Extract of ice plant (*Mesembryanthemum crystallinum*) ameliorates hyperglycemia and modulates the gut microbiota composition in type 2 diabetic Goto-Kakizaki rats

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Supplemental methods

1. Identification of D-pinitol in IPE

Identification of D-pinitol in IPE was performed using a GCMS-QP2010 SE (Shimadzu, Japan) equipped with a Rtx-5MS column (30 m × 0.25 mm i.d. × 0.25 μm). The lyophilized IPE and D-pinitol standard were then dissolved in 800 μL pyridine prior to reacting with 200 μL TMSI at 90°C for 1 h. [1] The supernatant was filtered and used as the sample solution for GC-MS analysis. The oven temperature programme was as follows: temperature at 150°C held for 3 min and raised to 200°C at a rate of 5°C/min before it was heated to 300°C at a rate of 10°C/min, and was finally upheld there for 17 min. Nitrogen was used as the carrier gas at a flow rate of 1.0 mL/min in split mode (split ratio 1:30). The temperature of the FID was 320°C. The range of mass scan was from 150 m/z to 600 m/z.

2. In vitro anti-diabetic assays

α-glucosidase and α-amylase inhibition assays were performed using the procedures detailed in a previous work.[2] For α-amylase inhibition assay: 20 μL of IPE (serial dilutions) and 40 μL of 2 g/L starch solution were mixed with 20 μL of α-amylase (0.1 mg/mL). All solutions were prepared in 0.1 M phosphate buffer. After incubation at 37 °C for 20 min, the reaction was stopped by the addition of 80 μL of 0.4 M HCl followed by 100 μL of 5 mM I_2 (in 5 mM KI) and the absorbance was read at 620 nm.

α-glucosidase inhibition assay: 50 μL of IPE (serial dilutions) was combined with 50 μL of enzyme solution (0.1 mg/mL) and 50 μL of 5 mM *p*NPG solution. All solutions were prepared in 0.1 M phosphate buffer. The mixture was incubated at 37 °C for 20 min in the dark. Finally, 100 μL of 0.1 M Na_2CO_3 solution was added and the absorbance was read at 405 nm.

3. Short chain fatty acids analysis

The concentration of short chain fatty acids (SCFAs) was measured by gas chromatography (GC) as previously described.[3] Briefly, fecal samples of 100 mg were soaked with 500 μL distilled water and vortexed until suspended. Samples were then centrifuged (9,000×g for 20 min), and supernatant fluid was collected for SCFAs determination.

Supplemental figures

Figure S1. Total ion chromatogram of pure D-pinitol standards derivatization with trimethylsilylimidazole (TMSI) (A); Full MS spectra of D-pinitol standards peak (RT=11.708) (B); chromatogram obtained from ice plant extract (IPE) (C); MS spectra of D-pinitol extracted from ice plant (RT=11.708) (D).

