Short Communication

Influence of corn variety, drying temperature and moisture content at harvest on the saccharides released during an *in vitro* pepsin-pancreatin digestion†

Sylvanus Odjo[1*], François Huart[1], François Béra[1], Nicolas Jacquet[2], Aurore Richel[2], Christophe Blecker[3], Paul Malumba[1]

[1]University of Liege, Gembloux Agro-Bio Tech, Food Process Engineering Laboratory, Passage des Déportés, 2, B-5030 Gembloux, Belgium

[2]University of Liege, Gembloux Agro-Bio Tech, Department of Industrial Biological Chemistry, Passage des Déportés, 2, B-5030 Gembloux, Belgium

[3]University of Liege, Gembloux Agro-Bio Tech, Department of Food Sciences and Formulation, Passage des Déportés, 2, B-5030 Gembloux, Belgium

*__Corresponding author__

Tel.: +3281622268/Fax: +3281601767

Food Process Engineering Laboratory

University of Liège, Gembloux Agro-Bio Tech,

Passage des Déportés 2, 5030 Gembloux, Belgium

E-mail: dpsodjo@doct.ulg.ac.be; sylvanusodjo@gmail.com .

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Abstract

High-Performance Anion-Exchange Chromatography Coupled with Pulsed Amperometric Detection (HPAEC-PAD) was used to quantify and to determine the nature of saccharides released after the digestion of two corn grains submitted to different drying and harvesting protocols. Five saccharides were quantified: glucose, maltose, isomaltose, maltotriose and glucosyl-maltotriose. After a subsequent hydrolysis with amyloglucosidase of the released sugar, the amount of total glucose recovered increased with the increase in drying temperature and the moisture content of grain at harvest. These results suggest that structural changes induced within corn starch granules during drying affect their hydrolysis by amylases. These changes may lead to different pathways of sugar absorption in the small intestine of monogastrics. Thus, corn grains with a similar composition may lead to different levels of metabolizable energy upon gastro-intestinal digestion.

Keywords: corn starch; drying; *in vitro* digestion; HPAEC-PAD; saccharides

1. Introduction

Corn is the most grain crop produced in the world and is heavily used as food and feed mainly because of its high and readily available starch content. Starch granules consist of linear and branched polymers of α-D-glucose linked by α-1,4 and α-1,6 glycosidic linkages. The digestion of starch granules occurs through several sequential steps. The main part of the starch hydrolysis is carried out by the pancreatic α-amylase, an endoenzyme which cleaves α-1,4 glycosidic bonds of starch polymers in maltose, maltotriose and maltotetraose [1].

When starch is submitted to different levels of heat treatment, the features of the glucopolymers that compose its structure undergo different forms of restructuring, which can modify the ability of digestive enzymes to cleave them in specific oligosaccharides [2].

Previous studies have shown that a high drying temperature and a high initial moisture content of grain results in dried corn that is digested at different rates using pepsin-pancreatin digestion schemes [3].

It is likely that structural changes within starch granules during high temperature treatments of grain may modulate the action of α-amylase and may lead to different patterns of starch hydrolysis. Indeed, partial gelatinization and glass transition are among the factors usually called upon to explain such internal restructuring of starch granules during heat treatments that impact starch digestibility [4]. All of these changes may modify the accessibility of digestive enzymes towards their active site and may lead to the production of oligosaccharides with different degrees of polymerization as well as the release of granule ghosts of different sizes [5].

Even if it has been well accepted that the structural changes occurring within starch granules during heat treatments influence the rate of granules digestion, further insights are still required regarding the effect of post-harvest grain treatment on the nature of saccharides released after the pepsin-pancreatic digestion.

Some recent studies have revealed that High-Performance Anion-Exchange Chromatography Coupled with Pulsed Amperometric Detection (HPAEC-PAD) is a powerful method that is particularly effective in the determination and characterization of saccharides [6]. This method gives qualitative information about the structure of the saccharides released and may be useful in assessing the effect of drying temperature on corn starch digestion [7].

In the present study this analytical technique was used to assess the effect of drying temperature, moisture content at harvest and corn variety on the nature and the amount of saccharides released after an *in vitro* pepsin-pancreatin digestion of corn grain.

