Corn distillers solubles as a new source of bioactive peptides with ACE and DPP-IV inhibition activity: characterization, in-silico evaluation, and molecular docking

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Abbreviations:

ACE - Angiotensin I-converting enzyme
DPP IV - Dipeptidyl peptidase IV
DPP III - Dipeptidyl peptidase III
AAs - Amino acids
CDS - Corn distillers solubles
PHs – Protein hydrolysates
PCA - Principle component analysis
PC – Protein concentrate
PF – Protein fraction
LC-MS - Liquid chromatography coupled to mass spectrometry
M_w - Molecular weight
DS – Discovery Studio
DH – Degree of hydrolysis
H-bond – hydrogen bonding
PH-Ala – Protein hydrolysate prepared by alcalase
PH-Pap - Protein hydrolysate prepared by papain
PH-Tryp – Protein hydrolysate prepared by trypsin
PH-Fla – Protein hydrolysate prepared by flavorzyme
CGM – Corn gluten meal
Ala – alanine; Val – valine; Leu – leucine; Ile – isoleucine; Gly – glycine; Pro – proline; Ser – serine; Thr – threonine; Asn – asparagine; Tyr – tyrosine; Trp – trptophan; Phe – phenylalanine; Asp – aspartic acid; Glu – glutamic acid; Gln – glutamine; Lys – lysine; His – histidine; Arg – arginine; Met – methionine.

Section1.

Molecular weight analysis

A PLRP-S column (1000 Å, 5 µm, Agilent) was used for the separation of protein from detergents with the following solvents: water with 0.1% formic acid for A and acetonitrile with 0.1 % formic acid for B. The mobile phase gradient was as follows: initial conditions, 30% B for 5 min increasing to 85% B in 5 min and then to 95% B in 0.10 more min followed by column wash at 95% B and 5 min re-equilibration. The first 2 and last 5 min of the gradient were sent to waste and not the spectrometer. The flow rate and sample volume were maintained at 0.3 mL.min^{-1} and 2 µl. Further settings were similar to those described in **section 2.3.** Data were analyzed by MassHunter Qualitative Analysis software (version B.06.00, Agilent). Deconvolution of m/z spectrum was achieved using maximum entropy algorithm within BioConfirm software (Agilent).

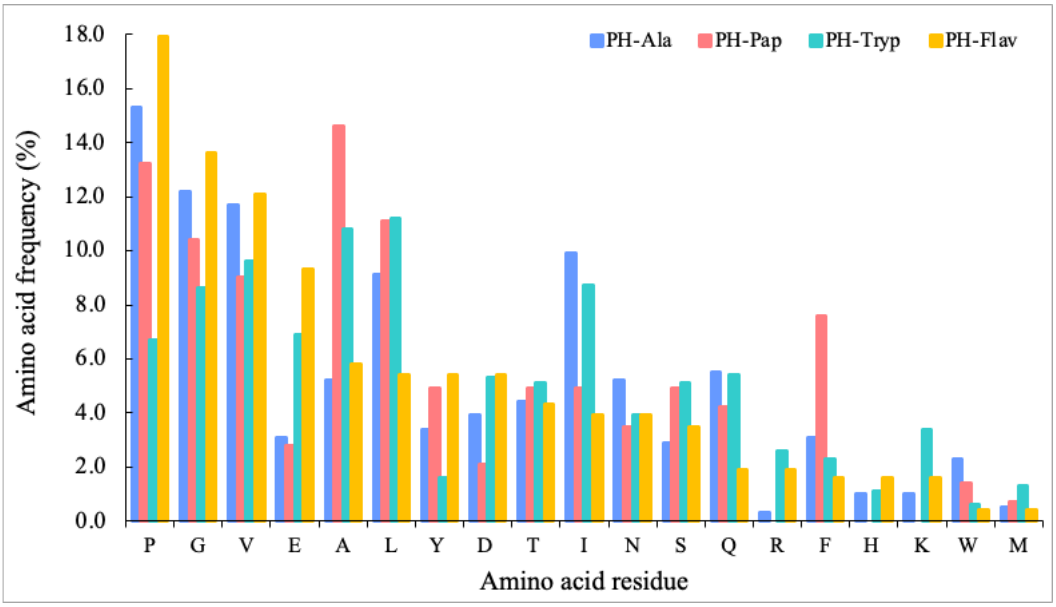

Fig. 1S Amino acid composition of PH-Ala, PH-Pap, PH-Tryp, PH-Flav built on the peptides characterized by mass spectrometry.

