

1 *Supplementary material for:*

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3 ***In vitro* gastrointestinal stability and intestinal absorption of**  
4 **ACE-1 and DPP4 inhibitory peptides from poultry by-product**  
5 **hydrolysate**

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20 *Table S1 – Selected genes with the corresponding Taqman primer/probes.*

Taqman primer/probe	Gene
Hs02800695_m1	<i>HPRT1</i>
Hs00192639_m1	<i>SLC15A1</i>
Hs00184500_m1	<i>ABCB1</i>
Hs00900408_m1	<i>CDH17</i>
Hs01030343_m1	<i>SLCO2B1</i>
Hs05465837_g1	<i>OCLN</i>
Hs01078080_m1	<i>CDX2</i>
Hs01113665_m1	<i>SLC15A2</i>
Hs00975961_g1	<i>NFE2L2</i>
Hs01551861_m1	<i>TJP1</i>

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22 *Table S2 – Peak area of the bioactive peptides VL, IY and YA in the in vitro digestion experiment. The peak areas are calculated*  
 23 *from ion counts of extracted ion chromatograms (EIC) corresponding to the molecular ion peaks [M+H]<sup>+</sup> of the respective*  
 24 *peptides.*

sample type	replicate	Peak area		
		VL	IY	YA
Digest	a	65299023	17630985	1703702
	b	63817034	16977368	1641682
	c	63944605	17068163	1653856
	average	64353554	17225505	1666413
	SD	821281	354078	32862
	SD relative to average (%)	1.3	2.1	2.0
LMWPF control	a	64391889	16516229	1448975
	b	64068245	16624540	1441466
	c	64810522	16834370	1473378
	average	64423552	16658379	1454607
	SD	372150	161748	16685
	SD relative to average (%)	0.6	1.0	1.1
enzymes' control	a	483605	190412	50245
	b	473166	182366	50658
	c	473554	201515	63155
	average	476775	191431	54686
	SD	5918	9615	7337
	SD relative to average (%)	1.2	5.0	13.4
digest (%) relative to LMWPF control		99.9	103.4	114.6
enzymes' control (%) relative to LMWPF control		0.7	1.1	3.8

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27 Table S3 – Peak area of the bioactive peptides VL, IY and YA from LMWPF identified in control transport experiment without  
 28 Caco-2 cells and in 1 mg/mL LMWPF stock used for the experiment.

Sample type		VL			YA			IY		
side	replicate	Area	relative to LMWPF stock (%)	AP+BL (%)	Area	relative to LMWPF stock (%)	AP+BL (%)	Area	relative to LMWPF stock (%)	AP+BL (%)
AP	a	11896975	52	68	170732	47	63	2502601	49	61
BL	a	3775127	16		54622	15		591611	12	
AP	b	12772183	55	70	161193	45	59	2551334	50	61
BL	b	3448020	15		52056	14		545039	11	
AP	c	12665801	55	71	155244	43	57	2191511	43	56
BL	c	3624464	16		48579	13		633087	12	
Average of replicates				70			59			59
LMWPF stock		23063220	100		360122	100		5073218	100	

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30 Table S4 – TEER measurements in ohm/cm<sup>2</sup> before and after Caco-2 monolayer transport experiments.