2. Materials and methods

2.1. Corn samples

Two corn varieties (Var I and Var II) were used in the current research. Var I was a flint-flint-dent cultivar and was harvested at two different dates after physiological maturity with two different moisture contents (33.4% and 28.8%, wet basis). Var II was only harvested at physiological maturity with moisture content of 33.0%. The proximal composition of the different corn grains used, determined on freeze-dried samples was presented in Table 1.

Table 1

Var I was dried in a fluidized-bed dryer at 54, 70, 90 and 130 °C respectively during 240, 120; 60 and 20 minutes. Var II was only dried at 54 and 130°C. Corn samples were then ground in an IKA Universalmühle M20 (Germany) and sieved through a 500 micron mesh.

2.2. *In vitro* digestion of corn grain

The corn flour samples obtained were *in vitro* digested following the procedure of Malumba et al. [3]. This digestive protocol includes a pepsin phase (using pepsin from porcine gastric mucosa; >250U/mg of solid powder P7000, Sigma-Aldrich, Germany) and a pancreatin phase (with pancreatin from porcine pancreas: 4xUSP/g of solid powder, P1750, Sigma-Aldrich, Germany). 500 mg of corn flour was accurately weighted in a 50 mL polypropylene tube together with 20.0 mL of phosphate buffer solution (pH 6.0) and 8 mL of HCl solution (0.2 M). The pH was adjusted to 2.0 and a fresh pepsin solution (1 mL, 25 g/L) were then added and the tubes were placed in a water-bath for 2 hours at 39±0.5 °C under gentle agitation. Following this step, which mimics the gastric phase of digestion, 8 mL of a phosphate buffer solution (pH 6.8) and 4 mL of a 0.6 M NaOH solution were added to the mixture. The pH was then adjusted to 6.and the resulting slurry was then carefully mixed with 1 mL of a freshly prepared pancreatin solution (100 g/L). Afterwards, the flasks were sealed and incubated under agitation at the same temperature in the same water-bath. After 4 h of incubation, the supernatants and the undigested fraction were separated after centrifugation at 3220 g during 10 minutes. The supernatants were then weighted, and stored at -18 °C for further analyses.

In order to determine the total concentration of D-glucose solubilized throughout the digestion, a supplemental hydrolysis of supernatants from the two-step pepsin pancreatin digestion scheme was performed using amyloglucosidase from *Aspergilus niger* (3260U/ml; Megazyme, Ireland), as proposed by Odjo et al. [7]. All of the digestibility trials were performed in triplicate.

2.3. Determination of oligosaccharides contained in supernatants collected at the end of the *in vitro* trial

The pattern and amount of oligosaccharides released after the pepsin-pancreatin *in vitro* digestion phase was determined in the supernatants collected, as described by Odjo et al. [7], using a Dionex ICS-5000 system (Dionex corp., Sunnyvale, CA.). This device is equipped with a Dionex ICs-5000 pump configured for a standard gradient analytical pump, an Eluent Generator (EG), a detector/chromatography module thermally regulated with a 25 μL injection loop, an AS-AP autosampler and an ED40 electrochemical detector with an amperometric cell. Oligosaccharide separation was performed as described by Goffin et al. [8], and the data collected were analyzed using the Dionex Chromeleon 6.80 SP3 Build 2345 software.

2.4. Statistical analyses

Statistical analyses were performed using Minitab software (version 16, MINITAB Inc., State College, PA). The amount of saccharides quantified was related to the dry mater of flour used for analyses. The values recorded were summarized as mean ±standard deviation. Taking into account the total amount of saccharides released, the relative proportion of each saccharides was also calculated

Collected data were subjected to analysis of variance (ANOVA) and mean comparisons were performed using the Tukey's test (family error rate). The experimental unit was the individual dried corn sample. The effect of drying temperature, initial moisture content and their interaction on the amount of the different saccharides released was monitored on Var I using the General Linear Model procedure of Minitab following equation (1):

$$Y_{ij} = m + \propto_i + \beta_j + \alpha\beta_{ij} + \epsilon_{ij} \quad (1)$$

where Y_{ij} represents the dependent variable (amount of sugar released); m the overall mean; α_i, the effect of drying temperature; β_j, the effect of initial moisture content, $\alpha\beta ij$, the interaction between drying temperature and initial moisture content and ε_{ij}, the random error. Using the same model, the effects of corn variety, drying temperature and their interaction were also checked on Var I and Var II.

