

## Inhibition of pancreatic alpha amylase digestion of potato starch by chlorogenic acid *in vitro*

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Potatoes are the fourth most important crop in the world and the most consumed vegetable in the UK. Chlorogenic acid (5-caffeoylquinic acid, 5-CQA) is the predominant phenolic acid in potatoes<sup>(1)</sup> and it has been shown to inhibit the activity of alpha amylase when tested on artificial substrates<sup>(2)</sup>. The aim of this project was to investigate the inhibition of porcine pancreatic alpha amylase by 5-CQA at levels expected in potato tissue and also using potato starch as a substrate. The results could support the hypothesis that potato varieties with high 5-CQA levels would have lower glycaemic effects than those with lower levels.

The inhibitory effects of different concentrations of 5-CQA (0–2 mg mL<sup>-1</sup>) on the activity of porcine pancreatic  $\alpha$ -amylase (0.33 units mL<sup>-1</sup>) on potato starch (0.83–6.6 mg mL<sup>-1</sup>) was examined. 5-CQA was added to the enzyme-substrate mixture at the start of the reaction or was pre-incubated for 10 minutes with the enzyme prior to addition of the substrate. Reducing sugar released was measured using the 3,5-dinitrosalicylic (DNS) colorimetric method at two reaction times (5 and 20 min). Reactions were carried out at 37°C in 20 mM sodium phosphate buffer pH 6.9 containing 6.7 mM NaCl. All reactions were carried out in triplicate.

The percent of inhibition for various concentrations of 5-CQA (2, 1.5 and 1 mg/ml) without pre-incubation were 45.69%, 25.15% and 3.47% respectively after 5 min of enzymatic reaction. Fig. 1 shows that the inhibition was more pronounced at 5 min compared to 20 min reaction time. Pre-incubation of the enzyme with 5-CQA (at 1.5 mg mL<sup>-1</sup>) led to a small but significant decrease in activity, observed at both reaction times ( $P < 0.05$ ).

The mechanism of inhibition was investigated by kinetic analyses. The Hans-Woolf plot (Fig. 2) and kinetic parameters (table below) show that 5-CQA decreased the  $V_{max}$  and increased the  $K_m$  of pancreatic alpha amylase, indicating a mixed-type inhibition mechanism when using potato starch as a substrate.

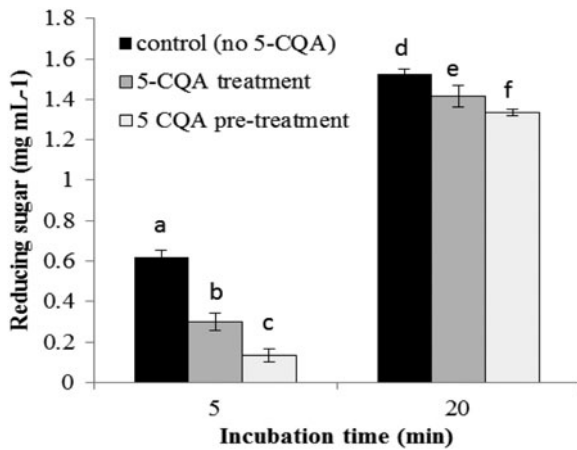


Fig. 1. Effect of 5-CQA (1.5 mg mL<sup>-1</sup>) on amylase activity;  $n = 3$ , error bars = SD, a,b,c  $p < 0.05$  and d,e,f  $p < 0.05$  at same time point and different treatments.

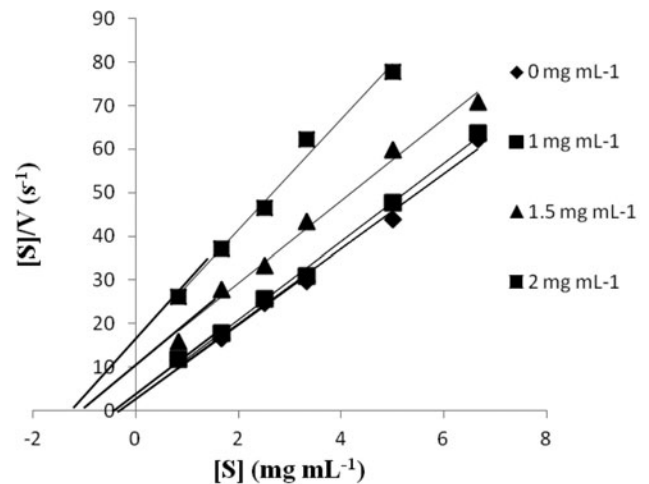


Fig. 2. Hans-Woolf plot at various 5-CQA concentrations.

The results of this study provide evidence that 5-CQA at concentrations found in food could reduce digestibility of potato starch with potential consequences on carbohydrate availability.

[5-CQA] mg mL <sup>-1</sup>	$k_m$ mg mL <sup>-1</sup>	$V_{max}$ mg mL <sup>-1</sup> sec <sup>-1</sup>
0	0.3495	0.1165
1	0.4635	0.1159
1.5	0.8712	0.1089
2	0.9750	0.0755

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