

Supplementary Materials for Almond Skin Extracts and Chlorogenic Acid Delay Chronological Aging and Enhanced Oxidative Stress Response in Yeast

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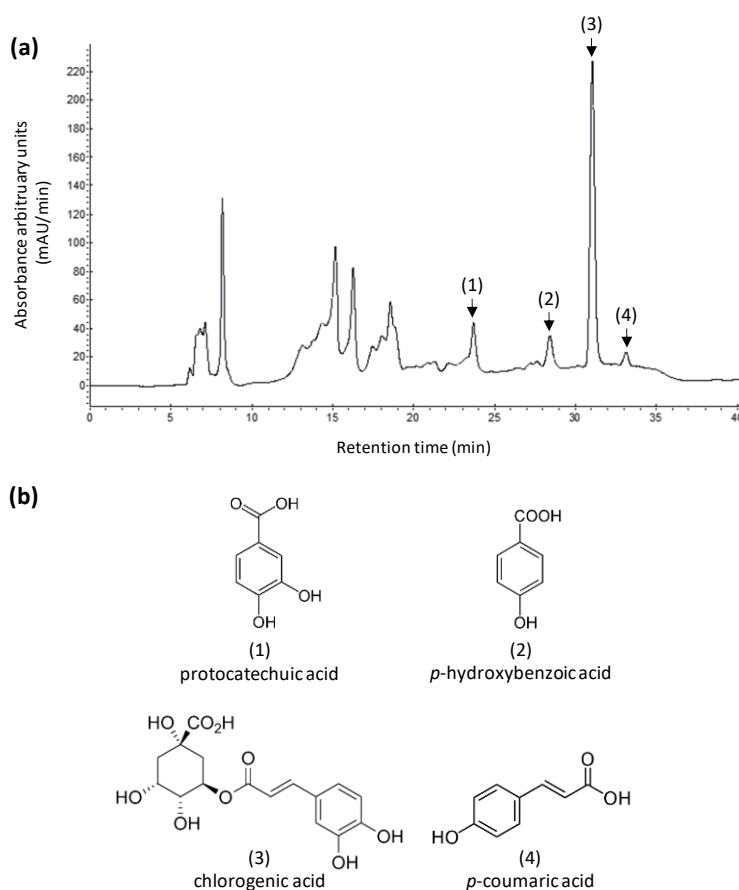


Figure S1. (a) HPLC chromatogram (detection set at 325 nm) of the almond skin extract (*Beldi* genotype grown in the Ain Sfa (34°46'42.4''N, 002°09'28.9''W) pilot location in the eastern Morocco) prepared by ultrasound-assisted extraction USAE. (b) Structures and corresponding numbers on the HPLC chromatogram of the main phenolic compounds considered in this study: protocatechuic acid (1), *p*-hydroxybenzoic acid (2), chlorogenic acid (3) and *p*-coumaric acid (4).

