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Inhibitory effect of chlorogenic acid on digestion of potato starch

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ABSTRACT

The effect of the chlorogenic acid isomer 5-O-caffeoylquinic acid (5-CQA) on digestion of potato starch by porcine pancreatic alpha amylase (PPAA) was investigated using isolated starch and cooked potato tuber as substrates. *In vitro* digestion was performed on five varieties of potato with varying phenolic content. Co- and pre-incubation of PPAA with 5-CQA significantly reduced PPAA activity in a dose dependent manner with an IC50 value of about 2 mg mL^{-1}. Lineweaver-Burk plots indicated that 5-CQA exerts a mixed type inhibition as k_m increased and V_{max} decreased. The total polyphenol content (TPC) of peeled tuber tissue ranged from 320.59 to 528.94 mg 100 g^{-1} dry weight (DW) in raw tubers and 282.03–543.96 mg 100 g^{-1} DW in cooked tubers. With the exception of Désirée, TPC and 5-CQA levels decreased after cooking. Principle component analysis indicated that digestibility is affected by multiple factors including phenolic, dry matter and starch content.

1. Introduction

Potato is the third most consumed food crop providing around 5–15% of dietary energy, primarily from starch, to various populations around the world. Potato has been labelled with a high glycaemic index (GI) because consumption of some potato products can cause a sharp increase of postprandial blood glucose concentration. However there is significant variation in the GI of various potato products (Weichselbaum, 2010). Many investigators have reported the effect of food processing (industrial or domestic) on the GI of potato (Ek, Brand-Miller, & Copeland, 2012). For example, it has been reported that freshly boiled potato (GI 78) and instant mashed potato (GI 87) have a higher GI than French fries (GI 63) (Atkinson, Foster-Powell, & Brand-Miller, 2008). Furthermore, the incremental area under curve (AUC) of freshly cooked potato decreased by 18% and 30% upon storage in the refrigerator for 1 and 5 days respectively. This observation is attributed to the retrogradation of amylose which reduces digestibility (Fernandes, Velangi, & Wolever, 2005). Henry, Lightowler, Strik, and Storey (2005) determined the GI of commercially available potatoes in Great Britain and demonstrated that varieties prepared by boiling for 15 min showed a wide variation in GI values, ranging from 56 for Marfona to 94 for Maris Peer. Henry postulated that the difference was related to their texture, with waxy potatoes having a medium GI and floury potatoes having a high GI. We postulate that some of these differences may be attributed to the presence of endogenous polyphenolic substances acting as α-amylase inhibitors in the digestive tract.

Pancreatic alpha amylase is an endoglycosidase enzyme that has a significant role in carbohydrate digestion. It has been shown that inhibitors of α-amylase reduce bioavailability of glucose (Bozzetto et al., 2015). Controlling blood glucose level by α-amylase inhibitors may play a role in preventing hyperglycaemia in patients with diabetes mellitus. Some α-amylase inhibitors are naturally present in foods. Potato tubers contain many plant secondary metabolites including phenolics, carotenoids and polyamines. Their content and composition vary according to the variety of potato, conditions of cultivation, cooking and processing methods (Ezekiel, Singh, Sharma, & Kaur, 2013; Reyes & Cisneros-Zevallos, 2003). Phenolics present in potatoes include phenolic acids, tannins, lignin, flavonoids, coumarins and anthocyanins (Reyes & Cisneros-Zevallos, 2003). 5-O-caffeoylquinic acid (5-CQA) is an isomer of chlorogenic acid (CGA) which makes up 90% of the phenolic content of potato (Malenberg & Theander, 1985). The concentration of 5-CQA is higher in the skin than in the medulla. Analysis has shown that cooked unpeeled potato contains between 9.1 and 12 mg 5-CQA per 100 g fresh weight compared to 0.86–6.6 mg for equivalent peeled samples (Mattila & Hellstrom, 2007). The variation in 5-CQA content suggests that

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different potatoes may inhibit pancreatic amylase to different extents.

Evidence from a number of *in vitro* and *in vivo* studies indicates inhibitory effects of polyphenols on enzymes involved in carbohydrate digestion. It was reported that 5-CQA, quinic acid (QA) and caffeic acid (CA) have mixed-type inhibitory effect against pure porcine pancreatic alpha amylase (PPAA) isomers I and II using *p*-nitrophenyl-α-d-maltoside as a substrate (Narita & Inouye, 2009). The most potent inhibitor was 5-CQA followed by CA and QA. In an *in vivo* animal study, oral intake of a 5-CQA solution (3.5 mg kg^{-1} body weight) during a glucose tolerance test lowered the height of glycaemia peaks at 10 and 15 min by 22 and 17% respectively compared to control (only glucose) (Bassoli et al., 2008). Rohn, Rawel, and Kroll (2002) derivatized PPAA with a number of phenolic compounds and showed that 5-CQA reacted covalently with the enzyme and decreased its activity by about 50%. However, the mode of inhibition and potential effect on digestion of native potato starch has not been shown. The aim of the present study was to characterise the effect of 5-CQA on PPAA activity *in vitro* using potato starch as a substrate and to determine the *in vitro* digestibility of steam cooked potatoes from varieties which vary in their phenolic content.

