

# Integrated evaluation of the multifunctional DPP-IV and ACE inhibitory effect of soybean and pea protein hydrolysates

Carlotta Bollati <sup>1</sup>, Ruoxian Xu<sup>1</sup>, Giovanna Boschin<sup>1</sup>, Martina Bartolomei<sup>1</sup>, Fabrizio Rivardo<sup>2</sup>, Jianqiang Li<sup>1</sup>, Anna Arnoldi<sup>1</sup> and Carmen Lammi<sup>1,\*</sup>

<sup>1</sup> Department of Pharmaceutical Sciences, University of Milan, Via Mangiagalli 25, 20133 Milan (Italy); carlotta.bollati@unimi.it (C.B.), ruoxian.xu@unimi.it (R.X.), giovanna.boschin@unimi.it (G.B.), Jianqiang.li@unimi.it (J.L.), anna.arnoldi@unimi.it (A.A.), carmen.lammi@unimi.it (C.L.)

<sup>2</sup> A. Costantino & C. Spa, Via Francesco Romana 11-15 - 10083 Favria (TO) – Italy; frivardo@acostantino.com (F.R.),

\* Correspondence: carmen.lammi@unimi.it; Tel.: +39 02/503019372

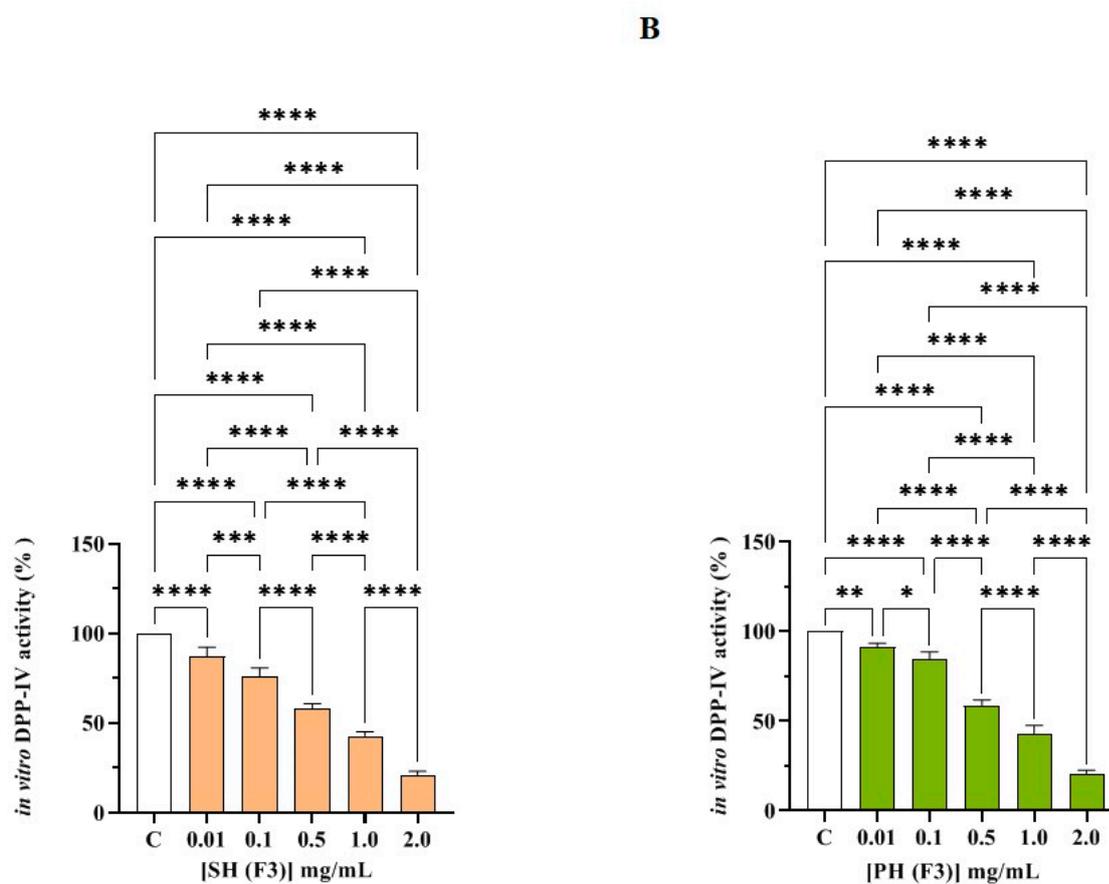
**Table S1:** LC-MS/MS based identification of SH and PH peptides.

