Analysis of kinetic parameters and mechanisms of nanocrystalline cellulose inhibition of α -

amylase and α -glucosidase in simulated digestion

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Fig.S1 Scanning electron microscopy micrograph of MCC morphology



Fig. S2 Linear least square fitting of the hydrolysis curve data obtained from simulated digestion of potato whey protein food model system in the presences and absence of NCC fractions and a acarbose using Graphpad prism software. Only data within the first 60 min of the hydrolysis was used for the linear model fitting.



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← Control ← NCC1 → NCC2 → NCC3 → NCC4



Fig. S3 Influence of size and concentration of NCC on kinetic behaviour of α -amylase. (A) Michaelis– Menten plots of substrate [s] (mg/mL) against initial velocity (v) (mg/mL/min) at fixed concentration of each in inhibitor (10mg/ml). (B) Michaelis-Menten plots of [s] against v at variable concentrations of each inhibitor indicated below Fig. S3B. Starch was used as the substrate. Data reported as mean measurements (n= 3).



Fig. S4 Influence of particle size and concentrations of NCC on kinetic behave of α -glucosidase. (A) Michaelis–Menten plots of substrate [s] (mM) against initial velocity (v) (mM/ml/min) at fixed concentration (10mg/mL) of each in inhibitor. (B) Michaelis-Menten plots of [s] against v at variable concentrations of each inhibitor indicate below Fig. S4B. p-NP-G was used as the substrate. Data reported as mean measurements (n= 3).

	α–Amylase			α-Glucosidase		
Inhibitor	[I] (mg/mL)	V _{max} (mg/mL/min)	K _m (mg/mL)	V _{max} (mM/min)	K _m (mg/mL)	
NCC1	0	0.337	64.33	17.15	0.97	
	5	0.272	45.42	16.87	0.88	
	10	0.196	48.21	15.81	0.87	
	20	0.127	47.47	12.74	0.86	
NCC2	5	0.23	43.12	16.45	0.93	
	10	0.159	45.11	14.57	0.921	
	20	0.103	41.31	10.92	0.906	
NCC3	5	0.192	33.22	15.58	0.997	
	10	0.136	32.32	13.03	0.99	
	20	0.085	33.31	10.51	0.984	
NCC4	5	0.181	21.12	14.57	1.49	
	10	0.125	22.23	12.35	1.52	
	20	0.078	21.33	9.12	1.32	

Table S1	1. Effects of NCC concentration on V_{max} and K_m for α -amylase and α -glucosidase using
starch an	d p-NPG substrates respectively. Lineweaver–Burk plots was used to extrapolate the
V _{max} and	$K_{\rm m}$ values using the same data for Figs 5B and 6B.

	α–Amylase		α-Glucosidase	
Sample	Dixon Plot	Modified Dixon plot	Dixon Plot	Modified Dixon plot
	7.83	7.83	22.38	23.15
NCC1	7.78	8.78	23.44	21.95
NCCI	7.88	9.88	22.43	24.76
	8.99	7.98	22.64	17.96
	6.42	6.42	19.26	18.69
NCC2	6.5	7.54	20.12	22.87
NCC2	5.27	7.27	18.35	19.95
	7.72	7.72	23.66	15.87
	5.5	4.49	20.04	15.77
NCC2	4.18	5.18	21.24	19.72
NCC3	3.29	4.92	17.44	17.18
	5.79	5.79	23.88	19.67
	2.71	3.71	20.38	14.17
NCC4	2.32	2.92	16.54	19.51
INCC4	2.27	4.28	17.95	18.5
	4.17	5.18	21.34	17.01

Table S2. List of Intercepts from Dixon and modified Dixon plots used to generate K_i and K_{ii} values

Sample	Alpha amylase			Alpha glucosidase		
	IC ₅₀	K _i		IC ₅₀	K _i	
	(mg/mL)	(mg/mL)	K _i /IC ₅₀	(mg/mL)	(mg/mL)	K _i /IC ₅₀
NCC1	6.72	7.84	1.2	11.6	22.94	2.0
NCC2	4.16	5.88	1.4	7.34	20.85	2.8
NCC3	3.01	3.74	1.2	4.66	19.63	4.2
NCC4	2.28	2.98	1.3	4.58	19.16	4.2

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Table S3. The ratios between K_i and IC_{50} for the two tested enzymes for each NCC fraction