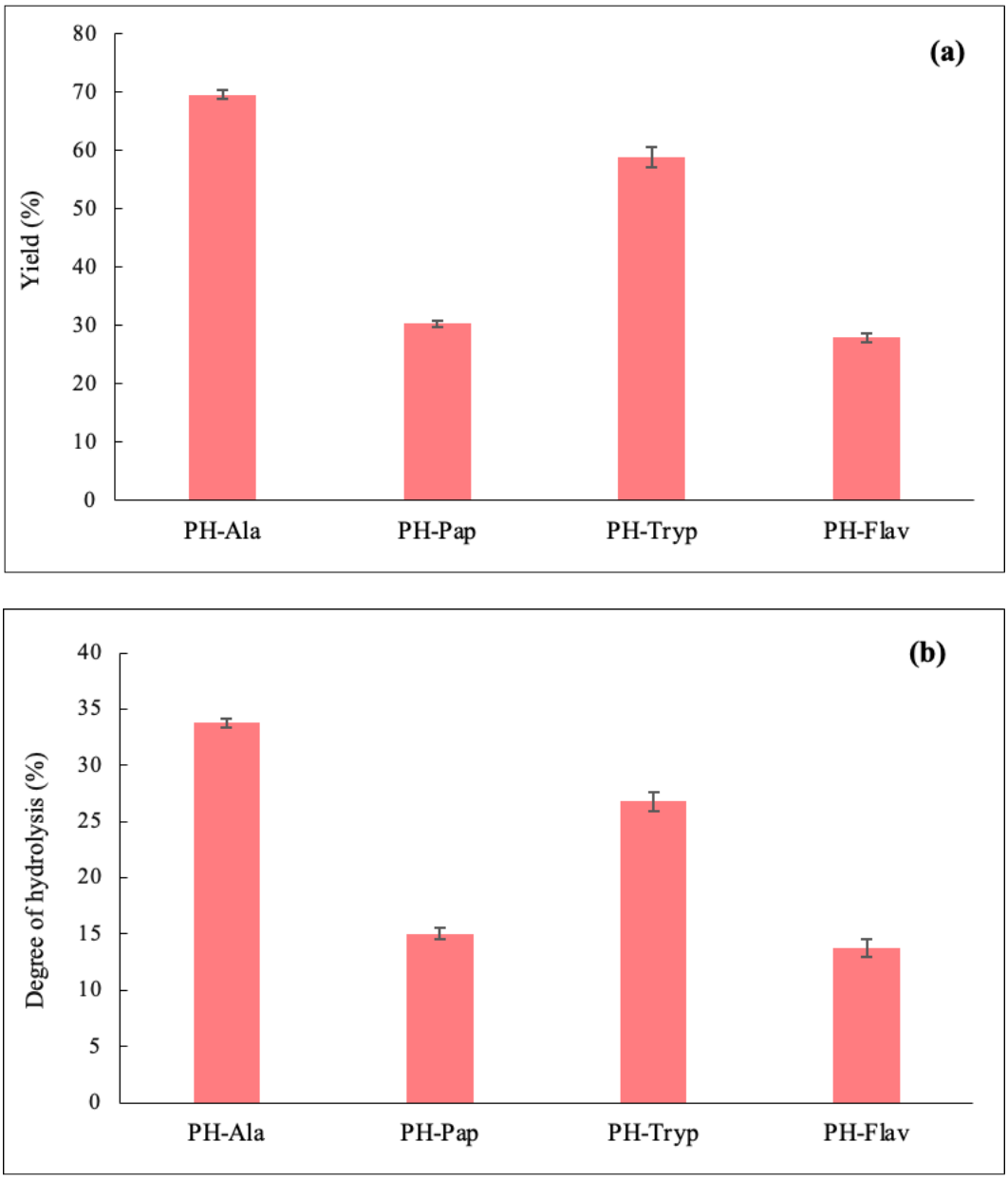

Fig. 2S. Variations in the (a) yields, (b) degree of hydrolysis of different protein hydrolysate prepared by four different proteases.

