

## Purification and characterization of hypoglycemic peptides from traditional Chinese soy-fermented douchi

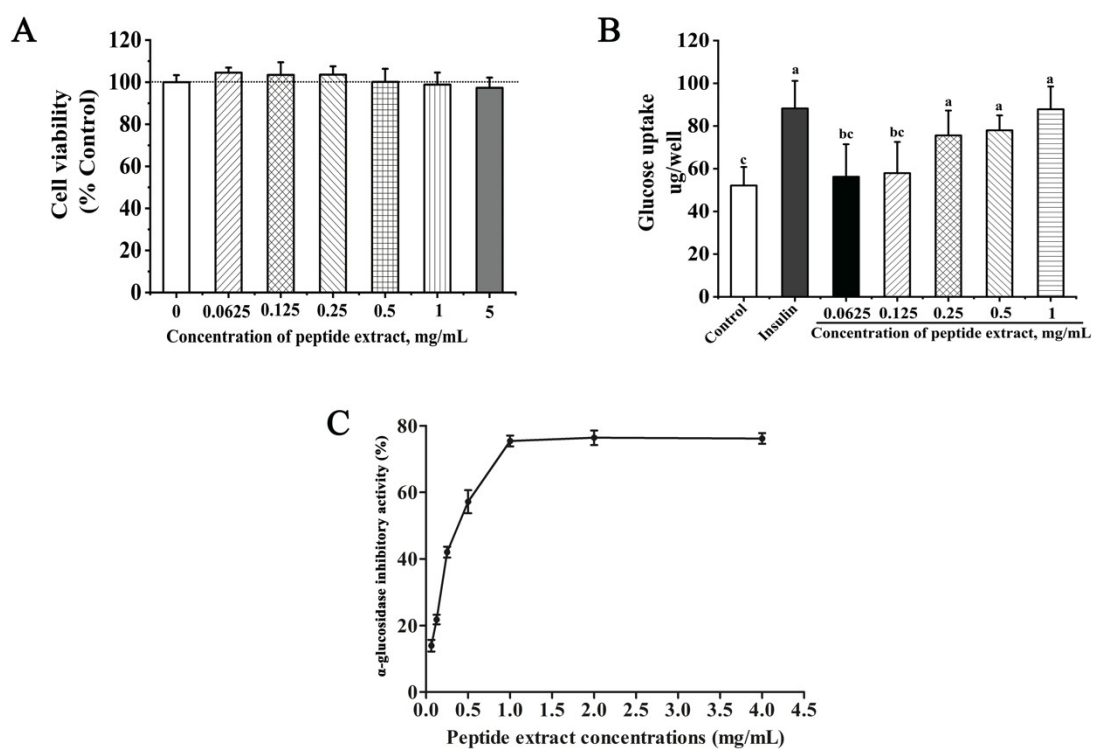


Figure S1. Effect of peptide extract on cell viability and glucose uptake in L6 myotubes.

(A) Effect of peptide extract on cell viability. The cells were incubated with peptide extract samples (0.0625 mg/mL, 0.125 mg/mL, 0.25 mg/mL, 0.5 mg/mL, 1 mg/mL and 5 mg/mL) for 24 h. The control group was set as DMEM medium containing cells and the DMEM medium without cells was set as the blank group at the same culture conditions for 24 h. Cell viability was measured using CCK-8 assay. (B) Effect of different concentrations of peptide extract on glucose uptake in L6 myotubes. (C) Inhibitory effects of peptide extract against  $\alpha$ -glucosidase.

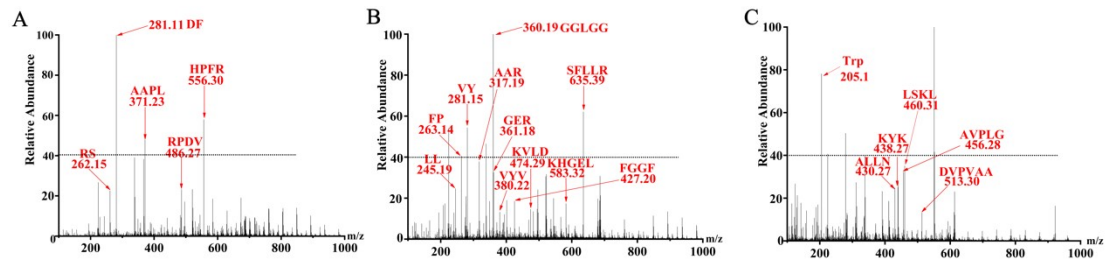


Figure S2. Identification and analysis of hypoglycemic peptides by LC-MS/MS. Spectra were recorded over the mass/charge ( $m/z$ ) ranges of 100-1000 in MS mode. (A) MS spectrum of fraction F4-1-6; (B) MS spectrum of fraction F4-1-9; (C) MS spectrum of fraction F4-3-12.