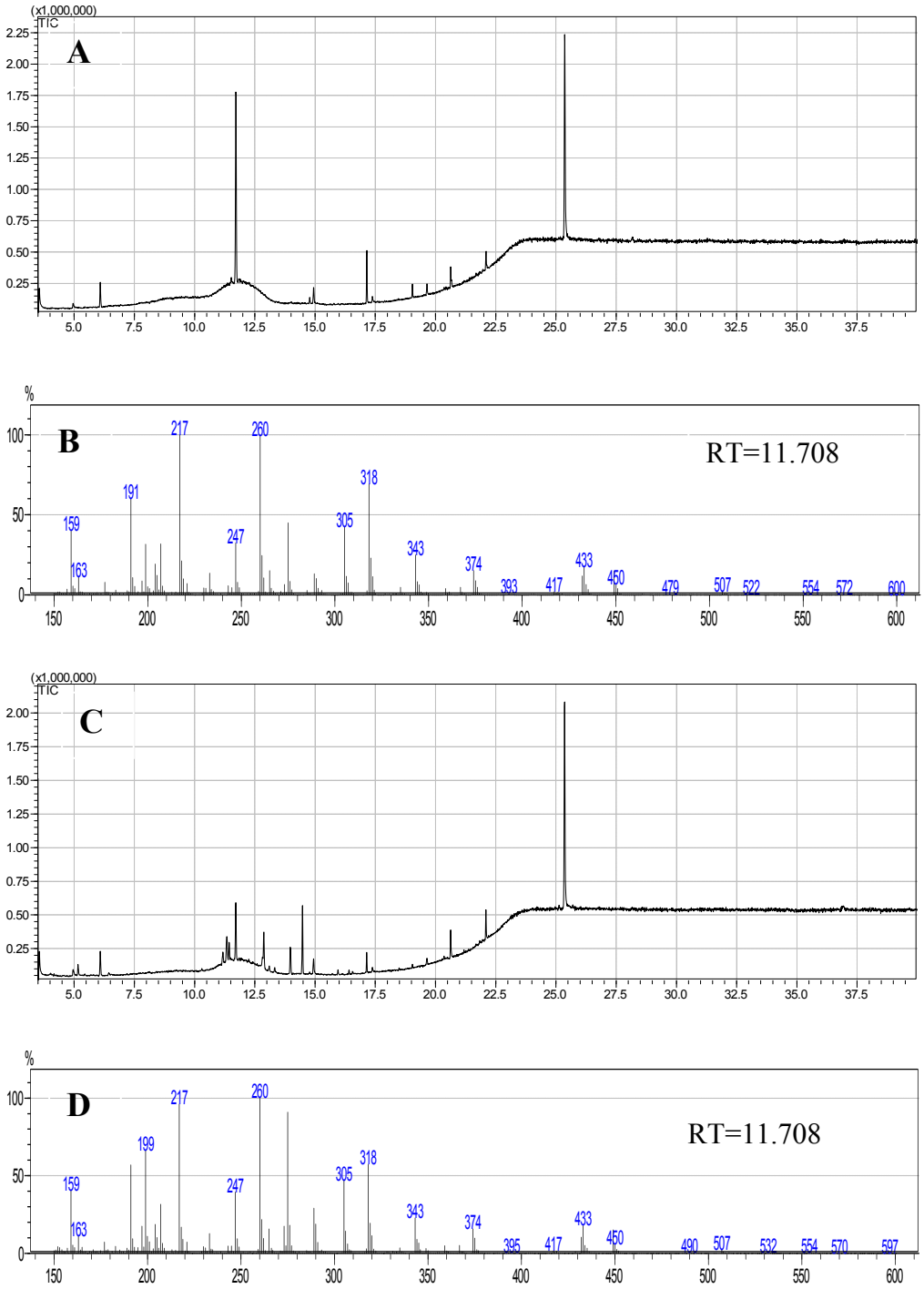


Figure S2. Response surface and contour plots for the effect of independent variables on D-pinitol extraction efficiency