Table 1S Protease products and their optimal conditions for producing CDS protein hydrolysates in this study.

Enzyme	pH, temperature	Source of enzyme	Activity	EC number	Activity[a]	Substrate specificity and preference[b]
Alcalase® 2.4 L	[pH 8, 55°C]	*Bacillus licheniformis*	Serine Endoprotease	EC: 3.4.21.62	≥ 2.4 U/g	Broad specificity; Ala, Ser at P1; Tyr, Trp, Phe, and Leu position
Papain	[pH 6.2, 25°C]	Papaya latex	Cysteine Endoprotease	EC: 3.4.22.2	1.5-10 units/mg solid	Fairly broad specificity; Arg and Phe at P1; Leu, Phe at P2; Gln at P10and P20; Asp at P4
Trypsin	[pH 8, 37°C]	Porcine pancreas	Serine Endoprotease	EC: 3.4.21.4	1,000-2,000 units/mg dry solid	Preferential cleavage of N-terminal Arg and Lys at P1 position
Flavorzyme	[pH 7, 55°C]	*Aspergillus oryzae*	Endo- and exo-protease	EC: 232-752-2	≥ 500 U/g	Preferential cleavage of a C-terminal dipeptide, oligopeptide position

[a]As specified by Sigma Sigma-Aldrich (Oakville, Ontario, Canada). [b]Information taken from previous studies (Zhu et al., 2019); (Cheung & Li Chan, 2017).s

Table 2Sa. The independent factors with levels (actual and coded) considered for optimizing alcalase hydrolysis.

Factors	Levels				
	-α	-1	0	+1	+ α
Incubation time (hrs)	0.14	2	6.5	11	12.86
Enzyme: Substrate (E:S) (%)	0.76	2	5	8	9.24

Table 2Sb. The independent factors with levels (actual and coded) considered for optimizing trypsin hydrolysis.

Factors	Levels				
	-α	-1	0	+1	+ α
Incubation time (hrs)	0.14	2	6.5	11	12.86
Enzyme: Substrate (E:S) (%)	0.76	2	5	8	9.24

Table 2Sc. The independent factors with levels (actual and coded) considered for optimizing papain hydrolysis.

Factors	Levels				
	-α	-1	0	+1	+ α
Incubation time (hrs)	0.6	1.5	3.8	6	6.9
Enzyme: Substrate (E:S) (%)	2.2	3	5	7	7.8

Table 2Sd. The independent factors with levels (actual and coded) considered for optimizing flavorzyme hydrolysis.

Factors	Levels				
	-α	-1	0	+1	+ α
Incubation time (hrs)	0.76	2	5	8	9.2
Enzyme: Substrate (E:S) (%)	1.17	2	4	6	6.83

Table 3S Predicted angiotensin-converting enzyme (ACE) inhibitory activity (IC50) of dipeptides, tripeptides and tetrapeptides encrypted in peptides[a] of ultrafiltered protein hydrolysate fraction (<3 kDa).