3. Results and Discussion

Table 1 presents the saccharide pattern of the supernatants collected after the complete pepsin-pancreatin digestion of corn flour while Figure 1 presents the relative proportion of the different saccharides released after the *in vitro* digestion.

Table 1

Figure 1

Following the pepsin-pancreatin digestion of corn grain dried at different temperatures, the main carbohydrates released into the supernatants were glucose, isomaltose, maltotriose, and glucosyl-maltotriose. The five major sugars identified in the supernatants collected after pepsin-pancreatin digestion are comparable with those reported elsewhere [7,9].

Glucose and maltose were the most abundant sugars released, with concentrations respectively ranging from 127 to 212 mg/g of dry matter and 102 to 250 mg/g of dry matter. As it can be seen from figure 1, these two saccharides represented more or less 80% of the released sugars. It is well accepted that porcine pancreatic α-amylase splits α-1,4-glycosidic linkages mainly into maltose, maltotriose and branched α-limit dextrins. The high amount of glucose recorded in supernatants collected in the present study may have resulted from the prolonged incubation time, which would have allowed porcine pancreatin α-amylase to produce glucose through a slow secondary hydrolysis of the primary maltodextrin products [10].

Statistical analyses showed a significant impact of the drying temperature on the amount of the different mono and oligosaccharides determined ($p<0.05$). The moisture content and the corn variety also significantly impacted the amount of saccharides released. However, the interaction between drying temperature and initial moisture content and the interaction between drying temperature and corn variety were also significant. This suggests that the effect of drying temperature on saccharide released depends on the level of moisture content and the corn variety.

Overall, a higher drying temperature and higher initial moisture content led to a significant increase in the amount of maltose and maltotriose. When comparing the amounts of glucose, and glucosyl-maltotriose released by grain dried at 54 °C and grain dried at 70 °C, a slight decrease was observed at 70 °C. Even though, the drying temperature had a significant impact on the amount of isomaltose released, no clear trend was observed with the increase in drying temperature and initial moisture content. A linear increase in the amount of D-glucose released according to drying temperature was also noticed regardless of the moisture content and the corn variety. This increase could be associated with a partial gelatinization of starch occurring during drying. As postulated by Odjo et al. [11], gelatinization of starch may take place within the corn grain during drying, most likely at the beginning of the process, when it is usually considered that the water activity in the upper layers is close to 1. Malumba et al. [3] have shown a significant positive correlation ($r>0.8$; $p<0.05$) between starch gelatinization parameters and the coefficient of the *in vitro* dry matter digestibility of starch. According to these authors, the collapse of the crystalline region coupled with a transition occurring in the amorphous phase of granules during the high-temperature drying of grains seem fundamental in explaining differences in the hydrolysis rate of granules and in the profile of the sugars subsequently released.

The oligosaccharide pattern revealed by HPAEC-PAD confirmed that the modifications in granules structure induced by heat treatment impacted the accessibility of enzymes to their site of attack and therefore the rate of release of different solubilized saccharides. The high rate of saccharides digested observed with a high drying temperature is illustrative of an improvement in α-amylase accessibility towards its site of attack. This accessibility may also be modulated by the proteins that surround starch granules. Even dough protein denaturation may occur during high drying temperature, its impact on starch digestion can be regarded as negligible. According to Warren et al. [12], the impact of others dietary components including protein on the interaction between α-amylase and starch granule may be considered insignificant, compared with the structural features of starch granules. According to these authors, the structural order of α-glucan chains at the enzyme–starch interface is fundamental in explaining differences in the hydrolysis rate of granules and therefore, the profile of the sugars subsequently released.

The highest value of maltose, maltotriose and glucosyl-maltotriose recorded with corn samples dried at 130 °C suggested that increasing the drying temperature may lead to the production of more complex saccharides. These structural features are likely to affect the viscosity of the bolus reaching the intestinal tract as well as the availability of the microbiome to residual starch ghosts and therefore the fermentation pathway and rate.