Table S1 Amino acid composition of the fractions of peptide extract.

Amino acid	Contents (g/100g dry sample)			
	F1	F2	F4	F6
Asp(Aspartic acid)A	1.27±0.13 <sup>ab</sup>	2.04±0.52 <sup>a</sup>	1.30±0.13 <sup>ab</sup>	0.94±0.04 <sup>b</sup>
Glu(Glutamic acid)E	2.29±0.10 <sup>b</sup>	4.39±0.37 <sup>a</sup>	2.40±0.08 <sup>b</sup>	1.53±0.07 <sup>c</sup>
Ser(Serine)S	0.64±0.01 <sup>b</sup>	1.00±0.04 <sup>a</sup>	0.51±0.05 <sup>c</sup>	0.47±0.01 <sup>c</sup>
Gly(Glycine)G	0.81±0.02 <sup>bc</sup>	0.98±0.06 <sup>b</sup>	1.28±0.10 <sup>a</sup>	0.72±0.04 <sup>c</sup>
His(Histidine)H	0.52±0.01 <sup>b</sup>	0.61±0.04 <sup>ab</sup>	0.62±0.05 <sup>a</sup>	0.63±0.01 <sup>a</sup>
Arg(Arginine)R	4.37±0.09 <sup>a</sup>	4.37±0.04 <sup>b</sup>	3.45±0.03 <sup>a</sup>	1.33±0.07 <sup>b</sup>
Thr(Threonine)T	3.22±0.27 <sup>a</sup>	3.11±0.03 <sup>b</sup>	1.90±0.04 <sup>d</sup>	2.48±0.17 <sup>c</sup>
Ala(Alanine)A	0.75±0.01 <sup>b</sup>	1.00±0.03 <sup>a</sup>	0.40±0.08 <sup>c</sup>	0.43±0.02 <sup>c</sup>
Pro(Proline)P	1.13±0.04 <sup>b</sup>	1.77±0.10 <sup>a</sup>	1.56±0.06 <sup>a</sup>	1.18±0.11 <sup>b</sup>
Tyr(Tyrosine)Y	1.24±0.04 <sup>b</sup>	1.33±0.014 <sup>b</sup>	4.89±0.07 <sup>a</sup>	0.47±0.03 <sup>c</sup>
Val(Valine)V	1.10±0.05 <sup>c</sup>	1.47±0.07 <sup>b</sup>	0.73±0.02 <sup>a</sup>	0.39±0.01 <sup>d</sup>
Met(Methionine)M	0.40±0.01 <sup>b</sup>	0.53±0.02 <sup>a</sup>	0.34±0.04 <sup>bc</sup>	0.27±0.03 <sup>c</sup>
Cys(Cysteine)C	1.66±0.09 <sup>a</sup>	1.61±0.04 <sup>a</sup>	1.36±0.03 <sup>b</sup>	1.22±0.01 <sup>b</sup>
Ile(Isoleucine)I	1.35±0.03 <sup>b</sup>	1.57±0.08 <sup>a</sup>	2.50±0.08 <sup>c</sup>	0.60±0.03 <sup>d</sup>
Leu(Leucine)L	1.89±0.14 <sup>ab</sup>	2.76±0.16 <sup>ab</sup>	3.72±0.07 <sup>a</sup>	0.53±0.04 <sup>b</sup>
Phe(Phenylalanine)F	2.21±0.08 <sup>b</sup>	1.80±0.12 <sup>bc</sup>	8.11±0.04 <sup>a</sup>	1.61±0.30 <sup>c</sup>
Lys(Lysine)K	0.49±0.01 <sup>d</sup>	1.50±0.09 <sup>b</sup>	1.12±0.09 <sup>a</sup>	0.85±0.02 <sup>c</sup>
HAA <sup>1</sup>	11.73±0.17 <sup>b</sup>	13.85±0.38 <sup>b</sup>	23.60±0.23 <sup>a</sup>	6.70±0.24 <sup>c</sup>
PCAA <sup>2</sup>	5.38±0.09 <sup>c</sup>	5.17±0.08 <sup>c</sup>	6.50±0.10 <sup>a</sup>	2.82±0.08 <sup>b</sup>
NCAA <sup>3</sup>	3.56±0.22 <sup>b</sup>	6.42±0.89 <sup>a</sup>	3.70±0.05 <sup>b</sup>	2.48±0.11 <sup>b</sup>
TAA <sup>4</sup>	25.35±0.63 <sup>c</sup>	30.53±0.48 <sup>b</sup>	37.48±0.36 <sup>a</sup>	15.67±0.18 <sup>d</sup>