Hydrolysate.	Protein name	Peptide sequence	Spectrum Intensity
SH	Uncharacterized protein	(W)FNIVGQWAVTT(S)	5.19 × 10 <sup>7</sup>
	Ankyrin repeat domain-containing protein 52	(A)IRSWIVQVMS(Q)	5.11 × 10 <sup>7</sup>
	Uncharacterized protein	(I)GKQASIIEDPRPGQGKN(L)	2.34 × 10 <sup>7</sup>
	Uncharacterized protein	(A)GMPVHVSVEDLPGAPFGDA(G)	2.57 × 10 <sup>7</sup>
	Glycinin G1	(A)VSIIDTNSLENQLDQ(M)	4.56 × 10 <sup>7</sup>
		(S)IIDTNSLENQLDQMPR(R)	2.07 × 10 <sup>7</sup>
		(G)ANSLLNALPEEVIQ(H)	2.25 × 10 <sup>7</sup>
	Hydrolase_4 domain-containing protein	(A)AAEGGGFSDPAPAPPRLAIPV(P)	1.45 × 10 <sup>7</sup>
	DNA-directed RNA polymerase (Fragment)	(L)FDIYRVMRPGEPPTMDSAEAMFNA(L)	1.48 × 10 <sup>7</sup>
	Heterokaryon incompatibility protein	(L)GGLVQPIQMSKSARADGGDVSAQLANLDL S(A)	1.64 × 10 <sup>7</sup>
	Uncharacterized protein	(Q)HGLGLEVIELGNMVDGFYLSSR(S)	4.81 × 10 <sup>7</sup>
	Phosphatidylinositol-specific phospholipase C	(H)DNDIATALSNLGIFTFSEQ(F)	1.15 × 10 <sup>7</sup>
	Uncharacterized protein	(P)LQRIGVGLVFSILAMVSAALI(E)	2.57 × 10 <sup>7</sup>
	Uncharacterized protein	(K)HKYVVPPIVIAMATGESG(E)	5.83 × 10 <sup>7</sup>
	C-x8-C-x5-C-x3-H type zinc finger protein	(N)TAVDRTLADFGRGFGRG(Q)	2.12 × 10 <sup>7</sup>
	PRONE domain-containing protein	(S)PQVPKSGLS(D)	1.34 × 10 <sup>7</sup>
	PH domain-containing protein	(P)PSISSQSRASSDSSSK(E)	9.54 × 10 <sup>6</sup>
	GMC_OxRdtase_N domain-containing protein	(N)AGFYSRADADFFARS(G)	2.08 × 10 <sup>7</sup>
	PH	Vicilin	(E)ITPEKNPQLQDLDFVN(S)
(E)KNPQLQDLDFVN(S)			5.96 × 10 <sup>7</sup>
Vicilin 47k		(F)EITPEKNQQLQDLDFVN(S)	2.26 × 10 <sup>7</sup>
		(E)KNQQLQDLDFVN(S)	7.09 × 10 <sup>7</sup>
		(K)NQQLQDLDFVN(S)	4.01 × 10 <sup>7</sup>
Legumin A2		(N)ALEPDNRIE(S)	1.53 × 10 <sup>7</sup>
		(S)SVINNPLDVVA(A)	4.96 × 10 <sup>7</sup>