4. Conclusion

This study aimed at assessing the effect of corn variety, drying temperature and initial moisture content on the amount and nature of saccharides released after a pepsin-pancreatin digestion of corn grain. Glucose, isomaltose, maltose, maltotriose and glucosyl-maltotriose were identified as the main saccharides present in supernatant collected. By modifying the structural feature of starch granules corn grain and probably the structure of other components surrounding starch granules, the drying process certainly affects the accessibility of pancreatic α-amylase towards its site of attacks and results in different rate and profiles of saccharides released. High temperature drying induces the production of saccharides with higher degree of polymerization. This may have consequences on the digestive physiology of consumers.

The impact of these modifications on animal performance is not fully elucidated. Further studies based on *in vivo* assessment are still needed to elucidate the effect of different saccharides released on the physiology of digestive tract as well as on the feeding value of grains dried at different temperatures and harvested at different drying stages.

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Conflict of interest statement

The authors have declared no conflict of interest.

Novelty statement

In the present study, the effect of structural modification induced within starch granules during high drying temperatures on the digestion of starch was assessed using HPAEC-PAD. Increasing the drying temperature was shown to result in a linear increase in glucose released.

Higher drying temperatures were shown to lead to the production of saccharides with a higher degree of polymerization, which may, in turn, affect the glycemic status of the consumers.

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Table 1: Average proximal composition of the corn samples used (g/100g of dry matter)

Corn varieties	Moisture content at harvest (wet basis)	Starch[1]	Crude protein[2]	Fats[3]	Ash[4]	Starch amylose content[5]
Var I	33.4	76.7	8.5	4.7	1.0	20.9
	28.8	76.7	8.6	4.7	1.1	21.0
Var II	33.0	74.6	8.2	5.4	1.2	19.6

Data presented are means of three replicates. 1: ISO 10520:1997; 2: Nx6.25, ISO 20483:2006; 3: AOAC 922.06; 1990; 4: ISO 2171, 1993; 5: Morisson & Laignelet, 1983.

Table 2: Patterns of saccharides released and total D-glucose solubilized after *in vitro* pepsin-pancreatin digestion of corn grain harvested at different moisture content after physiologic maturity and dried at different temperatures (n=3)