<sup>1</sup>HAA (hydrophobic amino acid): Ala; Cys; Ile; Leu; Met; Phe; Val; Tyr; Pro.

<sup>2</sup>PCAA (positively charged amino acid): Lys; His; Arg.

<sup>3</sup>NCAA (negatively charged amino acid): Asp and Glu.

<sup>4</sup>TAA: Total amino acid.

Different letters representing significant differences among the groups ( $p < 0.05$ ).

Table S2. Peptide sequences identified in fractions with glucose uptake stimulatory activity from the peptide extract of *douchi* by LC-MS/MS.

Fraction	Sequence <sup>1</sup>	Observed m/z	Accession number <sup>2</sup>	Description <sup>3</sup>
F4-1-6	RS	262.15	P04347	11S globulin [Glycine max]
F4-1-6	DF	281.11	P04347	Glycinin G5 [Glycine max]
F4-1-6	AAPL	371.23	gi 215398099	G13 protein [Glycine max]
F4-1-6	RPDV	486.27	gi 1635577150	ATP-dependent zinc metalloprotease FTSH [Glycine max]
F4-1-6	HPFR	556.30	gi 356541627	Sugar transportprotein 13 [Glycine max]
F4-1-9	LL	245.19	P04776	Glycinin G1 [Glycine max]
F4-1-9	FP	263.14	P04405	Glycinin G2 [Glycine max]
F4-1-9	VY	281.15	P13917	7S globulin [Glycine max]
F4-1-9	AAR	317.19	Q2TUW0	Anthocyanidin synthase [Glycine max]

Continued

Fraction	Sequence <sup>1</sup>	Observed m/z	Accession number <sup>2</sup>	Description <sup>3</sup>
F4-1-9	GGLGG	360.19	gi 574584690	ATP-dependent zinc metalloprotease FTSH 2 [Glycine max]
F4-1-9	GER	361.18	P25974	Beta-conglycinin beta subunit [Glycine max]
F4-1-9	VYV	380.22	D0VBL6	Isoflavone synthase [Glycine max]
F5-Fr10	FGGF	427.20	gi 356505340	1,4-alpha-glucan-branching enzyme 1 [Glycine max]
F4-1-9				Microtubule-associated protein futsch isoform X3 [Glycine max]
	KVLD	474.29	gi 95537486	
F4-1-9	KHGEL	583.32	gi 955317737	Glutamate synthase [Glycine max]
F4-1-9	SFLLR	635.39	gi 571503905	Root phototropism protein 3 isoform X1 [Glycine max]
F4-3-12	ALLN	430.27	gi 356553519	ABC transporter C family member 3 [Glycine max]
F4-3-12	KYK	438.27	Q39858	Soybean glycinin A3-B4 subunit [Glycine max]
F4-3-12	AVPLG	456.28	gi 363814589	Peroxidase precursor [Glycine max]
F4-3-12	DVPVAA	513.30	gi 356576361	Acyl-CoA-binding domain-containing protein 6 [Glycine max]

<sup>1</sup>Peptide sequences are expressed by one letter amino acid code. Ala-A; Cys-C; Asp-D; Glu-E; Phe-F; Gly-G; His-H; Ile-I; Lys-K; Leu-L;

Met-M; Asn-N; Pro-P; Gln-Q; Arg-R; Ser-S; Thr-T; Val-V; Trp-W; Tyr-Y.

<sup>2</sup>Accession numbers of matched proteins are from the National Center for Biotechnology Information nonredundant sequence (NCBI nr) databases or the database (UniprotKB/SwissProt) search limited to Glycine max.

<sup>3</sup>A detailed description of the protein name.