Supplementary Material

BIOACTIVE EXTRACTS FROM PERSIMMON WASTES: INFLUENCE OF EXTRACTION CONDITIONS AND RIPENESS

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Figure S1. Photos of the persimmon fruits at the two ripeness stages: A) Immature (I) and B) Mature (M)

Table S1. *C.elegans*-specific gene expression probes used for qPCR analyses*.

PrimeTime qPCR Probe Assays (Integrated DNA Technologies, Inc)			
***C.elegans* gene**	**Fw sequence (5' – 3')**	**Rv sequence (5' – 3')**	**Probe sequence (5' – 3')**
fat-5	CGTGTAGAAGGCGATGAAGG	GATTTGTACGAGGATCCGGTG	/56FAM/ACGACTGGA/Zen/ATGAAGGTGGGCA/3IABkFQ/
fat-7	ACAAGTTAAGGAGCATGGAGG	TCTCCTTCCAGAAATAAACGGG	/56-FAM/CAGAGAAAG/Zen/CACTATTTCCCACTGGTCA/3IABkFQ/
acox-1	AGCAAACTGGAAAGCGTAGG	AACAAGATACTCGGCAGTGAG	/56FAM/CGGGCGATG/Zen/AATGGGAAGAGACG/3IABkFQ/
maoc-1	CAGTAACCAATGTTTGTCCTGG	TCATGGATTGTGCAGTCTGG	/56FAM/CTGCTTGGG/Zen/CTGGAAATGATTCTGAC/3IABkFQ/
TaqMan® Gene Expression Assays (Thermo Fisher Scientific)			
***C.elegans* gene**	**TaqMan reference probe**	**Assay**	
daf-2	Ce02444349_m1	Made to order	
daf-16	Ce02422838_m1	Inventoried	
skn-1	Ce02407447_g1	Inventoried	
pmp-3 *	Ce02485188_m1	Inventoried.	

* The expression level of each gene was normalized comparing to the expression of the *pmp3* as housekeeping gene control.

Table S2. Analysis of variance for the predicted quadratic polynomial models for TPC and antioxidant capacity of extracts from persimmon fruit.

		IPE				MPE			
		TPC (mg GAE/g)		TEAC (μmol TE/g)		TPC (mg GAE/g)		TEAC (μmol TE/g)	
Source	d.f.	SS	p-value	SS	p-value	SS	p-value	SS	p-value
Model	9	1122.3	0	17267.9	0.088	260.552	0	22361.7	0
Linear	3	198.39	0.004	4991.8	0.159	174.132	0	16362.4	0
X_1 S/L	1	32.35	0.07	469.9	0.455	7.655	0.021	2101.3	0.002
$X_{2\ E/W}$	1	165.84	0.001	4137.1	0.044	30.633	0	2465.9	0.001
$X_{3\ T}$	1	0.21	0.875	384.8	0.498	135.844	0	11795.2	0
Square	3	679.67	0	4104.2	0.219	58.366	0	2156.4	0.016
X_1X_1	1	92.92	0.001	15.2	0.954	0.869	0.688	26.4	0.463
X_2X_2	1	505.1	0	4083.7	0.047	5.516	0.141	111.7	0.603
X_3X_3	1	81.65	0.009	5.3	0.936	51.981	0	2018.4	0.003
2-Way interaction	3	244.21	0.002	8171.9	0.058	28.055	0.003	3842.9	0.002
X_1X_2	1	126.77	0.002	2599.1	0.098	0.808	0.396	1.4	0.92
X_1X_3	1	3.16	0.54	2223.6	0.122	5.141	0.049	2232.4	0.002
X_2X_3	1	114.28	0.003	3349.2	0.065	22.105	0.001	1609.1	0.005
Lack of fit	5	34.93	0.592	6479.1	0.052	7.649	0.134	735.1	0.37
Pure error	5	43.43		1308.5		2.64		538	
Total	19	1200.7		25055.5		270.841		23634.8	
R^2		0.9347		0.6892		0.962		0.9461	
R^2-adj		0.876		0.4095		0.9278		0.8977	