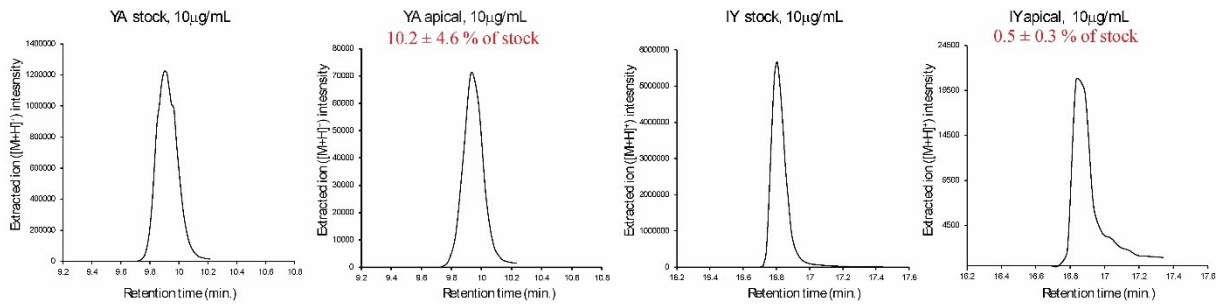
well	Transport experiment 1			Transport experiment 2			Transport experiment 3		
	day before	before experiment	after experiment	day before	before experiment	after experiment	day before	before experiment	after experiment
A1	762	869	1003	884	1004	1031	876	862	872
A2	813	966	1071	866	925	991	797	863	842
A3	768	939	1043	876	973	988	708	805	773
A4	741	947	1079	852	981	1020	845	876	835
B1	752	1006	1097	903	965	1013	794	889	885
B2	746	782	966	853	894	920	739	710	723
B3	726	839	993	845	904	917	739	812	782
B4	729	973	1049	867	947	982	805	811	786
C1	821	1104	1167	955	997	1081	913	927	954
C2	771	983	1073	955	1022	1075	866	876	920
C3	832	1033	1124	965	1050	1091	867	973	925
C4	813	1067	1138	950	1068	1066	916	939	913
Average	773	959	1067	898	977	1015	822	862	851

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32 Table S5 – Paracellular transport test across Caco-2 cell monolayers using 10 kDa FITC-d.

		FITC-d equivalent		
		Experiment 1	Experiment 2	Experiment 3
LMWPF	1 mg/mL	-0.5	-0.9	-1.3
IY	100 mg/mL	-0.4	-0.9	-1.2
YA	100 mg/mL	-0.5	-0.9	
control	DMEM	-0.5	-0.9	
No Caco-2	only filters	140.64		

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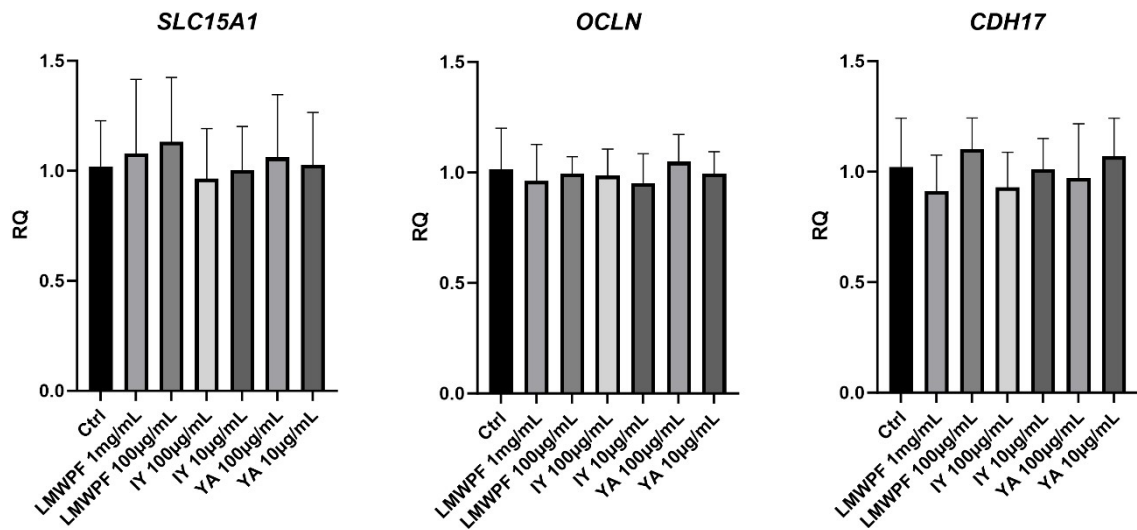


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35 Figure S1: Representative extracted ion chromatogram of 10µg/mL stock solution of peptides YA and IY as well as the  
 36 corresponding samples collected from the apical side after the transport experiment. On average 10.2 and 0.5% of the initial  
 37 stock concentration was adequately detected after on the apical side after 4 hours of incubation for YA and IY, respectively.

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41 Figure S2 – Relative gene expression of genes in Caco-2 cells encoding SLC15A1, OCLN, and CDH17 incubated with LMWPF  
 42 (100 µg/mL and 1 mg/mL) or synthetic peptides VL and YA (10 and 100 µg/mL) in comparison to the control treatment with  
 43 culture medium (Ctrl). Differences between treatments are determined by one-way ANOVA.

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