Sequence	IC^{b}_{50} (µmol/L)	Sequence	IC^{b}_{50} µmol/L)	Sequence	IC^{b}_{50} µmol/L)
AA	**51.40**	IA	153	PG	17000
AAP	30	IG	1200	PPL	>1000
AF	190	IP	130	PT	n.a.
AG	2500	IPP	5	TG	9900
AI	690	ILP	270	TGP	n.a.
ALP	240	IL	n.a.	RL	746.40
ALPP	280	KF	1160	RP	21
AP	322	**KP**	**22**	SF	130.2
AV	800	KG	n.a.	SY	66.3
AVL	1.67-930	KGP	n.a.	TF	18
DF	360	LA	n.a.	TP	290
DG	12.3	LF	349	VAA	13
EG	10000	LLF	79.8	**VAP**	**2**
FG	3700	LLP	57	VM	n.a.
FQ	<20 mM	LQ	n.a.	VF	49.7
FNQ	335	LG	8800	VMP	29
GA	2000	**LQP**	**2**	VP	420
GD	9200	LPF	10.59	VLP	n.d.
GG	7200	LPP	9.60	VG	n.a.
GE	<20 mM	LR	n.a.	VSP	10
GF	277.90	**LSP**	**1.70**	VW	3.1
GL	<20 mM	LSPA	315	VWP	n.a.
GLY	1.67-930	LY	38.5	WL	29.90
GP	360	LY	38.5	WG	5900
GPP	n.a.	NG	12000	YA	460
GPL	2.55	**QP**	**65.80**	YL	82
GI	1300	PL	n.a.	YY	n.a.
GPV	4.67	PLP	430	YP	720
GQ	1910	PP	n.a.	YK	610

GS	3800	GW	30	HLL	22.20
GT	5700	HG	6300	HP	n.a.
GV	4600	HL	3200		

[a]Peptide fragments (alphabetical order) with ACE inhibitory activity were identified by BIOPAP database as mentioned in Table 2a and 2b. [b]IC50 is the quantity of peptides needed to inhibit 50% of the enzymatic activity. Values were computed AHTPDB databases [c]n.a. means IC50 value not available. A = alanine, D = aspartic acid, F = phenylalanine, G = glycine, H = histidine, I = isoleucine, K = lysine, L = leucine, M = methionine, P = proline, Q = glutamine, R = arginine, S = serine, T = threonine, V = valine, W = tryptophan.

Table 4S Predicted DPP IV inhibitory activity (IC50) of dipeptides, tripeptides and tetrapeptides encrypted in peptides[a] of ultrafiltered protein hydrolysate fraction (<3 kDa).