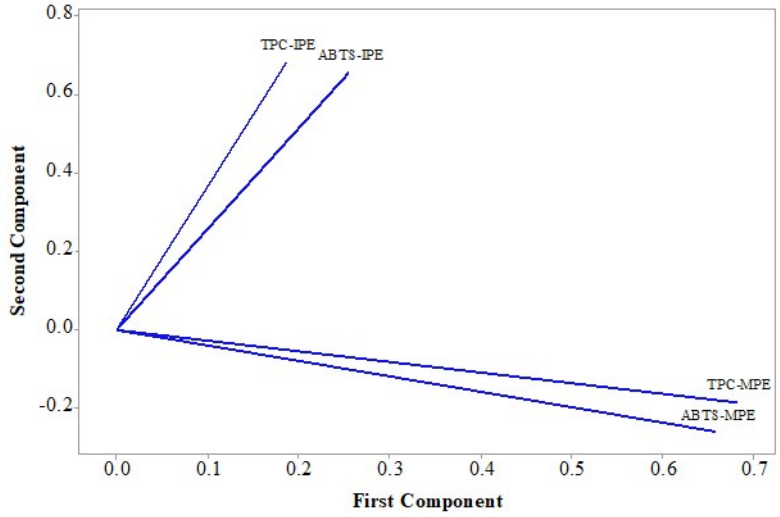
Principal Components Analysis (PCA)

A PCA was carried out to evaluate the variance related to the extraction conditions and the results are presented as scatterplots showing the relationship between the factors and variables (score plot A) and samples (loading plot B) in Figure S2. If variables are highly related, they can be incorporated into a component that accounts for the most variance in the observations. The second component explains the second highest amount of variance and will not be correlated to the first component [1].

The multivariate treatment of the data obtained for the samples permitted the reduction of the variables to two principal components, which together explained 88.5 % of the total variability. The first axis accounted for 58.8% and the second axis for 26.7%. According the factor loadings plot (Fig. S2b), the bioactive extracts from the mature fruits-MPE (TPC and antioxidant activity), were positively correlated to the PC1 axis which was highly represented by temperature, whereas the IPE extracts were positively correlated to the PC2 axis highly correlated to E/W(%) ratios and thus, supporting the differences between factors evaluated over the effect of extraction process of polyphenol content which was also significantly affected by the maturity stage of the fruit. Phenolic compounds extracted at low temperatures, and high E/W (%) (8, 17, 3) were rated low in TPC, indicating a high amount of polyphenolic compounds easily extracted by polar solvents. However, the center points conditions, had the best values allowing higher extraction degree of phenolic compounds and indicating that the E/W of 75 % with moderate temperatures, were the best conditions for IPE. Similarly, this E/W ratio was also reported as the best in polyphenolic extraction from propolis [2].

In summary, the discrimination of samples based on the scatter-plots highlighted a correlation with the response surface methodology results commented on in the manuscript.

a)



b)

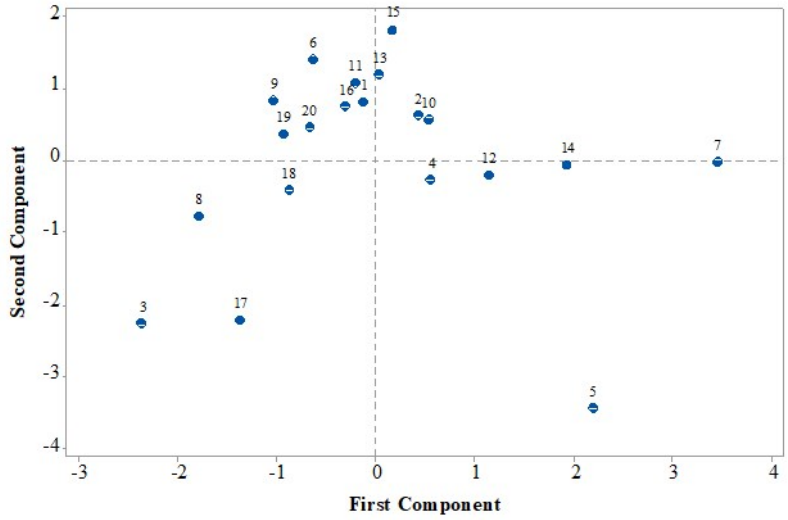


Figure S2. a) Principal component analysis of the extracted samples for TPC and antioxidant activity (for the codes of the samples, see Table 1) b) Factor loadings for principal component analysis.

Carbohydrate composition

Table S3. Monosaccharide composition of the persimmon extracts.

mg/g extract	Fuc	Rha	Ara	Gal	Glc	Xyl	GalA
lab-scale IPE	2±1	4±1	15±2	12±2	286±27	25±9	155±31
Up-scaled IPE	<2	<2	3±0	<2	459±15	<2	<2

Fuc: Fucose, Rha: rhamnose, Ara: arabinose, Gal: galacose, Glc: glucose, Xyl: xylose, GalA: Galacturonic acid. Mannose and Glucuronic acid were not detected or detected in trace amounts.

