

SUPPLEMENTARY INFORMATION

Evaluation of the relationship between the peptide profile and the lipid-lowering properties of olive seeds hydrolysates as a tool for tuning hypocholesterolemic functionality

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Table S1. Sequence, range of retention times, m/z, molecular mass, mass accuracy (ppm), range of average local confidence (ALC), isoelectric point, water solubility and varieties of peptides identified using *de novo* sequence in the hydrolysate from each variety.

Peptide sequence	Retention time range	Experimental m/z	Theoretical molecular mass	Error (ppm)	Alc range	Isoelectric point*	Water solubility*	Varieties**
ADLY	9.5 – 10.1	481.2290	480.2220	0.4	90 – 94	0.74	Good	BA, CAL, KA, LS, MA, OC, PI, RE, VE
AEAF	6.8 – 7.8	437.2031	436.1958	0.1	94 – 96	0.99	Good	BA, BO, CA, CN, CB, CAL, CR, KH, KA, LS, MJ, NA, OC, PI, RJ, RE, SA, VE
AGSDVTE	1.9 – 2.0	678.2941	677.2868	0.0	90 – 92	0.71	Good	MJ, VE
AGYAE	2.1	510.2199	509.2122	0.9	90 – 93	1.00	Good	CB, VE
ALMAPH	7.1 – 8.9	639.3281	638.3210	0.2	93 – 95	7.88	Poor	BA, BO, CA, CB, CAL, CR, KA, MJ, OC, RJ, RE, VE
ALMSPH	5.3 – 6.2	655.3231	654.3159	0.1	90 – 97	7.88	Poor	CAL, CR, KH, KA, MJ, NA, PI, RJ, RE
ALTLF	25.7 – 25.8	564.3378	563.3318	2.3	90 – 93	3.77	Poor	SA
AQVL	8.1 – 9.0	430.2660	429.2587	0.1	89 – 92	3.70	Poor	BA, CAL, KH, MJ, OC, RJ
AVFDD	13.5 – 14.8	566.2458	565.2883	0.3	90 – 97	0.73	Good	CB, KA, MA, PI, SA
AVVK	1.2 – 1.4	416.2867	415.2794	0.1	90 – 96	10.19	Good	CA, CB, CO, CAL, KA, NA, OC, RE
AVVR	3.5 – 3.7	444.2926	443.2856	0.6	90 – 92	10.90	Good	LS
DDLPR	3.7 – 4.1	615.3097	614.3024	0.1	90 – 96	3.71	Good	BA, CA, CN, CB, CAL, KA, LS, NA, OC, PI, RJ, RE, SA
DFVVLK	22.3 – 22.8	720.4293	719.4218	0.4	93 – 95	6.66	Good	MJ, RJ
ELLL	24.3 – 24.8	487.3119	486.3053	1.5	90 – 97	0.92	Good	MJ, NA
ESGESPLNN	5.7 – 6.2	946.4118	945.4039	0.6	90 – 95	0.85	Good	OC, RJ, RE
FAVV	21.3 – 21.5	435.2598	434.2529	0.9	90 – 95	3.41	Poor	MA
FAVVK	14.7 – 15.0	563.3539	562.3478	2.1	94 – 95	9.88	Poor	NA
FDGEVK	4.1 – 4.7	694.3406	693.3333	0.0	91 – 97	3.93	Good	BA, BO, CA, CN, CB, CAL, KH, KA, LS, MJ, MA, NA, OC, PI, RJ, RE, SA, VE

