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The influence of amylose-LPC complex formation on the susceptibility of wheat starch to amylase

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ABSTRACT

This study was aimed to assess the role of lysophosphatidylcholine (LPC) in the development of slowly digestible starch (SDS). The influence of LPC, on the enzymatic degradation of diluted 9% wheat starch suspensions (w/w) was investigated, using an *in vitro* digestion method. Wheat starch suspensions containing 0.5–5% LPC (based on starch) were heated in a Rapid Visco Analyser (RVA) till 95 °C and subjected to enzyme hydrolysis by porcine pancreatic α-amylase at 37 °C for several digestion periods. *In vitro* digestion measurements demonstrated that complexing starch with 5% LPC leads to a 22% decrease in rate of reducing sugar compared to the reference while the samples containing 0.5% LPC showed an equal digestibility comparable to the control. A clear decrease in the formation of reducing sugars was observed in presence of 2–5% LPC, since the results after 15 min digestion imply the formation of SDS due to the formation of amylose-LPC inclusion complexes. The DSC measurements proved the presence of amylose-LPC inclusion complexes even after 240 min digestion demonstrating the low susceptibility of amylose-V complexes to amylase.

1. Introduction

Public awareness on the relation between human health and nutrition has increased with the increased attention for obesity and diabetes type II. With respect to the latter, particularly a focus has been given to starch and starchy foods. Starch is the largest source of carbohydrates in the human diet. In the West, it constitutes 27% of the total food energy sources and it reaches to above 50% in Southeast Asia (Cui & Oates, 1997). In this respect, the rate and extent of starch digestion is of great interest as it affects the glycemic response (Tufvesson, Skrabanja, Björck, Elmstahl, & Eliasson, 2001). Starch is a homopolymer of glucose units that consists of two fractions, amylose and amylopectin, assembled in a cluster structure. Amylose is a linear polysaccharide of α-1→4 D-glucose and amylopectin is a branched polymer of α-1→4 D-glucose and α-1→6 at the branching points.

The source, amount and form of consumed carbohydrates determine the digestibility and subsequently the rate of glucose release to the blood stream, called Glycemic Index (GI) (Guraya, Kadan, & Champagne, 1997). The GI describes the level of the postprandial glucose rise in blood as compared to ingestion of a standard dose of glucose (Zhang, Ao, & Hamaker, 2006; Zhang, Venkatachalam, & Hamaker, 2006). High peaks in blood glucose are considered a risk factor in diabetes type II. Hence, there is an increased interest in controlling the rate of release of glucose from starch.

In the human body, starch is hydrolyzed to glucose by enzymes through several steps (Singh, Dartois, & Kaur, 2010). Upon ingestion, starch is exposed to salivary α-amylase. Glucose absorption mainly occurs in the small intestine (Lehmann & Robin, 2007) where the pancreatic α-amylase hydrolyses amylose/amylopectin to maltose and larger oligosaccharides (maltose, maltotriose and maltotetraose) (Hasjim, Lavau, Gidley, & Gilbert, 2010). The α-amylases hydrolyze α-(1–4) glycosidic bonds (Singh et al., 2010). Maltose-glucoamylase and sucrose-isomaltase, two brush border enzymes, degrade oligosaccharides to glucose which then passes the blood stream (Gray, 1992).

Starch, based on its digestibility, can be classified into three categories: RDS (rapidly digestible starch – starch that is digested to glucose after 20 min), SDS (slowly digestible starch – starch that is digested to glucose between 20 and 120 min) and RS (resistant starch – starch that cannot be digested but is fermented in the large intestine) which are characterized by the rate and duration of the glycemic response (Englyst, Englyst, Hudson, Cole, & Cummings, 1999; Englyst, Kingman, & Cummings, 1992).

Generally, the digestion of starch is a complex process that is strongly dependent on the substrate, enzyme adsorption by the

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substrate and presence of other components like lipids and proteins (Lehmann & Robin, 2007).

It is possible to increase the resistance of starch components to enzyme hydrolysis. For instance, endogenous lipids and phospholipids in cereal starches have the ability of complexation with amylose (Kwasniewska-Karolak, Nebesny, & Rosicka-Kaczmarek, 2008) thus rendering the amylose less susceptible to amylolytic enzymes (Nebesny, Rosicka, & Tkaczyk, 2002; Zhang, Ao, et al., 2006; Zhang, Venkatachalam, et al., 2006). *In vivo* and *in vitro* digestibility studies on the effect of these components have shown that they can considerably slow down the enzymatic digestibility (Singh et al., 2010).

In our previous study (Ahmadi-Abhari, Woortman, Hamer, Oudhuis, & Loos, 2013), we have evaluated the influence of LPC on the structuring properties of wheat starch and have shown that it is possible to form considerable quantities of amylose-LPC complexes or V-complexes while maintaining part of the thickening function of starch.