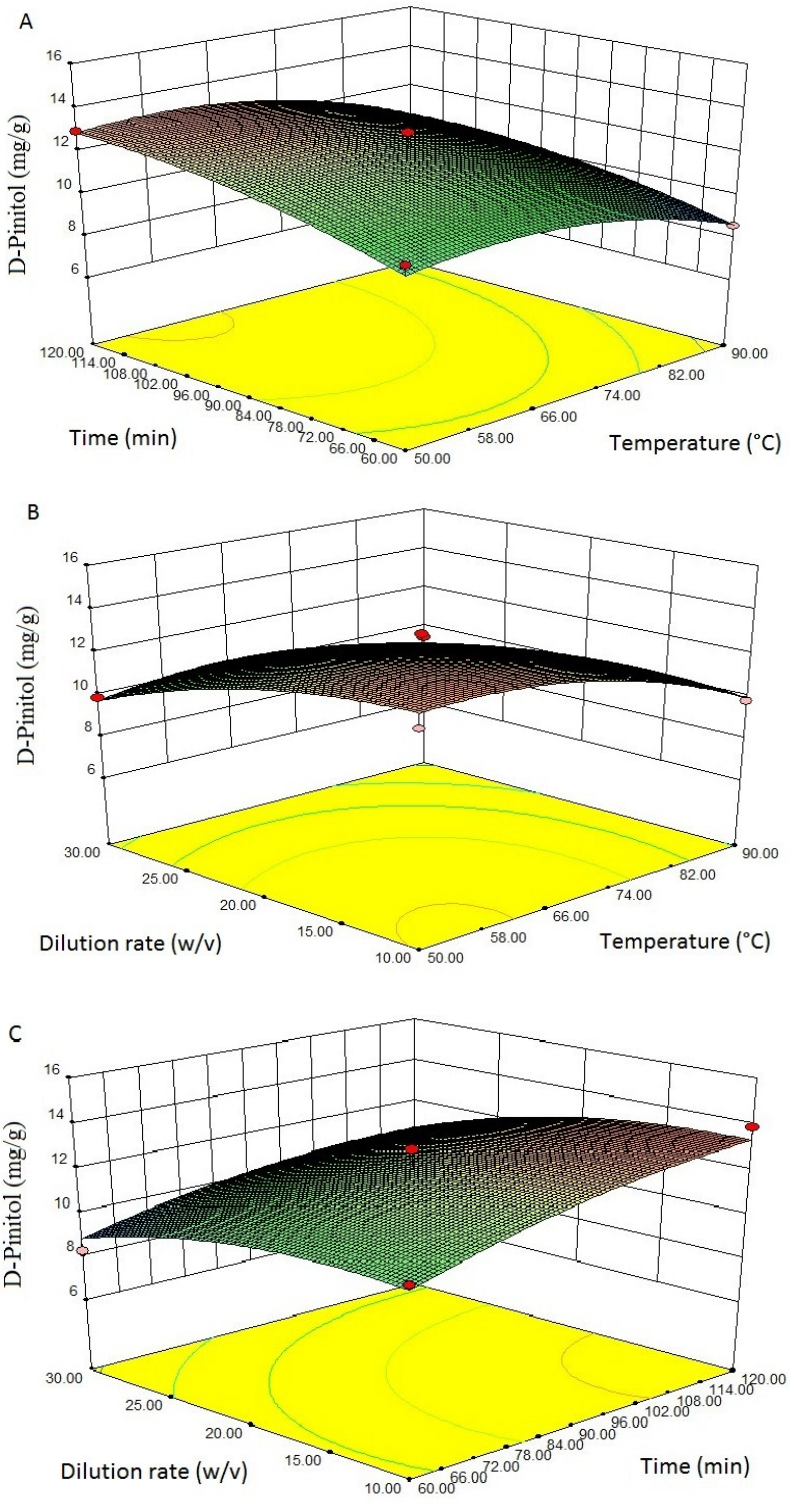


Figure S3. In vitro inhibitory activities of IPE towards α-amylase (A) and α-glucosidase (B)