FFPF	28.4 – 29.0	557.2725	556.2686	5.9	90 – 95	3.45	Poor	CB
FLLF	28.7 – 29.2	539.3225	538.3155	0.5	92 – 97	3.45	Poor	CA, CB, CO, CR, KH, LS, MJ, SA, VE
FLPH	8.7 – 11.5	513.2819	512.2747	0.0	90 – 96	7.56	Poor	BA, BO, CA, CN, CB, CAL, CR, KH, KA, LS, MJ, MA, NA, OC, PI, RJ, RE, SA, VE
FLPR	6.8 – 6.9	532.3240	531.3169	0.4	90 – 92	10.59	Good	CB
FVVL	24.4 – 24.8	477.3061	476.2999	2.1	91 – 94	3.38	Poor	LS, MJ
FVVLK	20.0 – 20.1	605.4020	604.3948	0.2	90 – 98	9.88	Poor	CO, CR, LS, MJ, NA, SA, VE
GESPLNN	3.9 – 4.2	730.3362	729.3293	0.4	90 – 91	1.00	Good	BA, KH
GFEDL	21.8 – 21.9	580.2615	579.2540	0.3	90 – 94	0.63	Good	CB, SA
GFVK	2.9 – 3.5	450.2711	449.2638	0.0	90 – 94	10.12	Good	BA, BO, CA, CN, CB, CO, CAL, KH, KA, LS, MJ, NA, OC, SA, VE
GLGNY	11.9 – 13.0	523.2513	522.2438	0.6	90 – 92	3.61	Poor	CO, KA, RJ, RE
GVLH	2.4	425.2509	424.2434	0.6	91 – 92	7.81	Poor	BO
HTLY	4.6 – 5.3	533.2719	532.2645	0.2	90 – 95	7.54	Poor	BA, CA, CN, CB, CAL, CR, KH, KA, LS, MJ, MA, NA, OC, PI, RJ, RE, SA, VE
KALM	3.2 – 3.5	462.2736	461.2672	1.9	91 – 97	9.91	Good	CAL, MA
KFVEGDE	8.5 – 9.0	823.3842	822.3759	1.2	90 – 94	3.69	Good	MA
KGAL	1.5 – 1.6	388.2555	387.2481	0.2	92 - 94	9.91	Good	BO, MA
KLGNF	14.1 – 16.3	578.3297	577.3224	0.0	90 – 94	9.91	Good	BA, BO, CA, CB, CO, CR, KH, KA, LS, MJ, MA, NA, PI, RE, SA, VE
KLPL	18.6 – 19.4	470.3336	469.3264	0.2	91 – 96	9.91	Good	BA, BO, CA, CN, CB, CO, CR, KH, KA, LS, MJ, MA, NA, RJ, RE, SA, VE
KLPLL	23.6 – 24.5	538.3179	582.4105	0.1	90 – 96	9.91	Poor	CB, CO, CR, KH, LS, MJ, MA, NA, SA, VE
KVSSPL	10.3 – 13.0	630.3821	629.3748	0.1	91 – 98	9.91	Good	BA, BO, CA, CN, CB, CO, KH, KA, LS, MJ, MA, NA, OC, PI, RJ, RE, SA, VE
LAFK	9.0 – 9.4	478.3023	477.2951	0.1	91 – 96	10.12	Good	KA, MA, NA
LAGY	7.9 – 9.0	423.2238	422.2165	0.1	91 – 96	3.61	Poor	BA, BO, CA, CB, CAL, CR, KH, KA, LS, MJ, OC, PI, RJ, RE, VE
LALLGL	27.1	599.4103	498.4054	3.9	90 – 93	3.63	Poor	MJ
LELL	24.3 – 24.5	487.3127	486.3053	0.1	91 – 93	0.92	Good	LS, SA