Characterization of the polyphenolic profile of the persimmon extracts

Table S4 compiles the different phenolic compounds identified in the lab scale and upscaled immature persimmon extract. Quantification of gallic acid and naringenin was performed with the response of the corresponding pure standards at 280 nm. Tentatively identified peaks (2-11) were quantified with the response of pure standards of their aglycones. Vanillic acid hexoside, naringin and hesperidin were quantified at 280 nm with the response of vanillic acid, naringenin and hesperetin, respectively (Figures S3-S4). Galloyl hexoside was quantified at 254 nm with the response of gallic acid (Figure S5). Quercetin glycosides (two hexosides) were quantified at 350 nm with the response of quercetin, while kaempferol glycosides (two hexosides and two hexoside-gallates) were quantified with the response of kaempferol (Figures S6 and S7).

Table S4. UPLC-DAD-MS information of phenolic compounds detected in the persimmon extracts (IPE) obtained at lab scale (A) and industrially upscaled (B).

Peak	Tentative identification	RT (min)	Extracts	UV-vis (nm)	[M-H]- *m/z*	MS^2
1	Gallic acid	3.7	A, B	270	169	125 (100%), 79 (20%)
2	Galloyl hexoside	7.6	B	254	331	169 (100%)
3	Vanillic acid hexoside	12.2	A, B	280	329	167 (100%), 191 (30%)
4	Naringin	16.5	B	283	579	271 (100%), 170 (30%), 296 (20%)
5	Hesperidin	16.9	B	284	609	301 (100%)
6	Quercetin hexoside I	17.0	A, B	254, 353	463	301 (100%)
7	Quercetin hexoside II	17.1	A, B	254, 353	463	301 (100%)
8	Kaempferol hexoside I	17.8	A, B	266, 352	447	285 (100%), 227 (20%)
9	Kaempferol hexoside II	18.2	A, B	266, 352	447	285 (100%)
10	Kaempferol hexoside gallate I	18.7	B	268, 352	599	285 (100%)
11	Kaempferol hexoside gallate II	19.0	B	268, 352	599	285 (100%)
12	Naringenin	22.9	B	280	271	151 (100%), 119 (30%)

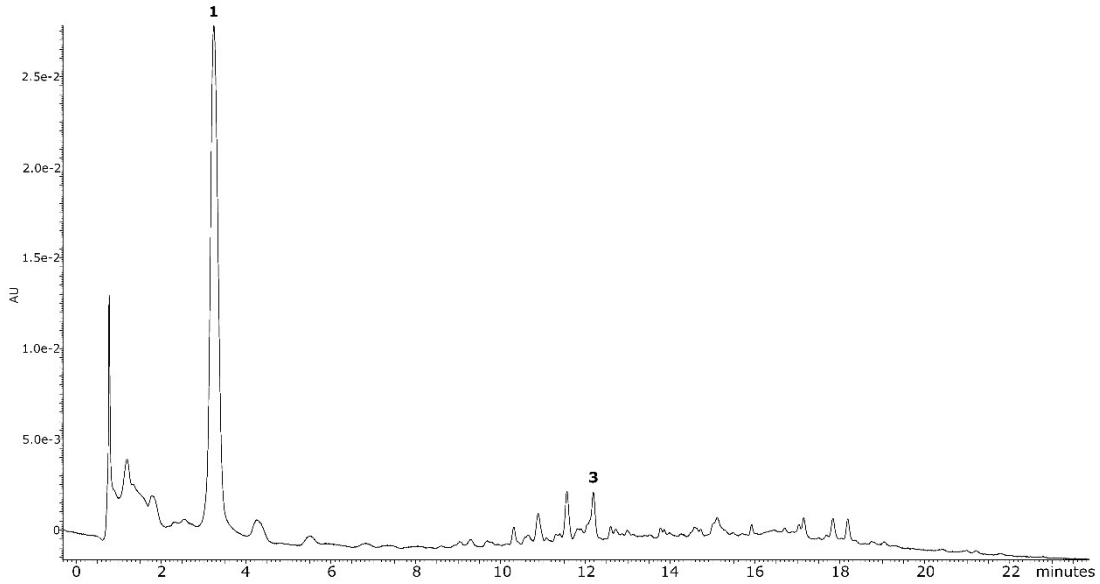


Figure S3. UPLC chromatogram of lab scale IPE (extract A) at 280 nm. For peaks identification see Table S4.