2. Materials and methods

2.1. Potato samples

Tubers from five commercial varieties that differ in skin colour were purchased from food markets in Leeds, UK. Maris piper has creamy flesh and golden yellow skin; Maris peer has creamy flesh and skin; Désirée, Rooster and Mozart have reddish pink skin and light yellow flesh. Three potatoes from each variety were rinsed with water and dried with a paper towel. Then the potatoes were peeled to remove skin and cortex, cut into cubes (1 cm^3) and separated into 100 g batches. Potatoes cubes were placed into a steam pan and cooked for 30 min at boiling temperature. Cooked potatoes were mashed by using a fork and used for enzymatic digestion. The remaining raw and steam cooked mashed potato was immediately frozen at −80 °C, freeze dried and stored at −80 °C for analysis of TPC and phenolic acid composition. Analyses were repeated at least three times with three batches of potato.

2.2. In vitro digestion of potato starch in presence of chlorogenic acid

The activity of PPAA (16 U/mg, Sigma Aldrich) enzyme on hydrolysis potato starch in the presence and absence of 5-CQA (Sigma Aldrich; PubChem CID 12310830) was examined by the method of Brenfeld (1955) and Kazeem, Adamson, and Ogunwande (2013) with some modifications. One percent (w/v) soluble potato starch (Sigma Aldrich) was suspended in 20 mM sodium phosphate buffer pH 6.9 buffer containing 6.7 mM NaCl and gelatinized for 15 min at 90 °C then allowed to cool to 37 °C before addition of PPAA at a concentration of 0.33 U ml^{-1}. The reaction was followed at 37 °C for up to 20 min. 5-CQA (final concentration 1.5 mg mL^{-1}) was either added to the enzyme-substrate mixture at the start of the reaction or was pre-incubated for 10 min with the enzyme prior to addition of the substrate. All reactions were carried out in four replicates. Reducing sugar released was measured at two reaction times (5 and 20 min) using the 3,5-dinitrosalicylic acid (DNS; Sigma Aldrich) colorimetric assay (Fei et al., 2014; Miller, 1959). An enzymatic kit was not used due the inhibition of enzymes in the kit by chlorogenic acid. Brayer has shown that maltose is the preferred leaving group for PPAA (Brayer et al., 2000), and therefore maltose was used for generating standard curve to quantify the reducing sugar released. The enzymatic activity of PPAA was determined in the presence of various concentrations of 5-CQA (0.08–2 mg mL^{-1}). IC_{50} was calculated as the concentration of 5-CQA required to inhibit 50% of enzyme activity.

2.3. Enzymatic kinetics and mode of inhibition

Michaelis-Menten kinetic parameters and mode of inhibition of PPAA by 5-CQA was determined from a Lineweaver-Burk plot. One mL of 5-CQA at concentrations ranging from 0 to 2 mg mL^{-1} was added to a mixture containing 1 mL of starch solution at concentrations from 0 to 6.6 mg ml^{-1} the same buffer solution as described in the previous section. The reaction was initiated by addition of a fixed concentration of PPAA (0.33 unit mL^{-1}). The solution mixture was incubated for 5 min at 37 °C. The reducing sugar produced was determined by the DNS colorimetric method as described previously.

2.4. Determination of total starch content

Total starch (TS) was determined enzymatically according to the method of Goñi, Garcia-Alonso, and Saura-Calixto (1997) with some modifications. Raw potato (50 mg) was homogenized in 6 mL of 2 M KOH and then agitated using a shaking vortex at room temperature for 30 min. The agitation step was very important to ensure complete solubility of the starch. 3 mL of 0.4 M sodium acetate buffer (pH = 4.75) was added to the suspension and pH was adjusted to 4.75 by using 3 M acetic acid. 60 μl of amyloglucosidase (AMG) from *Aspergillus niger* (70 U/mg, Sigma Aldrich) was added to the solubilized starch and hydrolysed for 45 min at 60 °C in a shaking water bath. The digestion mixture was centrifuged for 5 min and pH was neutralised with 0.2 M NaOH. Glucose in the supernatant was measured using the DNS method. Glucose amount was converted to starch by multiplying by a factor 0.9.

2.5. Determination of free sugar content

Free sugar content was determined in order to correct the TS value in potato samples. Potato samples, 200 mg of raw or cooked tuber, were homogenized in 6 mL sodium acetate buffer (pH = 4.75) and then centrifuged for 10 min. Soluble sugars were determined using the DNS method and high performance anion exchange chromatography with pulsed amperometric detection.

2.6. High performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD)

Sugar solutions (glucose, fructose, sucrose, maltose and maltotriose, all from Sigma Aldrich) were used as standards at concentrations of 0–0.2 μg mL^{-1}. Samples and standards were spiked with internal standard (fucose, final concentration of 0.05 μg mL^{-1}). Samples were filtered through PTFE membrane filters (0.2 μm pore size, Chromacol Ltd) and analysed by HPAEC-PAD (Thermo Fisher DX500 instrument equipped with a GP40 gradient pump, ED40 electrochemical detector including gold working and silver reference electrodes and a LC20 column oven set at 30 °C). The analytical column used was CarboPac PA20 (3 × 150 mm) with guard (3 × 30 mm) with anion exchange capacities of 65μeq/column. The mobile phase was 200 mM NaOH and the flow rate was 0.4 mL/min. Injections (10 μL) were made by an AS500 autosampler. The elution program was as follows: isocratic elution with 60 mM NaOH from 0 to 8 min, followed by increasing gradient up to 140 mM NaOH to 17 min. The concentration was reduced back to 60 mM and equilibration was carried out for 6 min.