of maltopentaose and maltohexaose, whereas peak 7 was present at a position between maltohexaose and maltoheptaose. This is a sign of the presence of a branch point in oligosaccharides [23], and the structure of small, branched limit dextrins produced by the pancreatic amylase are known [24]. Table 1 gives the proportions of the major oligosaccha-

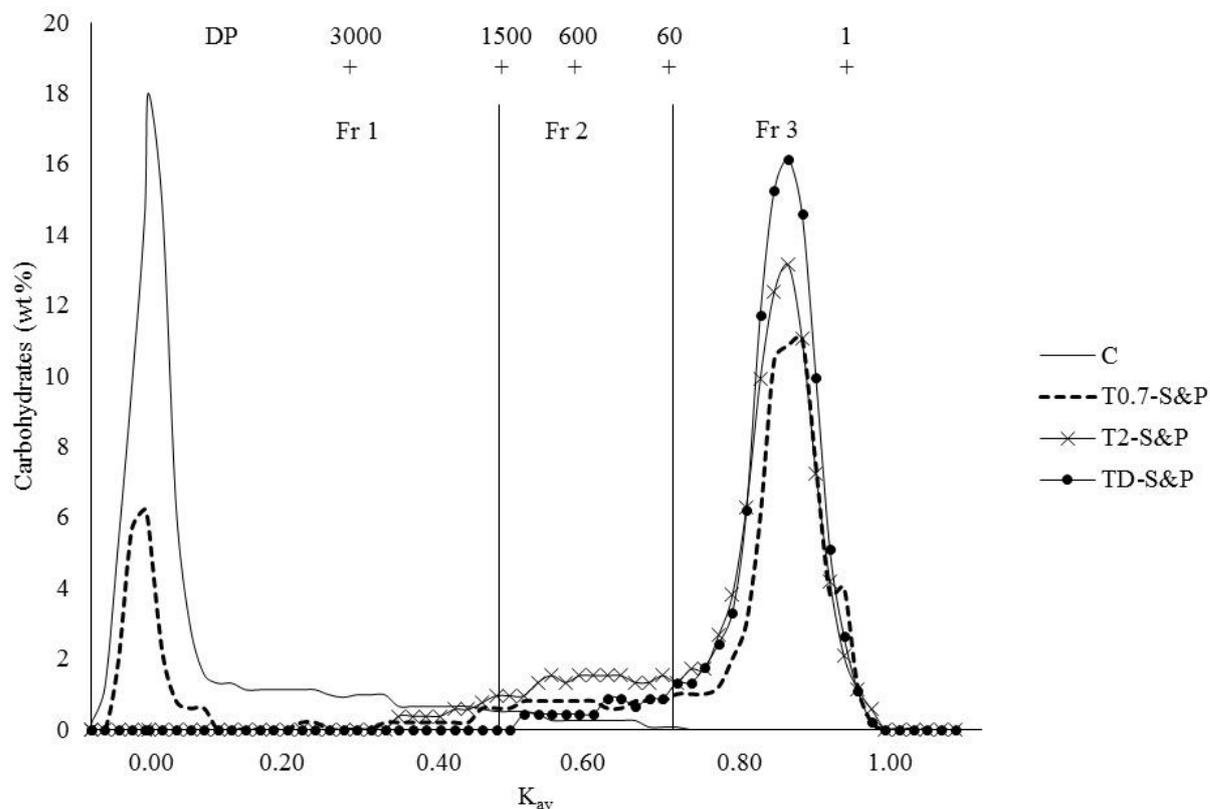


Figure 3: Gel-permeation chromatograms of salivary and pancreatic amylase hydrolysates of normal potato starch digested for 20 min. C = control; T0.7-S&P = starch cooked in 1:0.7 starch: water and subjected to salivary and pancreatic amylases hydrolysis. T2-S&P = starch cooked in 1:2 starch: water and subjected to salivary and pancreatic amylases hydrolysis. TD-S&P = starch dispersed with DMSO and subjected to salivary and pancreatic amylases hydrolysis. DP = degree of polymerization. Fr 1-3 represents divisions of DP fractions; fraction 1 (DP >1500), fraction 2 (DP 1500-60) and fraction 3 (DP < 60). K_{av} = gel partition coefficient.

