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Assessment of the influence of amylose-LPC complexation on the extent of wheat starch digestibility by size-exclusion chromatography

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ABSTRACT

Amylose forms inclusion complexes with lysophosphatidylcholine (LPC), that decrease the susceptibility of amylose to amylase degradation. This study on the influence of complexation on starch susceptibility to amylase explains the nature of this protective effect. Wheat starch suspensions (9% w/w) containing 0.5–5% LPC were subjected to hydrolysis by porcine pancreatic α-amylase at 37 °C for several digestion times. The digesta were analysed by size-exclusion chromatography (SEC). The molar mass distribution was closely dependent on the digestion time and amount of LPC. This study precisely demonstrates the alteration of the digestion profile of starch on a molecular level, influenced by amylose-LPC complexation; however the effect depends on the digestion time. During 15 and 30 min digestion, inclusion complexes not only protect amylopectin in the initial hydrolysis stage, but also demonstrate lower susceptibility of the molecular amylose complexes to amylase hydrolysis. Digestion for 240 min resulted in a lower oligosaccharide peak concentration, in the presence of a high LPC concentration, which is related to less degradation of complexed amylose fraction.

1. Introduction

Starch is a widely used component to provide functional properties to food and is considered as the major source of energy in human nutrition, supplying more than 50% of the caloric energy. It is a major component of many food plants like wheat, potato, maize and rice (BeMiller & Whistler, 2009). Starch is composed of two polymers: amylose and amylopectin. Amylose is linear and has a molecular weight of *ca.* 10^6 Da and amylopectin is branched with a molecular weight of about *ca.* 10^8 Da. Enzymes are able to hydrolyse the α-1 → 4 and the α-1 → 6 glycosidic bonds, resulting in maltodextrins, maltose and glucose. The source of starch and the amylose:amylopectin ratio determine not only the functional properties but also the digestibility of starch and subsequently the amount of glucose release into the blood stream, known as the glycaemic index (GI) (Guraya, Kadan, & Champagne, 1997). Starch, based on its digestibility, can be classified into three categories: RDS (rapidly digestible starch – starch that is digested within 20 min), SDS (slowly digestible starch – starch that is digested between 20 and 120 min) and RS (resistant starch – starch that cannot be digested but is fermented in the large intestine), which are characterised by the rate and duration of the glycaemic response (Englyst, Englyst, Hudson, Cole, & Cummings, 1999; Englyst, Kingman, & Cummings, 1992). Starch digestibility has a big impact on human health. Rapid postprandial glucose increase in the blood stream, due to the rapid digestible starches, is considered as a risk factor, which may cause obesity and type II diabetes; while slow and resistant starches are suggested to help in preventing metabolic disorders and colon cancer (Hasjim, Lavau, Gidley, & Gilbert, 2010).

Starch digestion is a complex process that is highly dependent on several factors, such as the source of starch, enzyme activity and presence of other components like lipids and proteins. In our previous studies, we followed the formation of amylose inclusion complexes with lysophosphatidylcholine (LPC) (Ahmadi-Abhari, Woortman, Hamer, Oudhuis, & Loos, 2013a and Ahmadi-Abhari, Woortman, Oudhuis, Hamer, & Loos, 2013c). The formation of inclusion complexes is reported to decrease water ingression into the starch granules at the temperature of gelatinisation (Putseys et al., 2010; Gelders, Duyck, Goesaert, & Delcour, 2005). In a previous paper, we demonstrated the effect of inclusion complex formation on the physical properties of starch, such as viscosity and swelling power (Ahmadi-Abhari, Woortman, Hamer et al., 2013a). Also, we evaluated the influence of inclusion complexation on the degradability of starch with an *in vitro* digestion method (Ahmadi-Abhari, Woortman, Oudhuis, Hamer, & Loos, 2013b). We were able to show that amylose-LPC complexation decreases the

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susceptibility of starch to α-amylase. The influence is stronger at higher LPC concentrations and best observed during shorter digestion times. At longer incubation times (>60 min), the effect is notably less. Our previous study provided reproducible results, showing the overall influence of the formation of amylose inclusion complexes on the degradability of starch; however more insight into the molar mass and size distribution of starch polymers after each digestion time (in relation to the influence of amylose inclusion complexation with LPC) was required. We therefore, in this study, used size-exclusion chromatography (SEC) to determine the molar mass (MM) of the starch polymers after enzyme treatmentduring different incubation times. Hence, the combination of our *in vitro* method and SEC allowed us to study the influence of LPC on the whole samples after each digestion time. While standard methods only assess the amount of reducing sugars after digestion; we are able to gain more detailed information on the nature of the protective effect of inclusion complex formation, assessing both the amount of reducing sugars and the molar mass of amylopectin, amylose and low molar mass sugars after amylase hydrolysis.