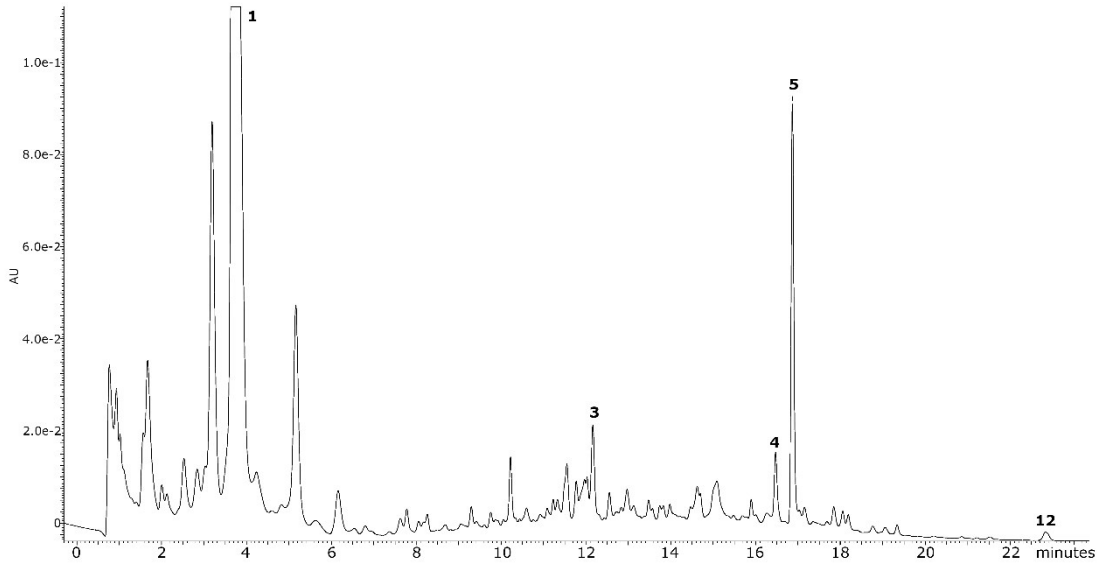


Figure S4. UPLC chromatogram of upscaled IPE (extract B) at 280 nm. For peaks identification see Table S4.

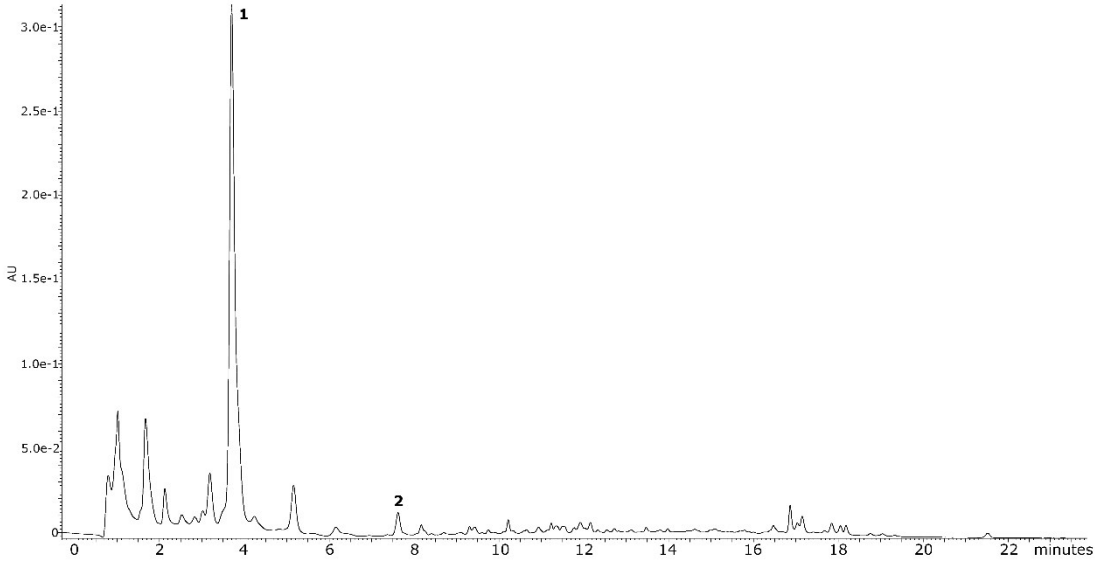


Figure S5. UPLC chromatogram of upscaled IPE (extract B) at 254 nm. For peaks identification see Table S4.