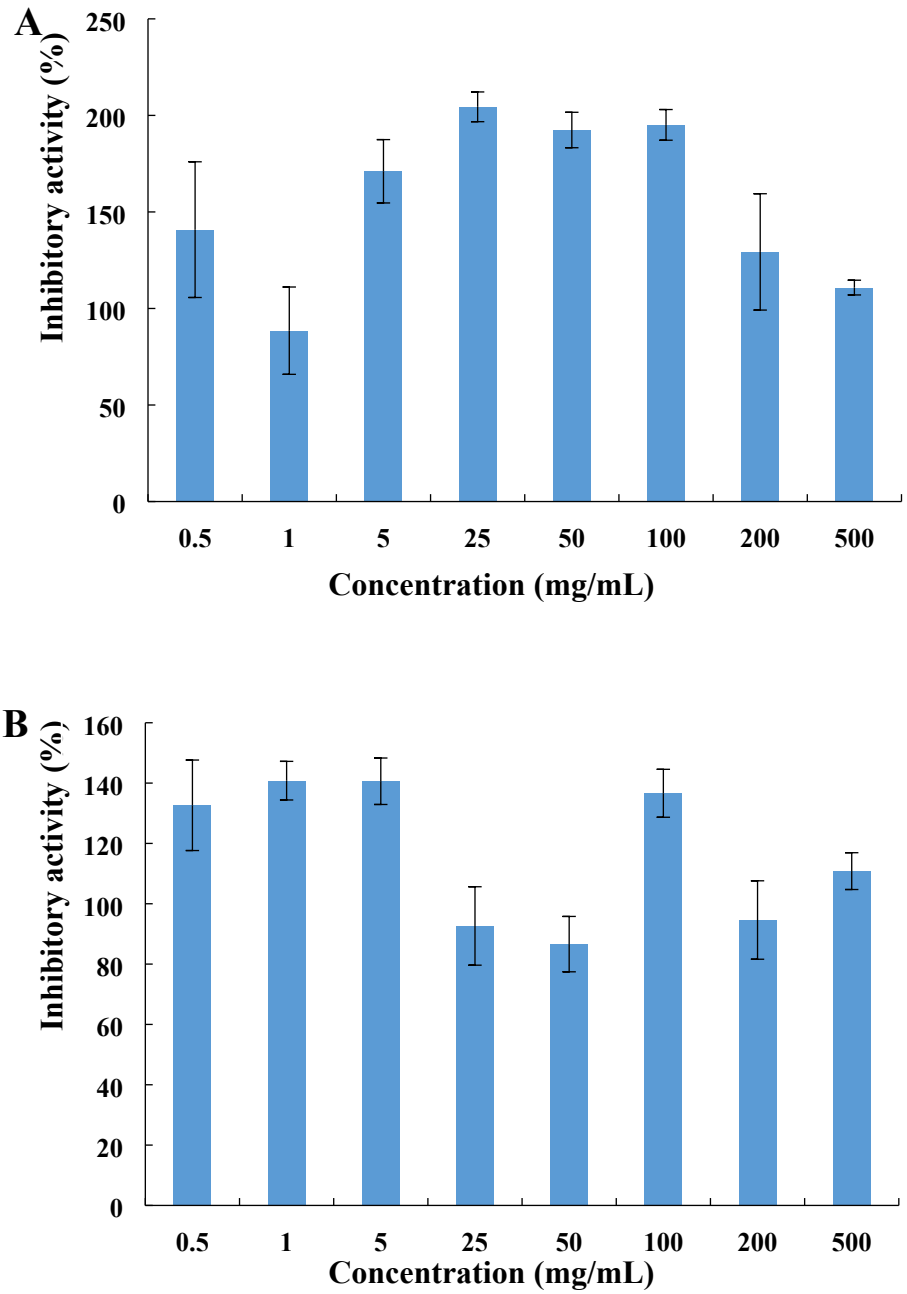
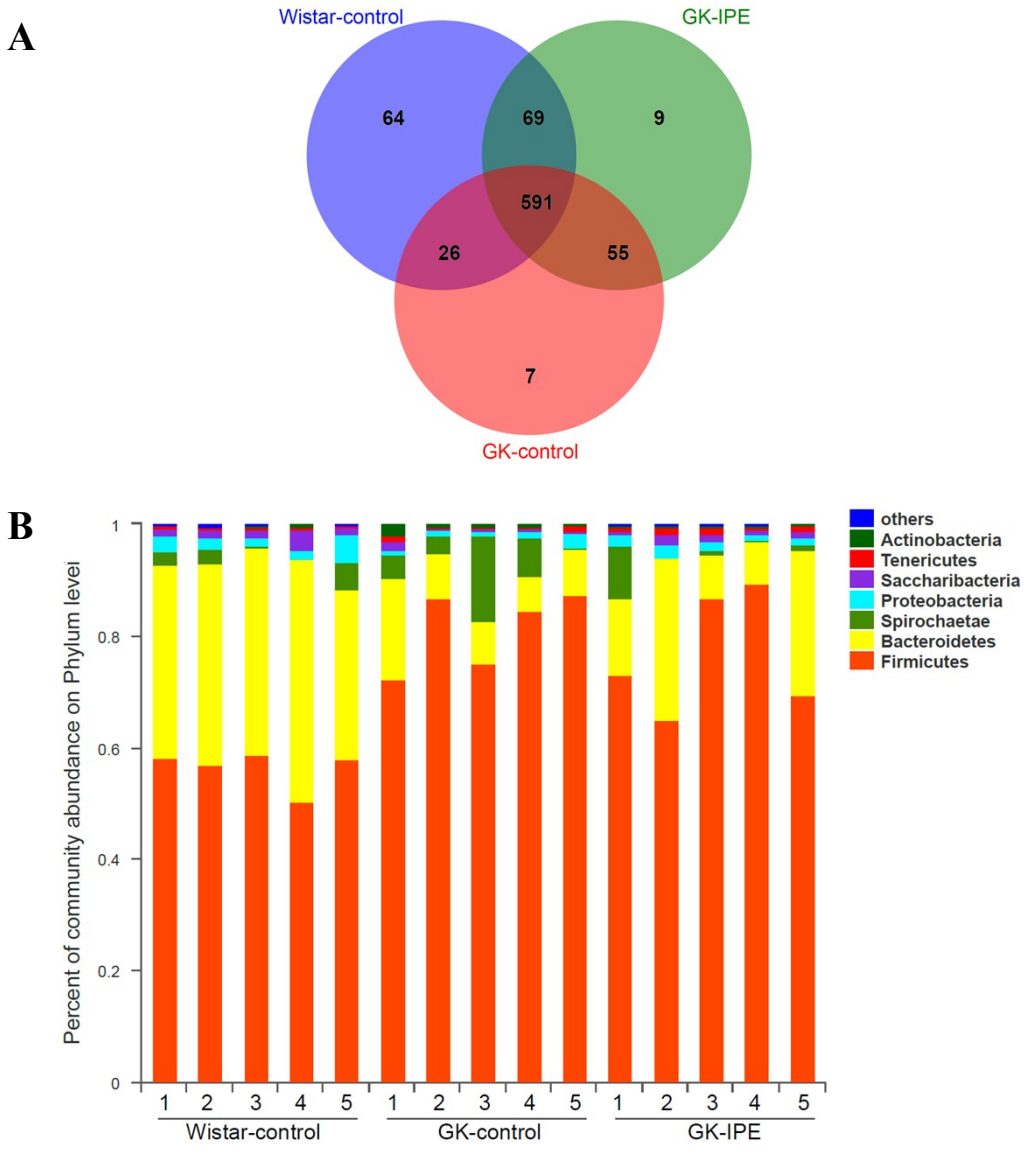
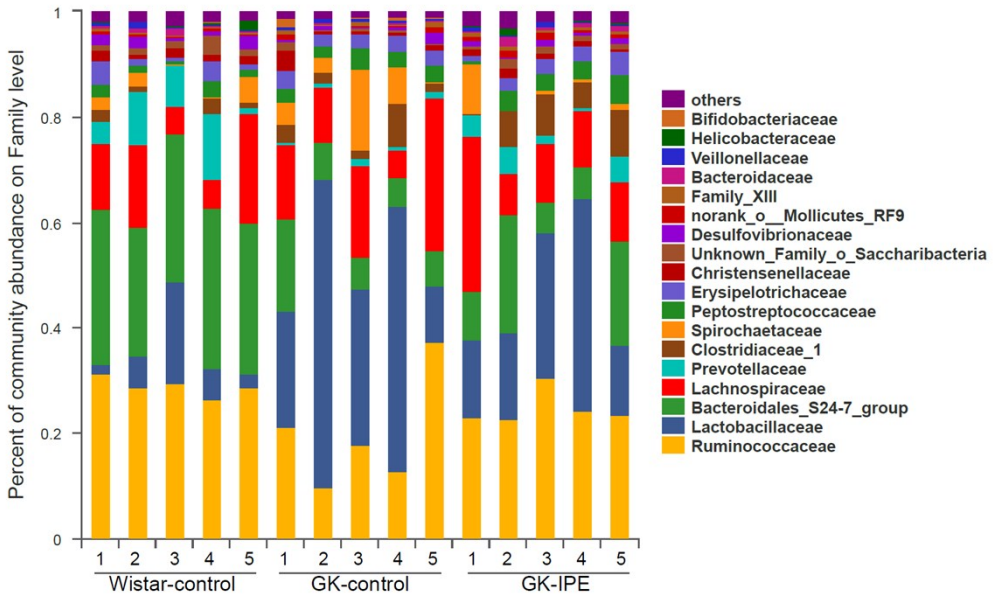