LGFLPH	22.9 – 23.6	683.3878	682.3802	0.4	90 – 94	7.81	Poor	CO, CR, KH, LS, MJ, NA, SA, VE
LLDA	8.9 -9.4	431.2501	430.2427	0.3	90 – 97	0.69	Good	BO, KA, OC
LLDAA	10.5 – 11.5	502.2869	501.2798	0.4	91 – 97	0.69	Good	CR, LS, RE
LLDTA	12.2 – 12.5	532.2971	531.2904	1.2	94 – 97	0.69	Good	KA
LLGL	23.2 – 23.5	415.2907	414.2842	1.9	91 – 97	3.63	Poor	CB, KH, MA
LLLPQ	21.3 – 21.4	583.3809	582.3741	0.8	90 – 91	3.70	Poor	CB
LLPVNTL	24.5 – 24.7	769.4806	768.4745	1.6	90 – 93	3.63	Poor	MJ, SA
LLVPLL	29.5 – 29.8	667.4718	666.4680	5.1	90 – 94	3.63	Poor	CR, SA
LMAPH	4.2 – 4.8	568.2911	567.2839	0.0	90 – 95	7.81	Poor	BA, BO, KH, KA, MJ, RJ, RE
LPLL	24.8 -24.9	455.3217	454.3155	2.2	90 – 98	3.63	Poor	CB, KH, VE
LPVNTL	22.0 – 22.2	656.3962	655.3904	2.3	90 – 92	3.63	Poor	MA
LSPL	16.8 – 19.0	429.2711	428.2635	0.8	90 – 93	3.63	Poor	BA, BO, KA, OC, PI, RE, VE
LTYL	21.9 – 22.2	509.2969	508.2897	0.1	91 – 94	3.57	Poor	BO, CB, CR, KH, MJ, NA
LVLFF	26.2 – 27.2	491.3225	490.3155	0.5	90 - 96	3.70	Poor	CO, CR, LS, MJ, SA
LVTPH	3.6 – 4.3	566.3297	565.3224	0.1	95 – 98	7.81	Poor	BO, CAL, KH, KA, OC, PI, VE
LVVD	7.2 – 8.1	445.2657	444.2584	0.2	90 – 95	0.88	Good	BA, BO, CAL, CR, KA, LS, OC, PI, RE, VE
MDMS	3.1 – 3.3	483.1577	482.1508	0.1	90 – 97	0.79	Good	BA, CA, CN, CAL, KH, KA, OC, PI, RJ, RE
MKLADVPLC LVN	26.9 – 27.0	658.3602	1314.7039	1.5	90 – 92	5.90	Poor	CAL
NDGFE	4.5 – 4.9	581.2200	580.2129	0.3	90 – 97	0.71	Good	BA, BO, CA, CN, CB, CAL, KH, KA, MJ, OC, RJ, SA
NDVFK	5.5 – 6.4	622.3190	621.3122	0.0	93 – 95	6.22	Good	BA, BO, CA, CN, CB, CAL, CR, KH, KA, LS, MJ, MA, NA, OC, PI, RJ, RE, VE
NFAVVK	17.7 – 18.1	677.3983	676.3908	0.3	90 – 92	9.70	Poor	BA, BO, KH, MJ, RE
NFVVLK	21.1 – 21.4	719.4444	718.4377	0.9	91 – 93	9.70	Poor	CB, CO, MA, NA
NLLN	7.6 – 8.1	473.2720	472.2645	0.5	90 – 92	3.29	Poor	CB, LS, NA, OC, RE
NLPAE	4.5 – 5.2	543.2773	542.2700	0.0	90 – 93	1.00	Good	CN, CB, CAL, KA, LS, NA, OC, PI, RJ, RE