Recent studies have reported the low digestibility of V-complexes (Putseys et al., 2010). V-complexes are characterized by a specific X-ray diffraction pattern and are formed between the aliphatic chains of lipids and the amylose molecules. Lysophosphatidylcholine (LPC) is a complexing agent that has shown high complexing ability with amylose, as indicated by DSC (Ahmadi-Abhari et al., 2013; Cui et al., 1997). Also, the poor digestibility of the amylose-lipid complexes has been demonstrated (Seneviratne & Biliaderis, 1991). However it is not clear if this complexation only affects the digestibility of complexed amylose chains or also the overall rate of digestion of starch. Therefore, the purpose of this study was to establish an understanding of the digestion of wheat starch and the influence of LPC on hampering enzyme hydrolysis, revealing additionally the difference between an overall effect of LPC on starch digestibility *versus* the degradation of the amylose inclusion complexes.

Various *in vitro* starch digestion methods exist which are designed to simulate starch digestion in the human body (Hasjim et al., 2010). The Englyst method (Englyst et al., 1992) is a widely used method for *in vitro* hydrolysis of starchy foods. Most studies have found a good correlation between the results of the Englyst method and *in vivo* results. The Englyst method is designed to assess whole meals, while in this study we work with a purified system and need to analyze the rate of starch digestion in considerable detail. This requires a slightly different setup. Therefore, in this study we demonstrate an alternative method that is established based on the optimum conditions to investigate the digestibility of well-defined starch–LPC mixtures under controlled time–temperature–shear conditions in a diluted suspension.

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 Chemical Company. 12.63% moisture content was measured by a moisture analyzer (Sartorius MA35M, Sartorius AG, Germany) and 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, lyophilized powder, purity >99% and fatty acid content of 16:0 69%, 18:0 27% and 18:1 3%, from Sigma Chemical Company (St. Louis, Missouri, USA) was used.

The α-amylase from Porcine Pancreatic (150,000 U/g), free flowing powder, partially purified, from Megazyme International Ireland (Wicklow, Ireland) was employed.

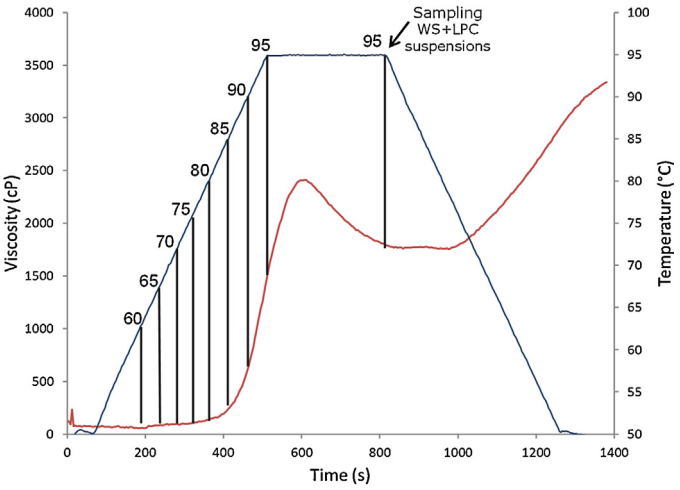

Fig. 1. RVA profile indicating the temperature–time profile and sampling point.

LPC and α-amylase were kept at −20 °C and wheat starch at room temperature under dark and dry conditions.

Monosodium phosphate monohydrate, sodium phosphate dibasic, sodium chloride, sodium hydroxide, 3,5-dinitrosalicylic acid (DNSA), potassium sodium tartrate and maltose monohydrate purchased from Sigma were of analytical grade or better.

2.2. Starch gelatinization/complexation

A 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 deionized water was prepared by mixing starch with 0%, 0.5%, 1%, 2%, 3% and 5% LPC (based on starch dry matter content), previously dissolved in deionized water. The suspensions were kept 10 min at room temperature to equilibrate. The RVA was programmed in three steps. The temperature of the suspensions was first equilibrated at 50 °C for 60 s, increased to 95 °C at a rate of 6 °C/min and held at 95 °C for 300 s. The reference (pure starch) was subjected to the same temperature gradient.

Another series of 9% (w/w) wheat starch suspensions, without LPC, were prepared in deionized water and after 10 min equilibration at room temperature, heated in the RVA first at 50 °C for 60 s. Then the temperature increased to 60 °C, 65 °C, 70 °C, 75 °C, 80 °C, 85 °C, 90 °C, 95 °C at the same rate of 6 °C/min (see Fig. 1).

2.3. Preparation of DNSA reagent

The DNSA solution was prepared by dissolving 1 g DNSA (3,5-dinitrosalycylic acid) in 20 mL 2 N sodium hydroxide solution and 50 mL, 30% (w/v), potassium sodium tartrate solution. The solution was stirred while gently heating until a clear solution was obtained. This solution was diluted with deionized water to 100 mL. The DNSA solution was flushed with N_2 and stored in a dark place until use.

2.4. In vitro enzymatic digestion

5 g of each sample from the RVA was 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 each day), was added to each suspension.