Fragments with DPP IV inhibition combined with their Frequency of occurrence
AA [A = 0.50, B = 5.32 × 10^{-5}], **AF** [A = 0.50], **AG** [A = 0.50], **AE** [A = 0.50], **AL** [A = 0.50, B = 5.67 × 10^{-4}], **AS** [A = 0.50]
DN [A = 0.50], **DA** [A = 0], **DP** [A = 0.50]
EG [A = 0.50], **EP** [A = 0.50], **ES** [A = 0.50]
FL [A = 0.50, B = 125 × 10^{-5}], **FN** [A = 0.50], **FP** [A = 0.50, B = 137 × 10^{-5}], **FQ** [A = 0.50]
GF [A = 0.50], **GA** [A = 0.50], **GI** [A = 0.50], **GE** [A = 0.50], **GP** [A = 0.50, B = 5.16 × 10^{-5}], **GPM** [A = 1.0, B = 83.20 × 10^{-5}], **GG** [A = 0.50], **GL** [A = 0.50, B = 19.12 × 10^{-5}], **GW** [A = 0.50], **GV** [A = 0.50]
HP [A = 0.50, B = 17.73 × 10^{-5}], **HL** [A = 0.50, B = 341.19 × 10^{-5}], **HT** [A = 0.50, B = 19.12 × 10^{-5}], **HI** [A = 0.50]
IA [A = 0.50]**, IN** [A = 0.50]**, IL** [A = 0.50]**, II** [A = 0.50]**, IP** [A = 0.50, B = 21.95 × 10^{-5}]
KG [A = 0.50]
LA [A = 0.50]**, LP** [A = 0.50, B = 21.10 × 10^{-5}]**, LM** [A = 0.50]**, LPL** [A = 1.0, B = 83.20 × 10^{-5}], **LN** [A = 0.50]**, LL** [A = 0.50]**, LQP** [A = 0.67, B = 28.22 × 10^{-5}]**, LV** [A = 0.50]
MP [A = 0.50, B = 57.47 × 10^{-5}], **MK** [A = 0.50]
NP [A = 0.50], **ND** [A = 0.50], **NG** [A = 0.50], **NQ** [A = 0.50], **NL** [A = 0.50]
PA [A = 0.50], **PF** [A = 0.50], **PG** [A = 0.50], **PI** [A = 0.50], **PL** [A = 0.50**]**, **PM** [A = 0.50], **PK** [A = 0.50], **PT** [A = 0.50], **PPL** [A = 1.0, B = 91.13 × 10^{-5}], **PV** [A = 0.50], **PP** [A = 0.50, B = 8.53 × 10^{-5}], **PPG** [A = 1.00, B = 20.49 × 10^{-5}], **PS** [A = 0.50], **PY** [A = 0.50], **PPPP** [A = 1.00, B = 12.80 × 10^{-5}], **PW** [A = 0.5]
QP [A = 0.50], **QL** [A = 0.50], **QH** [A = 0.50], **QQ** [A = 0.50], **QF** [A = 0.50]
RP [A = 0.50, B = 22.32 × 10^{-5}], **RN** [A = 0.50]
SL [A = 0.50, B = 19.86 × 10^{-5}], **SF** [A = 0.50], **SY** [A = 0.50], **SP** [A = 0.50, B = 8.36 × 10^{-5}]
TG [A = 0.50], **TR** [A = 0.50], **TP** [A = 0.50, B = 2106 × 10^{-5}], **TA** [A = 0.50], **TL** [A = 0.50], **TF** [A = 0.50], **TN** [A = 0.50]
VA [A = 0.50, B = 297.20 × 10^{-5}], **VD** [A = 0.50], **VF** [A = 0.50], **VI** [A = 0.50], **VG** [A = 0.50], **VM** [A = 0.50], **VL** [A = 0.50, B = 675.68 × 10^{-5}], **VP** [A = 0.50, B = 56.82 × 10^{-5}], **VV** [A = 0.50], **VW** [A = 0.50]
WG [A = 0.50], **WH** [A = 0.50], **WN** [A = 0.50, B = 336.70 × 10^{-5}], **WP** [A = 0.50, B = 11.04 × 10^{-5}], **WY** [A = 0.50, B = 177.94 × 10^{-5}], **WV** [A = 0.50, B = 761.15 × 10^{-5}]
YP [A = 0.50, B = 15.77 × 10^{-5}], **YQ** [A = 0.50], **YL** [A = 0.50], **YE** [A = 0.50], **YPY** [A = 1.00, B = 147.30 × 10^{-5}], **YA** [A = 0.50], **YG** [A = 0.50], **YK** [A = 0.50]

[a]Peptide fragments (alphabetical order) with DPP IV inhibitory activity were identified by BIOPEP database as mentioned in Table 2a and 2b. A = alanine, D = aspartic acid, F = phenylalanine, G = glycine, H = histidine, I = isoleucine, K = lysine, L = leucine, M = methionine, P = proline, Q = glutamine, R = arginine, S = serine, T = threonine, V = valine, W = tryptophan. A peptide's activity is predicted using two quantitative parameters: the occurrence frequency value (A) and the prospective biological activity (B). To evaluate the bioactivity of a peptide included in the query sequence, the A value, which is the frequency of bioactive fragments occurring in a protein sequence, is analyzed. The B value, which is a possible biological activity of protein fragments, is derived based on the IC50 or EC50 value of peptides available in the literature. As a result, peptides with higher A and B values, particularly B values, are expected to inhibit DPP IV more effectively. Reference- Nong, N.T.P. and Hsu, J.L., 2021. Characteristics of Food Protein-Derived Antidiabetic Bioactive Peptides: A Literature Update. *International Journal of Molecular Sciences*, *22*(17), 9508.