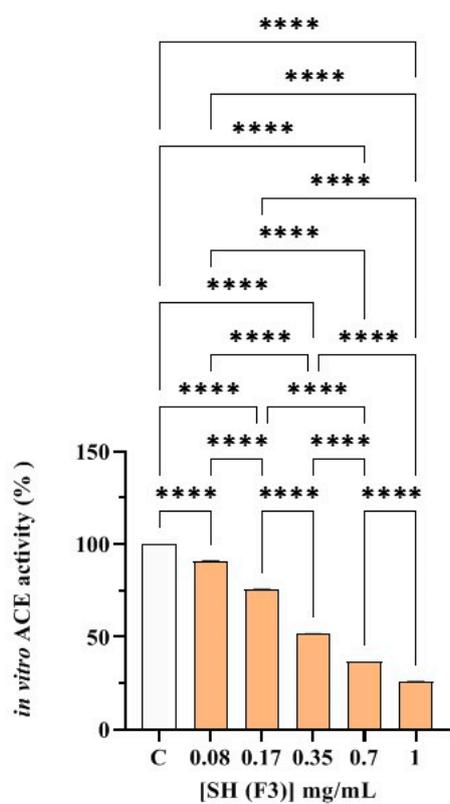
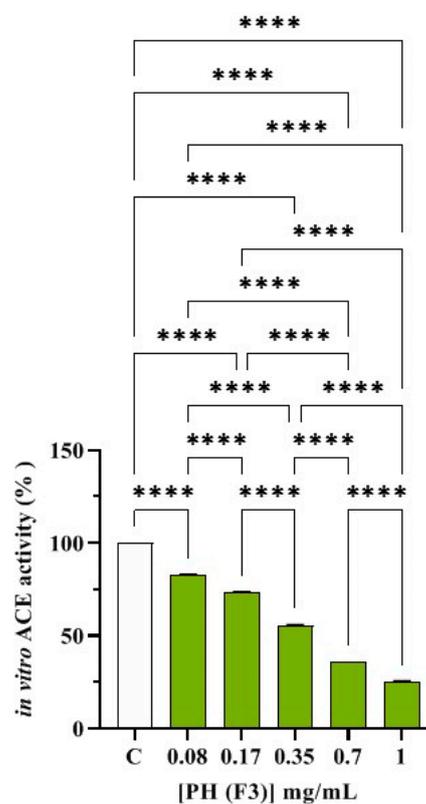
---

Mannonate dehydratase	(T)GATNIVSSLHQVPIGRAWT(E)	$3.28 \times 10^7$
LysR family transcriptional regulator	(K)HLFILGGLGWGGLPASVVKDDL(A)	$1.04 \times 10^8$
Aldehyde dehydrogenase	(T)GATAQWAAINCGLGADILREAA(A)	$1.70 \times 10^7$
Leucine-rich repeat receptor-like protein	(F)GIDLSNNLLHGEIPRGLFGLAGLE(Y)	$3.11 \times 10^7$
AsmA family protein	(S)GGLSFDRKAAKTTASGGLTLKADA(G)	$2.73 \times 10^7$
TP-binding protein	(I)LFGQAGLDPLPVDVGVANGRL(T) (L)DRMFCGIIDRDGGAPGTDRIF(P)	$1.80 \times 10^7$ $3.23 \times 10^7$
Putative aromatic aminotransferase protein	(A)TFIQAAVPRIIT(Q)	$3.03 \times 10^7$
Argonaute 2	(Q)WPCLQVGNPQRPNYLPMEVCKIVEG(Q)	$2.95 \times 10^7$
ABC transporter substrate-binding protein	(G)WAGAAFGFEESPELKALVDAGKLPPVE(K)	$6.51 \times 10^7$
ABC transporter substrate-binding protein	(S)GGGTWEAAQKKAFFDPFTRDTGIKVV(L)	$5.85 \times 10^7$
Mannonate dehydratase	(I)RGGKLSFMETFPDEGDMDMVRS(V)	$5.80 \times 10^7$
LysR family transcriptional regulator	(K)HLFILGGLGWGGLPASVVKDDL(A)	$2.92 \times 10^7$
Clink	(F)SQLPEELKEKIMNEHLKEI(K)	$2.79 \times 10^7$
Peptidoglycan-associated protein	(K)KPPNSAGDLGLGTGAGGAATPGSAQDFTV NV(G)	$6.60 \times 10^7$
Sporulation protein	(P)ITPAPQQVA AVSPRPAPVFA(P)	$1.82 \times 10^7$
Dioxygenase RAMOSUS5	(P)KPVPAPAPIPTTDVVIPGRILQPVPFI(D)	$1.36 \times 10^7$
Putative DNA modification/repair radical SAM protein	(L)NIELPTDSGITRF(A)	$2.14 \times 10^7$
Hydantoinase/oxoprolinase family protein	(Y)EGDVLVSTSIGGCNQISDVISKPIQLAK(S)	$3.06 \times 10^7$
Aspartate/tyrosine/aromatic aminotransferase	(F)IDLAYQGLGDGLEQDAAPARM(V)	$1.59 \times 10^7$
DNA replication licensing factor MCM3	(G)THLRGDINMMMVGDPVSAKS(Q)	$2.79 \times 10^7$
L-threonine 3-dehydrogenase	(V)PMVVGHEFSGEIAEIGSAVTRY(H)	$4.36 \times 10^6$