NVDLE	9.4 – 11.2	589.2828	588.2775	0.0	91 – 97	0.71	Good	BA, BO, CA, CN, CB, CAL, CR, KH, KA, LS, MJ, NA, OC, PI, RJ, RE, SA, VE
PNYQTPR	19.5 – 19.6	486.7477	971.4825	1.8	90 – 91	10.27	Good	CA, NA
QGLYE	9.4 – 9.6	609.2872	608.2806	1.1	90 – 93	1.20	Good	OC
QLSPN	2.2	558.2778	557.2809	0.2	90 – 91	3.42	Poor	KA
SPLL	20.1 – 20.2	429.2693	428.2635	3.4	90 – 92	3.37	Poor	OC, PI
SQQDL	2.9	590.2785	589.2708	0.8	92	0.69	Good	KA
SSPL	4.9 – 5.3	403.2188	402.2114	0.1	90 – 93	3.37	Poor	CA, CN, CAL, KH, KA, LS, MA, OC, RJ
SSPLL	20.1 – 20.4	516.3029	515.2955	0.1	92 – 98	3.37	Poor	BO, CB, CO, CR, KH, LS, MJ, MA, NA, SA, VE
STLF	21.2 – 21.7	467.2501	466.2427	0.1	91 – 96	3.43	Poor	BA, CA, CB, CO, CR, KH, LS, MJ, MA, NA, RJ, SA, VE
SVLY	17.3 – 17.8	481.2657	480.2584	0.1	90 – 93	3.39	Poor	BA, BO, KH, MA, PI, RE, VE
SVNDL	7.7 – 8.3	547.2721	546.2650	0.2	93 – 95	0.69	Good	CA, CB, KA, RE
TDGESPLNN	5.6 – 6.1	946.4110	945.4039	0.2	90 – 93	0.71	Good	CA, CN, CAL, SA
TLLF	25.0 – 25.3	493.3022	492.2948	0.4	90 – 94	3.39	Poor	CO, CR, LS, MJ
TLPLL	25.1 – 26.1	556.3703	555.3632	0.2	90 – 93	3.32	Poor	BO, CA, CB, CO, CR, KH, LS, MJ, SA, VE
TLPLLN	23.3 – 23.6	670.4138	669.4061	0.6	90 – 93	3.40	Poor	CO, KH, MJ, SA, VE
TLVY	17.7 – 18.5	495.2813	494.2740	0.0	91 – 95	3.35	Poor	BA, BO, CA, CN, CB, CO, CR, KH, KA, LS, MJ, MA, NA, PI, RJ, RE, SA, VE
TYSSPLL	22.6	780.4112	779.4065	3.3	97 – 98	3.30	Poor	BA, KH
VDLE	7.0 – 7.8	475.2398	474.2325	0.0	92 – 97	0.71	Good	BA, CA, CN, CB, CO, CAL, CR, KH, KA, LS, MJ, MA, NA, OC, PI, RJ, RE, SA, VE
VFDGE	6.7 – 7.5	566.2456	565.2383	0.0	90 – 96	0.71	Good	BA, BO, CA, CN, CB, CO, CAL, CR, KH, KA, LS, MJ, MA, NA, OC, PI, RJ, RE, SA, VE
VFDGEVK	15.6 – 16.5	793.4091	792.4017	0.1	92 – 95	3.93	Good	BO, CO, KA, LS, MJ, VE
VLAL	21.6 – 21.7	415.2910	414.2842	1.1	90 – 93	3.60	Poor	CA, CB
VLPR	17.0 – 18.4	484.3243	483.3169	0.4	90 – 93	10.81	Good	CR
VPLSPT	14.5 – 16.0	613.3554	612.3483	0.2	90 – 96	3.66	Poor	BA, BO, CN, CB, KH, KA, MA, NA

VVLK	2.3 – 2.7	458.3337	457.3264	0.0	96 – 98	10.10	Good	BA, BO, CAL, CR, KH, KA, LS, MA, OC, PI, RE, VE
VVLLT	20.0 – 21.5	544.3708	543.3632	0.7	91 – 92	3.66	Poor	MA
VVLQ	6.9 – 7.1	458.2980	457.2900	1.5	91 – 92	3.67	Poor	MJ, SA
VVPH	1.4 – 1.6	451.2663	450.2591	0.0	92 – 96	7.78	Poor	BO, OC, PI
VVVPH	7.0 – 7.5	550.3356	349.3275	1.5	90 – 96	7.78	Poor	MA
VVVVPH	16.4 – 17.2	649.4034	648.3959	0.5	90 – 96	7.78	Poor	BA, BO, KH, PI, RE
VYLE	14.8 – 16.1	523.2762	522.2689	0.0	90 – 95	1.00	Good	BA, BO, CA, CB, CO, CR, KH, KA, LS, MJ, MA, NA, PI, RJ, RE, SA, VE
WDPN	5.6 – 6.2	531.2198	530.2125	0.0	90 – 92	0.78	Good	KA, LS, MA, OC
WGAPE	11.8 – 12.1	559.2524	558.2438	2.4	92 – 94	1.00	Good	CR
WLAF	26.6 – 27.0	536.2870	535.2795	0.5	90 – 92	3.58	Poor	CB, CO, CR, LS, MJ, SA
WNVN	12.8 – 15.7	532.2515	531.2441	0.2	91 – 96	3.59	Poor	BA, BO, CA, CN, CB, CO, CAL, CR, KH, KA, LS, MJ, MA, NA, OC, PI, RJ, RE, SA, VE
WSMH	11.4 – 12.9	560.2310	559.2213	4.3	92 – 93	7.69	Poor	RE
WVAF	25.1 – 25.5	522.2703	521.2638	1.4	90 – 94	3.58	Poor	BA, MJ, NA, VE
YMDM	18.5 – 18.6	559.1892	558.1818	0.2	92 – 97	0.78	Good	CA, OC
YTSSPL	17.9 – 18.4	667.3297	666.3224	0.0	90 – 95	3.34	Poor	BA, BO, CA, CN, CB, CO, CR, KH, KA, LS, MJ, MA, NA, PI, RJ, RE, SA, VE