rides detected in the hydrolysates of processed starches after 120 min of hydrolysis. Maltose and maltotriose constituted the majority by weight (together around 85-87%) of the carbohydrates. The presence of glucose and the high amounts of maltose and maltotriose is in agreement with data reported by Jones et al. [25] of hydrolysate composition of pre-digested corn starch (Caloreen) following a 120 min hydrolysis by porcine pancreatic amylase.

In this study, the amount of maltose and maltotriose did not change between the treatments for each of the starches. But there were differences ($p < 0.05$) in amounts of other oligosaccharides between the processing treatments. Peak 5 was significantly lower in T2

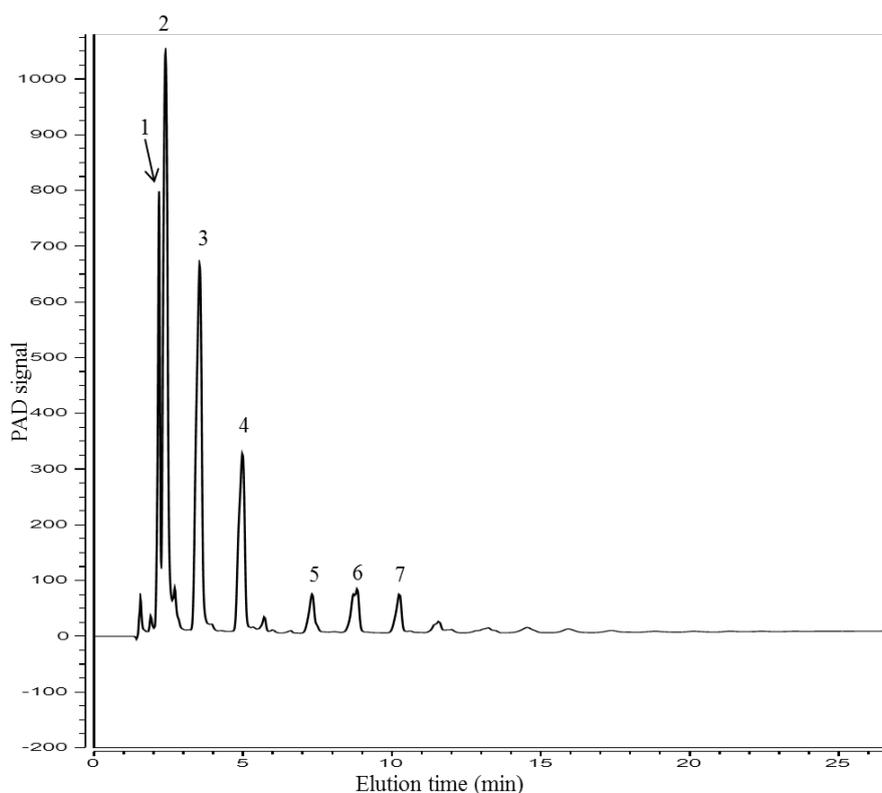


Figure 4: HPAEC chromatograms of oligosaccharides of normal wheat starch cooked with 1:2 starch:water ratio and then subjected to salivary and pancreatic amylase for 120 min. Peaks: 1 corresponded to glucose, 2 corresponded to isomaltose, 3 corresponded to maltose, 4 = DP 3, 5 = DP 5, 6 = DP 6, 7 = DP 7. Note: Peaks 1 and 2 also include materials present in porcine pancreatic amylase solution.

for NCS than in the other treatments. For NWS, this peak was lowest in TD and highest in T2. For NPS, peak 5 was lower in T0.7 than in the T2 and TD. The amount of peak 6 was highest in T2 than in the other treatments for NWS but no significant differences were observed between the treatments for the other starches. NCS gave a higher amount of peak 7 in T0.7 than in the other treatments. NWS had a higher amount of peak 6 in T2 than in TD. No significant differences were observed for this peak in NPS between the treatments.