Figure S4. Comparison of the OTUs and the composition of gut microbiota in the three groups. The number of observed OTUs sharing >97% nucleotide sequence identity. (A) Venn diagram was generated to describe the common and unique OTUs among the control group; (B) Relative abundance of fecal microbiota at phylum level; (C) Relative abundance of fecal microbiota at family level.

Taxa with average relative abundance less than 1% in all diet groups were labeled as "Others".



C


Percent of community abundance on Family level
1
0.8
0.6
0.4
0.2
0
1 2 3 4 5 1 2 3 4 5 1 2 3 4 5
Wistar-control
GK-control
GK-IPE
others
Bifidobacteriaceae
Helicobacteraceae
Veillonellaceae
Bacteroidaceae
Family_XIII
norank_o__Mollicutes_RF9
Desulfovibrionaceae
Unknown_Family_o_Saccharibacteria
Christensenellaceae
Erysipelotrichaceae
Peptostreptococcaceae
Spirochaetaceae
Clostridiaceae_1
Prevotellaceae
Lachnospiraceae
Bacteroidales_S24-7_group
Lactobacillaceae
Ruminococcaceae

Supplemental tables

Table S1. ANOVA for response surface quadratic model.

Source	Sum of squares	df	Mean square	*F* value	*p*-Value
Model	38.21	9	4.25	10.91	0.0024[a]
Residual	2.73	7	0.39		
Lack of Fit	2.04	3	0.68	3.94	0.1091[b]
Pure Error	0.69	0.69	0.17		
Total	40.94	16			

R^2=0.9334, Adj. R^2=0.8478, Adeq Precision=9.787, CV=5.63%

[a] The p-value (0.0024) less than 0.01 indicates a statistical significance. This model was satisfactorily fitted.