* Determined using Innovagen's peptide property calculator.

** Abbreviations: Barnea (BA), Bouteillan (BO), Caballo (CA), Cañivano negro (CN), Cobrancosa (CB), Corbella (CO), Cordobeses de Arroyo de la luz (CAL), Cornicabra (CR), Kalokerida (KA), Khalkhali (KH), Lechín de Sevilla (LS), Majhol (MJ), Manzanilla (MA), Nevado azul (NA), Ocal (OC), Picual (PI), Racimal de Jaén (RJ), Reixonenca (RE), Sayfi (SA), Verdiell (VE)

Table S2. Sequence, range of retention times, m/z, molecular mass, mass accuracy (ppm), range of average local confidence (ALC), isoelectric point, water solubility and varieties of peptides identified using *database tool* sequence in the hydrolysate from each variety.

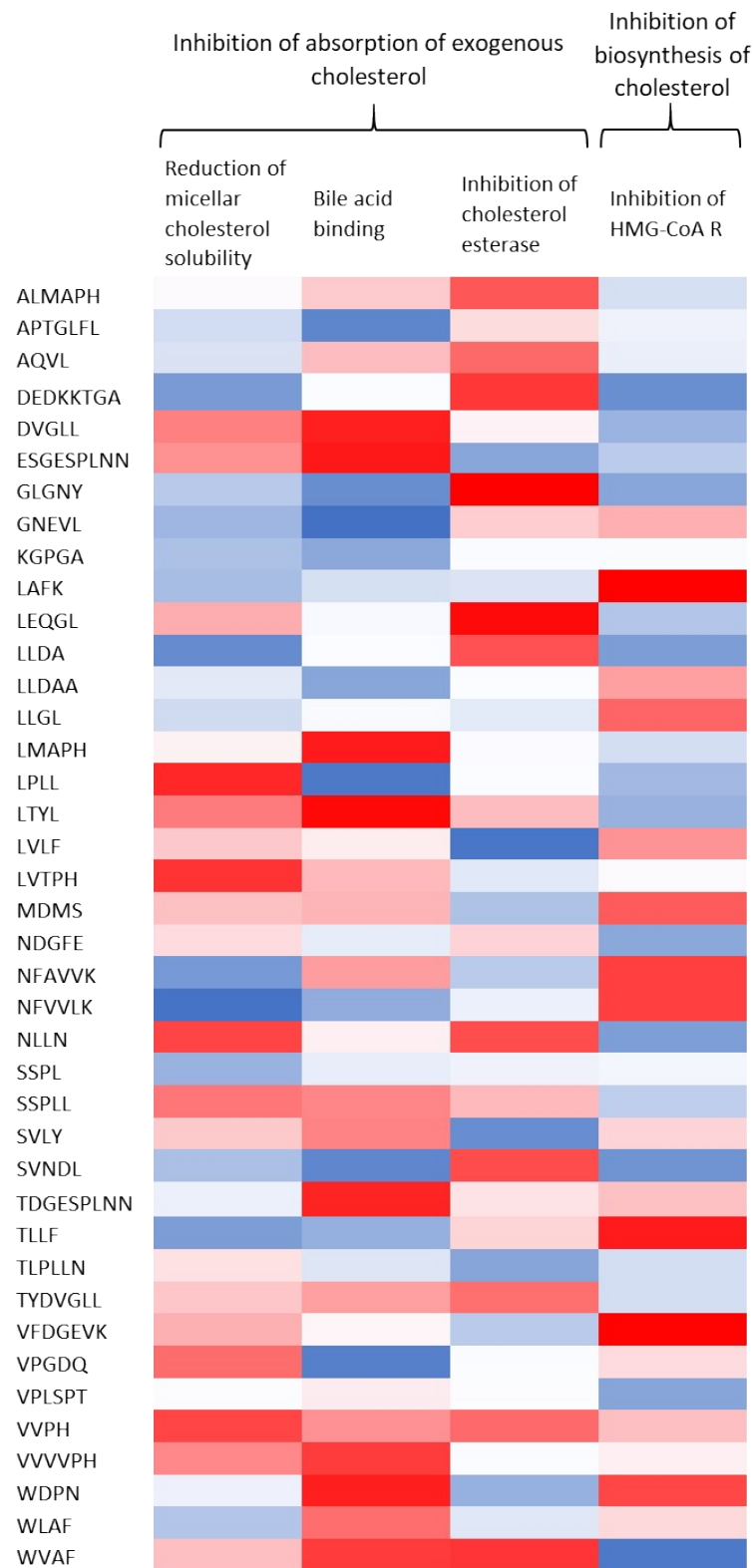
Peptide sequence	Retention time	Experimental m/z	Theoretical mass	Error (ppm)	Isoelectric point*	Water solubility*	Protein accession number	Varieties**
AGAVI	8.0 – 12.7	430.2664	429.2587	0.8	3.72	Poor	A4GDQ8 PROAL_OLEEU A4GDR9 PROAY_OLEEU A4GDS7 PROBB_OLEEU A4GDS9 PROBC_OLEEU A4GDT4 PROBG_OLEEU A4GE39 PROBP_OLEEU A4GE42 PROBQ_OLEEU A4GE45 PROBS_OLEEU A4GE50 PROBW_OLEEU P0DKF8 PROAQ_OLEEU	BA, BO CA, CN, CB, CO, CAL, CR, KA, KH, LS, MJ, MA, NA, OC, PI, RJ, RE, SA, VE