These results show differences in the proportions of oligosaccharides between starches and between the processing treatments. Surprisingly, hydrolysis for 120 min of T0.7 treatment yielded same ($p < 0.05$) amounts of oligosaccharides as those of TD treatment as evident from NPS (Table 1). These results therefore raise questions such as: Do differences in processing/water content matter in the digestion of starch in the small intestine? Do the

Table 1: Relative amounts of oligosaccharides of starches following salivary and pancreatic amylase hydrolysis¹

Starch type	Treatment	DP2	DP3	DP5	DP6	DP7
NCS	T0.7-S&P	52.8 ± 0.1 ^{bcd}	32.0 ± 1.0 ^{ab}	4.4 ± 0.1 ^d	6.0 ± 1.0 ^{bc}	4.7 ± 0.2 ^d
	T2-S&P	53.5 ± 0.0 ^{cd}	32.6 ± 0.0 ^{ab}	3.9 ± 0.0 ^c	5.9 ± 0.0 ^{bc}	4.1 ± 0.0 ^b
	TD-S&P	54.3 ± 1.5 ^d	31.1 ± 1.2 ^a	4.3 ± 0.0 ^d	6.1 ± 0.0 ^{bc}	4.2 ± 0.4 ^b
NWS	T0.7-S&P	50.8 ± 0.3 ^{abc}	34.6 ± 1.2 ^{abc}	3.9 ± 0.2 ^c	6.1 ± 0.5 ^{bc}	4.6 ± 0.2 ^{cd}
	T2-S&P	49.7 ± 0.8 ^a	34.8 ± 1.4 ^{bc}	4.3 ± 0.0 ^d	6.4 ± 0.5 ^d	4.8 ± 0.1 ^d
	TD-S&P	50.3 ± 0.7 ^{ab}	37.1 ± 0.0 ^c	3.2 ± 0.2 ^{ab}	5.2 ± 0.4 ^{ab}	4.2 ± 0.1 ^{bc}
NPS	T0.7-S&P	52.9 ± 2.3 ^{bcd}	36.1 ± 2.9 ^c	2.9 ± 0.3 ^a	4.5 ± 0.2 ^a	3.6 ± 0.1 ^a
	T2-S&P	52.4 ± 1.7 ^{abcd}	35.2 ± 1.3 ^{bc}	3.3 ± 0.0 ^b	5.2 ± 0.2 ^{ab}	3.9 ± 0.2 ^{ab}
	TD-S&P	53.7 ± 1.5 ^{cd}	34.5 ± 1.5 ^{abc}	3.2 ± 0.1 ^{ab}	4.9 ± 0.0 ^a	3.8 ± 0.1 ^{ab}

¹Results are means of two replicate experiments; mean ± standard deviation ($n = 2$).

Values with different letters in a column are significantly ($p < 0.05$) different from each other.

DP = degree of polymerisation. T0.7 = cooked at 1:0.7 starch to water ratio.

T2 = cooked at 1:2 starch to water ratio. TD = dispersed using DMSO.

NCS = normal corn starch. NWS = normal wheat starch. NPS = normal potato starch.

S & P = subjected to salivary and pancreatic α -amylases hydrolysis.

differences observed affect glucose homeostasis? A study subjecting the hydrolysis products characterized in this study is underway to shed some light on these questions with regard to rate of glucose production by sucrase-isomaltase and maltase-glucoamylase.

4 Conclusions

The amount of water present during cooking of starch affects the structures and composition of starch hydrolysates from luminal (salivary and pancreatic) amylases. The differences in structures and composition differ depending on botanical sources of the starch. These differences in structures of starch hydrolysates and in proportions of oligosaccharides may impact on starch digestion by mucosal enzymes in the small intestines and/or possibly influence glucose homeostasis *in vivo*.

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