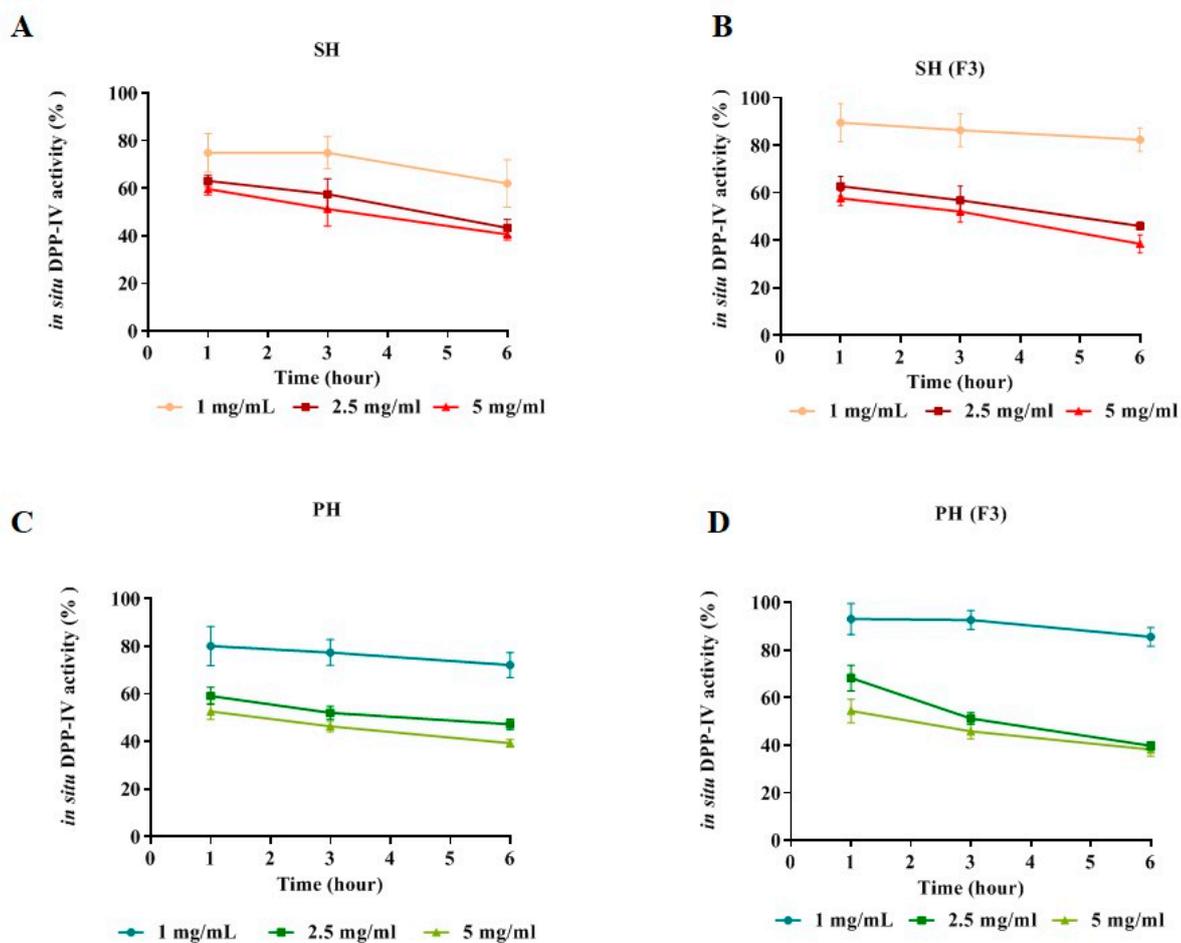
---



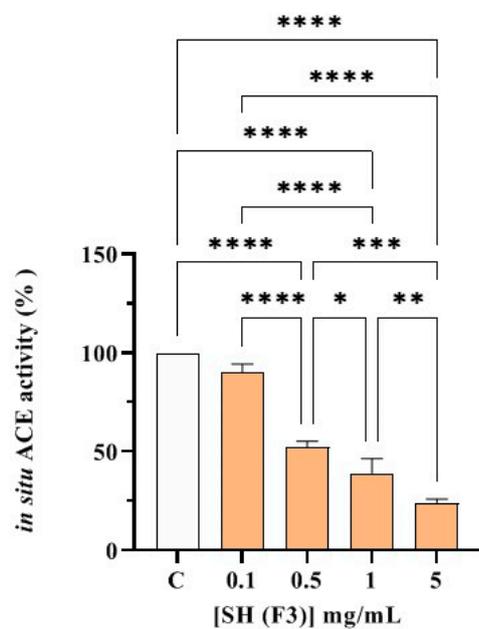
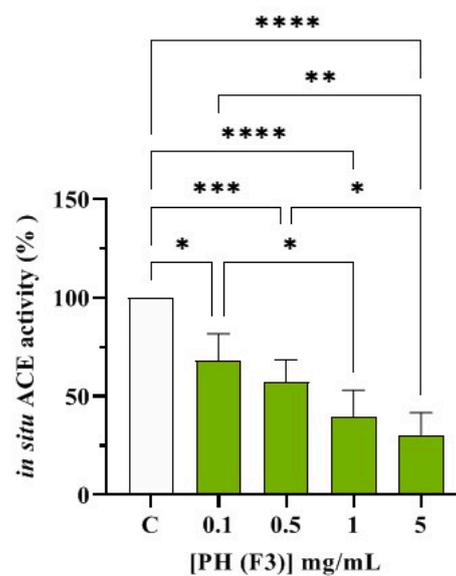
**Figure S1:** Evaluation of the *in vitro* inhibitory effects of SH (F3) (A) and PH (F3) (B) hydrolysates on human recombinant DPP-IV. Bars represent the average  $\pm$  s.d. of three independent experiments in duplicates. \*\*\*\*  $p < 0.0001$ , \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$ , \*  $p < 0.05$  versus control (C) sample (Activity).

**A****B**

**Figure S2:** Evaluation of the *in vitro* inhibitory effects of SH (F3) (A) and PH (F3) (B) hydrolysates on ACE. Bars represent the means  $\pm$  sd of three independent experiments in duplicate. \*\*\*\*  $p < 0.0001$  versus Control sample (C).



**Figure S3:** The kinetics of the inhibition of cellular DPP-IV activity after incubating Caco-2 cells with the SH (A), SH (F3) (B), PH (C) and PH (F3) (D) hydrolysates for 1, 3 and 6 hours at different concentrations. The data are represented as the means  $\pm$  s.d. of four independent experiments, performed in triplicate.

**A****B**

**Figure S4:** Evaluation of the inhibitory effects of SH (F3) (A) and PH (F3) (B) hydrolysates on ACE expressed on Caco-2 cells membranes. Bars represent the SD of three independent experiments in triplicate. \*\*\*\* p < 0.0001, \*\*\* p < 0.001, \*\* p < 0.01, \* p < 0.05 versus Control sample (C), non-significant (ns) is not shown.