APTGLFL	24.6 – 25.1	718.4135	717.4061	-0.2	3.70	Poor	A4KA54 PROCN_OLEEU	CN, CB, MJ, SA
DEDKKTGA	20.1 – 20.4	863.4183	862.4032	9.0	4.17	Good	Q94G86 ALL9_OLEEU	BO, KA, OC
DVGLL	20.1 – 20.5	516.3002	515.2955	-5.0	0.69	Good	Q94G86 ALL9_OLEEU	BO, CB, CR, KH, LS, MJ, RJ, RE
EAKLA	7.7 – 11.0	531.3134	530.3064	-0.6	6.85	Good	P24820 VP3_ARMV	MA
EGQPI	6.5 – 7.3	543.2773	542.2700	0.1	0.94	Good	P24820 VP3_ARMV	BA, CAL
EVHVQ	21.7 – 22.4	611.3140	610.3075	-1.2	5.10	Good	Q43593 STAD_OLEEU	MA
FNRFD	29.2 – 30.0	698.3256	697.3184	-0.1	6.40	Good	Q9M7R0 ALL8_OLEEU	LS
GAGGI	2.2	374.2048	373.1961	3.9	3.66	Poor	A4GFC0 PROCE_OLEEU	CB
GFDEQVMELRK	29.3 – 29.8	1351.6691	1350.6602	1.2	4.32	Good	Q43593 STAD_OLEEU	CB, KH
GNEVL	9.0 – 10.6	531.2759	530.2700	-2.6	0.92	Good	Q94G86 ALL9_OLEEU	BO, KA, RJ, RE
GTVLAL	23.1 – 23.9	375.3578	572.3533	-4.8	3.63	Poor	Q43593 STAD_OLEEU	CB, CO, CR, KH, LS, MJ, MA, NA, RJ, SA
IEQGL	10.0 – 11.4	559.3080	558.3013	-1.1	0.92	Good	A4GE44 PROBR_OLEEU	BO, RJ
IIQPV	20.4 – 20.7	569.6368	568.3584	-3.2	3.66	Poor	B2VPR8 AL11B_OLEEU D8VPP5 AL11A_OLEEU	BA, BO, KH, LS, RE, VE