[b] The lack of fit value of the model was 0.1091 (>0.05), implies that the lack of fit is insignificant relative to the pure error.

Table S2. The raw data and alpha diversity index among different treatments.

	Samples	Sequence(*n*)	Bases(bp)	Mean length	Shannon	Simpson	Chao1	ACE
Wistar -control	1	55491	24126725	434.8	4.92	0.02	679.1	680.7
	2	64058	27898037	435.5	4.96	0.01	686.3	681.4
	3	60216	26377846	438.1	4.56	0.03	714.5	694.9
	4	63749	27849833	436.9	4.66	0.02	672.2	659.0
	5	56060	24340401	434.2	4.83	0.02	630.3	630.5
	Mean	59915 ± 4074	26118568.4 ± 1827589.2	435.9 ± 1.6	4.78 ± 0.17	0.02 ± 0.01	676.4 ± 30.4	669.3 ± 25.2
GK -control	1	51540	22506968	436.7	4.31	0.05	603.9	596.7
	2	50205	22192076	442.0	3.14	0.21	543.6	549.3
	3	69213	30305038	437.9	3.73	0.08	595.2	584.7
	4	49754	21940565	441.0	3.17	0.16	547.7	557.9
	5	64706	27911816	431.4	4.67	0.03	642.8	623.6
	Mean	57084 ± 9179	24971292.6 ± 3875492.4	437.8 ± 4.1	3.80 ± 0.68**	0.10 ± 0.07*	586.6 ± 41.5***	582.4 ± 30.0***
GK -IPE	1	61405	26710518	435.0	4.66	0.03	619.0	606.1
	2	60037	26203000	436.4	4.57	0.02	674.2	638.0
	3	60514	26325987	435.0	4.39	0.03	593.6	593.6
	4	57016	24926124	437.2	4.07	0.06	619.8	607.4
	5	64550	28129342	435.8	4.60	0.02	632.5	640.4
	Mean	60704 ± 2709	26458994.2 ± 1149591.3	435.8 ± 0.9	4.45 ± 0.24$^{\#}$	0.03 ± 0.02$^{\#}$	627.8 ± 29.5*	617.1 ± 20.9$^{**\#}$

Data are expressed as means ± SD.
$^{*}p < 0.05$ *vs* Wistar-control; $^{**}p < 0.01$ *vs* Wistar-control; $^{***}p < 0.001$ *vs* Wistar-control;
$^{\#}p < 0.05$ *vs* GK-control; $^{\#\#}p < 0.01$ *vs* GK-control; $^{\#\#\#}p < 0.001$*vs* GK-control.

Table S3. Concentrations of SCFAs in fecal samples among different treatments.

Group	Acetic acid (μmol/g)	Propionic acid (μmol/g)	Isobutyric acid (μmol/g)	Butyric acid (μmol/g)	Isovaleric acid (μmol/g)	Valeric acid (μmol/g)
Wistar-control	66.57±14.85	9.43±2.04	1.15±0.57	12.38±2.43	1.72±0.54	1.35±0.45
GK-control	39.95±8.39**	3.36±0.53***	1.11±0.15	7.93±2.09**	1.28±0.40	0.89±0.16
GK-IPE	33.28±9.60***	4.29±0.52***#	0.82±0.26#	4.38±0.42***##	1.32±0.28	0.72±0.21

Data are expressed as means ± SD.
*$p < 0.05$ *vs* Wistar-control; **$p < 0.01$ *vs* Wistar-control; ***$p < 0.001$ *vs* Wistar-control;
#$p < 0.05$ *vs* GK-control; ##$p < 0.01$ *vs* GK-control; ###$p < 0.001$*vs* GK-control.

References:

1 W.Q. Sun, X.P. Li, and B.L. Ong, Preferential accumulation of D-pinitol in Acrostichum aureum gametophytes in response to salt stress, *Physiol Plantarum*, 1999, 105, 51-57.

2 V. Spinola, J. Pinto and P. C. Castilho, Hypoglycemic, anti-glycation and antioxidant in vitro properties of two Vaccinium species from Macaronesia: A relation to their phenolic composition, J Funct Foods, 2018, 40, 595-605.

3 Q. Wu, X. Pi, W. Liu, H. Chen, Y. Yin, H. D. Yu, X. Wang and L. Zhu, Fermentation properties of isomaltooligosaccharides are affected by human fecal enterotypes, Anaerobe, 2017, 48, 206-214.