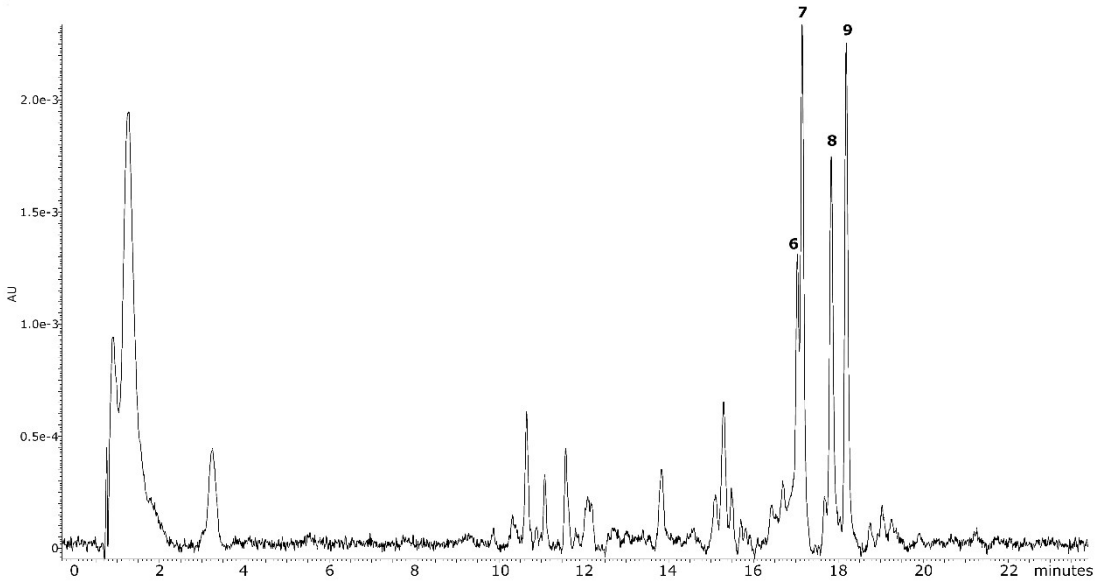


Figure S6. UPLC chromatogram of lab scale IPE (extract A) at 350 nm. For peaks identification see Table S4.

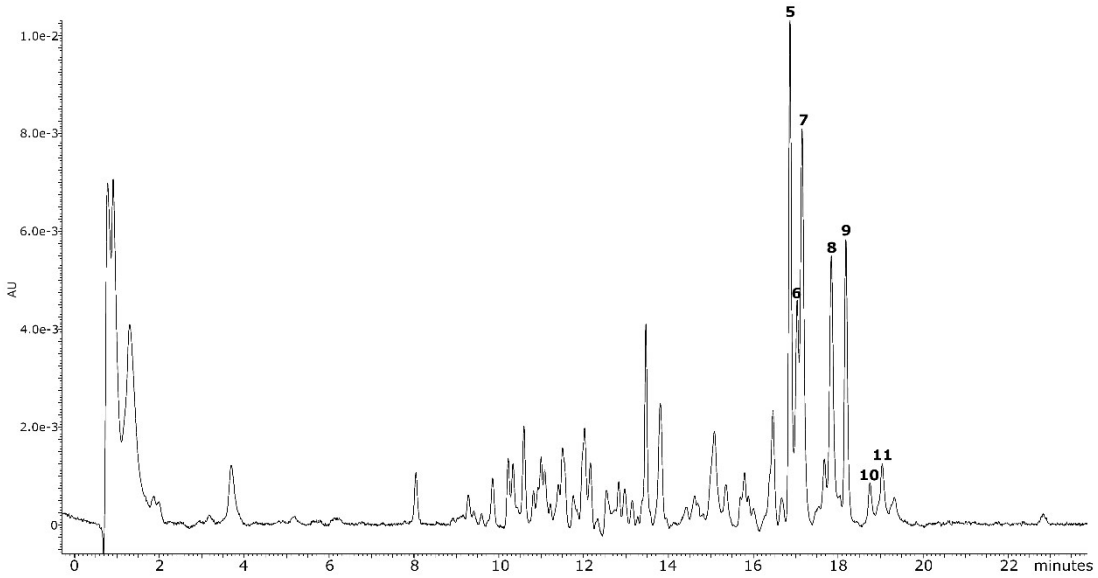


Figure S7. UPLC chromatogram of upscaled IPE (extract B) at 350 nm. For peaks identification see Table S4.