KGPGA	40.2 – 40.6	429.2453	428.2383	-0.6	9.91	Good	B2VPR8 AL11B_OLEEU D8VPP5 AL11A_OLEEU	BA, KH, LS, VE
KVSMMPA	15.2 – 15.5	632.3398	631.3363	-5.9	9.91	Good	Q43593 STAD_OLEEU	CB
LASNPN	3.7 – 4.2	615.3094	614.3024	-0.4	3.71	Poor	Q94G86 ALL9_OLEEU	BO, KA, OC, RJ, RE,
LEQGL	10.0 – 11.4	559.3080	558.3013	-1.1	0.92	Good	A4GDQ8 PROAL_OLEEU A4GDR9 PROAY_OLEEU A4GE39 PROBP_OLEEU	BO, OC, RJ
NFSSVAQ	17.0 – 17.5	752.3560	751.3500	-1.7	3.28	Poor	Q43593 STAD_OLEEU	CB, KH
NPSGAKSRM GA	22.9 – 23.1	1075.5273	1074.5240	-3.7	11.39	Good	P24820 VP3_ARMV	CN, MJ
PREVHVQ	25.9 – 26.1	864.4667	863.4613	-2.1	8.25	Good	Q43593 STAD_OLEEU	KH
QAGIY	11.9 – 13.5	551.2816	550.2751	-1.4	3.61	Poor	P24819 POL2_ARMV	BO, CA, CN, CB, CO, CAL, CR, KA, KH, LS, MJ, NA, OC, PI, RJ, RE, SA
QDDNL	36.5 – 37.8	604.2599	603.2500	4.4	0.54	Good	Q43593 STAD_OLEEU	CB
RLGDY	2.7 – 3.1	623.3142	622.3074	-0.8	6.34	Good	A4GDQ8 PROAL_OLEEU A4GDS9 PROBC_OLEEU A4GDT4 PROBG_OLEEU A4GE39 PROBP_OLEEU A4GE50 PROBW_OLEEU A4GFB9 PROCD_OLEEU A4KA54 PROCN_OLEEU P0DKF8 PROAQ_OLEEU	BA, BO, CA, CB, CO, CR, KA, KH, NA, OC, PI, RJ, RE, SA, VE
SKPPQ	2.7 – 3.7	556.3083	555.3016	-1.1	9.86	Good	P24820 VP3_ARMV	MA
TYDVGLL	22.5 – 22.9	780.4106	779.4065	-4.0	0.69	Poor	Q94G86 ALL9_OLEEU	BA, CO, CR, MJ, NA, RJ, RE, SA
VPGDQ	5.8 – 6.2	515.2457	514.2387	-0.6	0.76	Good	B2VPR8 AL11B_OLEEU D8VPP5 AL11A_OLEEU Q43593 STAD_OLEEU Q94G86 ALL9_OLEEU	BA, KH, LS, VE

* Determined using Innovagen's peptide property calculator.

** Abbreviations: Barnea (BA), Bouteillan (BO), Caballo (CA), Cañivano negro (CN), Cobrancosa (CB), Corbella (CO), Cordobeses de Arroyo de la luz (CAL), Cornicabra (CR), Kalokerida (KA), Khalkhali (KH), Lechín de Sevilla (LS), Majhol (MJ), Manzanilla (MA), Nevado azul (NA), Ocal (OC), Picual (PI), Racimal de Jaén (RJ), Reixonenca (RE), Sayfi (SA), Verdiell (VE)

Figure S2. Heatmap representation of correlation coefficients of the 40 peptides identified in olive seed hydrolysates with the capacity to reduce the micellar cholesterol solubility, bind bile acids, inhibit the pancreatic cholesterol esterase, and inhibit the HMG R.



Correlations coefficients were calculated on Pearson's correlation. Blue indicates positive correlation and red negative correlation.