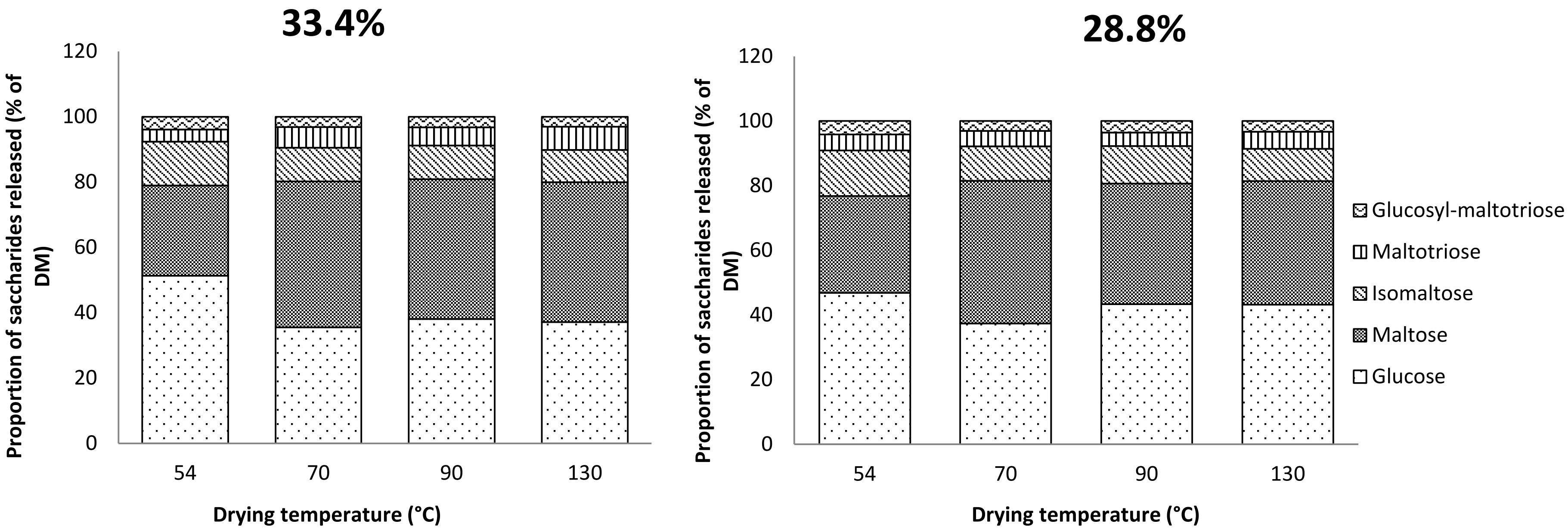
Corn variety	Harvesting date and moisture content	Drying temperature (°C)	Mono and oligosaccharides (mg/g of flour, dry basis)					
			Glucose	Maltose	Isomaltose	Maltotriose	Glucosyl-maltotriose	Total D-glucose solubilized
Var I	Date 1 (moisture content 33.4%, w.b)	54	190.1±2.1^{a}	102.2±5.5^{a}	49.7±0.6^{a}	13.9±0.9^{a}	14.4±1.2^{ab}	320.5±12.7^{a}
		70	134.9±1.6^{b}	169.7±7.6^{b}	39.0±0.8^{b}	23.9±0.8^{b}	12.1±1.1^{a}	375.1±21.6^{a}
		90	153.9±2.2^{c}	173.0±3.7^{b}	41.6±1.3^{b}	22.5±1.5^{b}	13.1±0.6^{ab}	459.4±25.1^{b}
		130	184.2±3.4^{a}	212.0±5.6^{c}	48.9±2.1^{a}	35.2±1.0^{c}	15.2±1.2^{b}	516.2±24.6^{c}
	Date 2 (moisture content 28.8%, w.b)	54	159.2±11.1^{a}	101.8±1.2^{a}	47.8±0.2^{a}	16.8±0.5^{a}	14.1±0.3^{a}	300.8±18.7^{a}
		70	127.1±2.1^{b}	150.0±2.2^{b}	36.3±1.3^{b}	16.5±1.6^{a}	10.3±0.4^{b}	362.7±12.0^{b}
		90	185.5±2.3^{c}	159.3±7.8^{b}	49.8±1.3^{a}	17.9±1.6^{a}	15.1±1.0^{ac}	393.6±34.3^{b}
		130	212.3±3.0^{d}	188.1±10.0^{c}	49.1±2.7^{a}	26.0±0.6^{b}	16.2±0.4^{c}	477.0±19.6^{c}
Var II	Date 1 (moisture content: 33.0% w.b)	54	164.0±1.3	201.8±10.1	50.5±2.1	23.8±1.9	14.8±0.2	324.5±23.3^{a}
		130	193.0±2.5	249.8±11.7	45.9±1.7	29.6±2.5	18.5±0.8	510.6±17.3^{b}
Model 1								
Temperature		P	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
Moisture content		P	0.012	p<0.001	0.148	p<0.001	0.546	0.002
Temperature X Moisture content		P	p<0.001	0.023	p<0.001	p<0.001	0.009	0.049
Model 2								
Temperature		P	p<0.001	p<0.001	0.001	p<0.001	p<0.001	p<0.001
Corn variety		P	p<0.001	p<0.001	0.176	0.022	p<0.001	0.032
Temperature X Corn variety		P	p<0.001	p<0.001	0.025	p<0.001	0.001	0.092

Different letters within the same column indicate statistically significant differences (p < 0.05).

▪ FIGURE CAPTIONS

Figure 1: Impact of drying temperature and moisture content at harvest on the proportion of saccharides released after an *in vitro* pepsin-pancreatin digestion

Figure 1:


33.4%
28.8%
Proportion of saccharides released (% of DM)
120
100
80
60
40
20
0
54
70
90
130
Drying temperature (°C)
Glucosyl-maltotriose
Maltotriose
Isomaltose
Maltose
Glucose