Genotoxicity screening assay

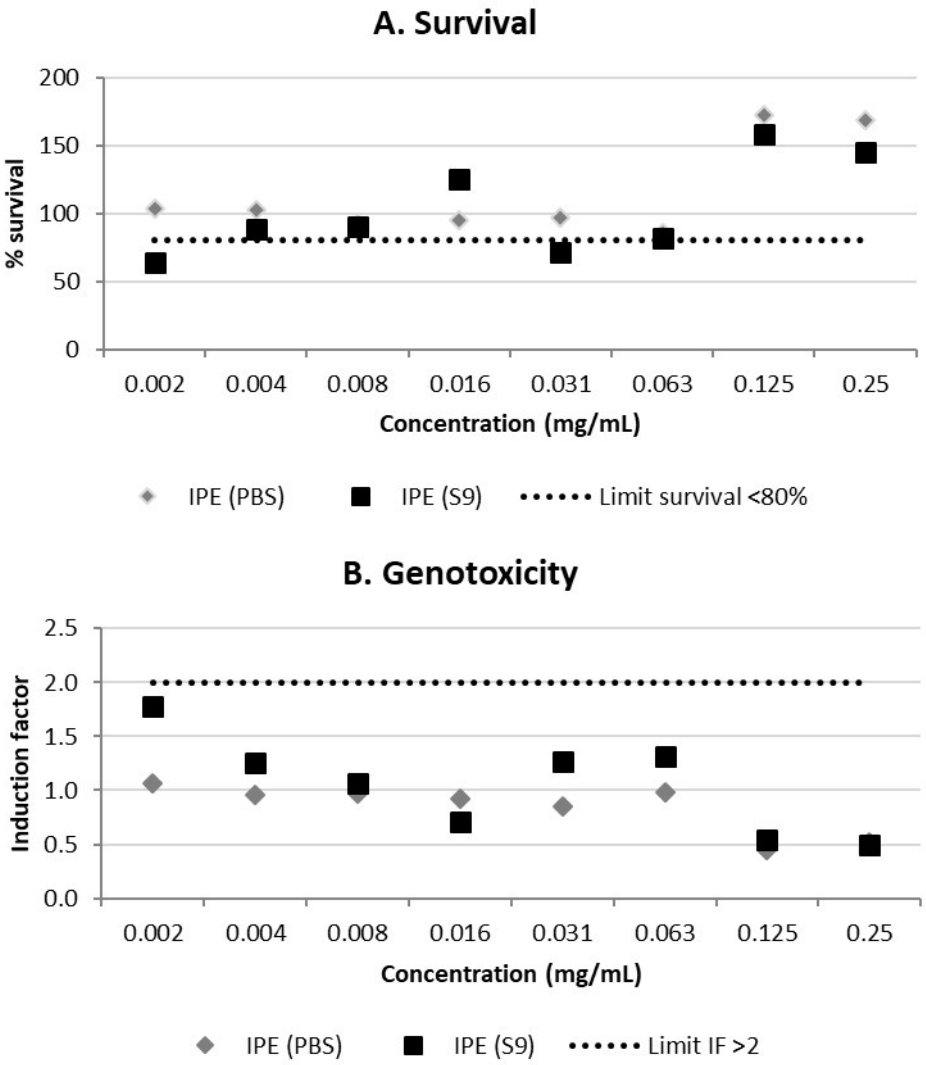


Figure S8. Results from SOS/umu test with (black) o without S9 (grey) activation. A) Bacterial survival is shown as percentage. Concentrations are considered non-toxic if survival is > than 80%. B) Genotoxicity. A compound is considered genotoxic if the induction factor is ≥2 at non-toxic concentrations for the bacteria in any of the conditions tested.

References

1 J. M. Lattin, J. D. Carroll, P. E. Green and P. E. Green, *Analyzing multivariate data*, Thomson Brooks/Cole, Pacific Grove, CA, 2003.

2 C. Sun, Z. Wu, Z. Wang and H. Zhang, *Evidence-based Complementary and Alternative Medicine*, , DOI:10.1155/2015/595393.