2. Materials and methods

2.1. Materials

Native wheat starch with a purity of 99% and a total lipid content of 0.4% was obtained from Sigma–Aldrich, St Louis, MO. 12.63% moisture content was measured by a moisture analyser (Sartorius MA35M; Sartorius AG, Goettingen, Germany). 2.8% damaged granules and 23.5% amylose content (wheat starch not defatted) were reported by Eurofins Food B.V. Egg yolk L-α-lysophosphatidylcholine (LPC; type XVI-E, lyophilised powder, purity >99% and fatty acid content of 16:0 69%, 18:0 27% and 18:1 3%) from Sigma–Aldrich was used. Porcine pancreatic (150,000 U/g, free flowing powder, partially purified) α-amylase was employed from Megazyme International (Wicklow, Ireland). LPC and α-amylase were kept at −20 °C and wheat starch at room temperature under dark and dry conditions. Maltose and maltotriose (DP2 and DP3 respectively), monosodium phosphate monohydrate, sodium phosphate dibasic, sodium chloride and dimethylsulphoxide (DMSO) (CHROMASOLV Plus, HPLC grade, ⩾99.7%) purchased from Sigma–Aldrich were of analytical grade or better. Anhydrous lithium bromide (99%) and pullulan (P 0.3–800) molar mass standards were obtained from Fisher Scientific and PSS (Polymer Standard Service, Mainz, Germany), respectively. Maltoheptaose (DP7) was previously synthesised as a standard (Van der Vlist et al., 2008).

2.2. Sample preparation

An RVA-4 Newport Scientific (NSW, Australia) Rapid Visco Analyzer was employed to prepare samples for the enzymatic hydrolysis. A series of 9% w/w wheat starch suspensions in deionised water was prepared by mixing starch with 0%, 0.5%, 1%, 2%, 3% and 5% LPC (based on starch dry matter content). The suspensions were kept 10 min at room temperature to equilibrate. The RVA was programmed in three steps. The temperature of all samples was first equilibrated at 50 °C for 60 s, increased to 95 °C at a rate of 6 °C/min and they were ultimately held at 95 °C for 300 s. The samples were cooled down to 37 °C in a water bath.

2.3. Amylase hydrolysis

Five grams of each sample from the RVA were diluted with phosphate buffer (17 g, 0.025 M, pH 6.9) to achieve a 2% (w/v) suspension. The phosphate buffer contained 6 mM sodium chloride to preserve the activity of the enzyme (Qian, Ajandouz, Payan, & Nahoum, 2005). The suspensions were equilibrated at 37 °C in a water bath to simulate body temperature. 0.5 mL of the enzyme solution (0.004% w/v), freshly prepared, was added to each suspension. Subsequently the suspensions were incubated while rotating in a modified ventilation oven (Thermo Scientific Heraeus 6000, Langenselbold, Germany) at 37 °C for 15, 30, 60 and 240 min. After each digestion time, the amylase hydrolysed sample was heated immediately in a boiling water bath for 5 min, to inactivate the enzyme, and freeze dried in a laboratory freeze dryer (VaCo 2; Zirbus Technology, Harz, Germany).

2.4. Size-exclusion chromatography

LiBr in DMSO (50 mM) was stirred at room temperature for 2 h. The solution was degassed for 5 min by an ultrasonic cleaner (Branson 2510; Branson, Danbury, CT). The freeze dried samples were dissolved in DMSO–LiBr at a concentration of 2 g/L while overnight rotation at ambient temperature was followed by 2 h rotation in a modified ventilation oven (Thermo Scientific Heraeus 6000, Langenselbold, Germany) at 80 °C, obtaining clear sample solutions. The samples were allowed to cool down slowly to room temperature and filtered through 5-μm Millex PTFE membrane (Millipore Corporation, Billerica, MA) and 1-μm PTFE membrane (Pall Corporation, Port Washington, NY) for the short (15 and 30 min) and long (60 and 240 min) degradation times, respectively. Each sample was made in duplicate. Size-exclusion chromatography equipment (SEC, often termed gel-permeation chromatography, GPC,) from PSS (Polymer Standard Service, Mainz, Germany) was used to analyse the molecular size distribution of the starch molecules after digestion, according to Ciric, Oostland, de Vries, Woortman, and Loos, (2012). The system was equipped with an isocratic pump and an online degasser. DMSO–LiBr was employed as the eluent. The samples were injected with a flow rate of 0.5 mL/min by an autosampler to a PFG guard column and the separation was carried out by three PFG-SEC columns with porosity 100, 300 and 4000 Å, purchased from PSS. The columns were held at 80 °C. The SEC setup consisted of a refractive index detector (G1362A 1260 RID; Agilent Technologies, Santa Clara, CA) and a MALLS detector (SLD 7000; PSS, Mainz). The refractive index detector was thermostatted at 45 °C. The MALLS signal was used to assess the molar masses up to the elution volume of 21 mL (a refractive index increment d*n*/d*c* of 0.072 was used). In the low molar mass region, in which light scattering becomes unreliable, pullulan calibration standards (PSS, Mainz, Germany; molecular weights of 342, 1320, 6000, 10,000, 21,700, 48,800, 113,000, 210,000, 366,000 and 805,000 Da for P0.3, P1, P5, P10, P20, P50, P100, P200, P400 and P800, respectively) were used. All samples were filtered through a 0.45-μm PTFE membrane from VWR (Radnor, PA), before injection. The resulting SEC chromatograms were analysed using WinGPC Unity software (PSS, Mainz, Germany).

3. Results

3.1. Relationship between molecular size and elution volume

Fig. 1 shows the SEC chromatograms of maltose, maltotriose, maltoheptaose and their mixture. In SEC, the elution volume is directly related to the hydrodynamic volume of the linear and branched molecules, which leads to the size distribution of a sample. The hydrodynamic volume is the volume occupied by an equivalent sphere in the eluent. It is important to recall that SEC separates by hydrodynamic volume (Cave, Seabrook, Gidley, & Gilbert, 2009).

Fig. 1 demonstrates that the used low molar mass maltodextrins can be separated and analysed at the employed conditions.