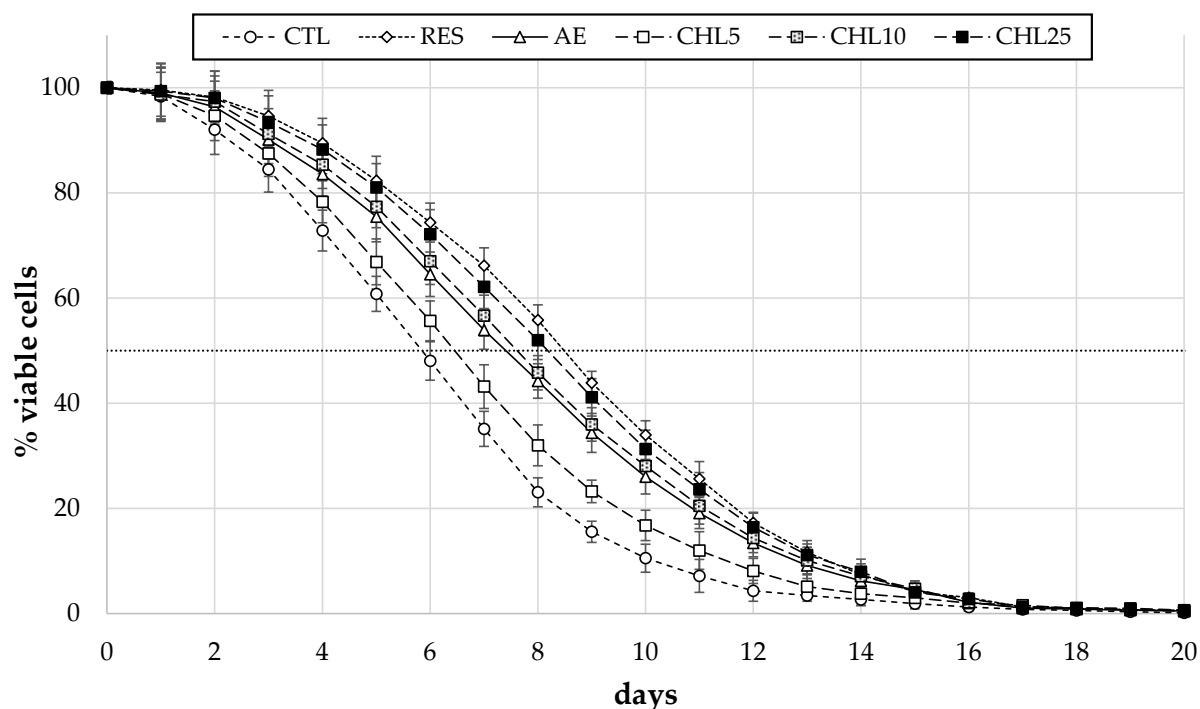


Figure S2. Survival plots used to determine chronological lifespan of yeast (strain DB746) presented in Figure 1a. percentage of viable cells was determined by the microcolony method on YPD plates as described by Hu et al. [27]. Values are means \pm standard deviations (SD) of 6 independent experiments.

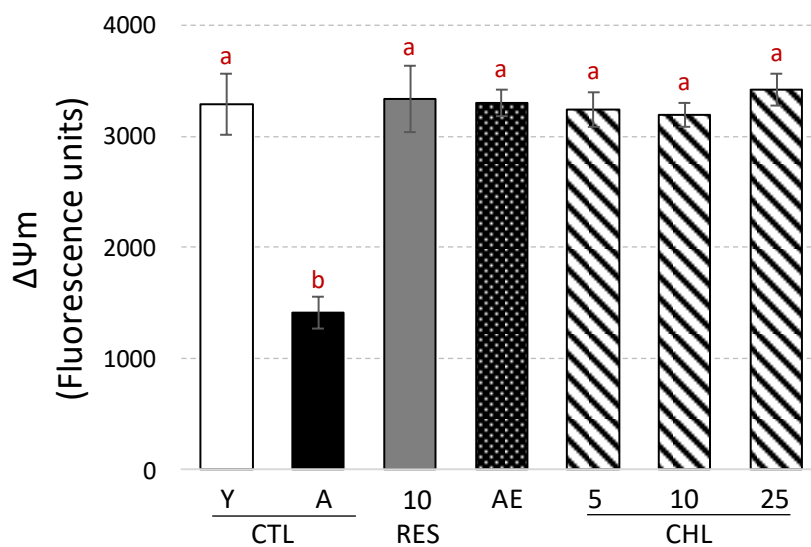


Figure S3. Mitochondria integrity estimated by mitochondrial potential ($\Delta\Psi_m$) variation.

The evaluation of mitochondria membrane potential ($\Delta\Psi_m$) was carried out by treating cells with 3,30-dihexyloxycarbocyanineiodide (DiOC6(3), Sigma-Aldrich, Saint-Quentin Fallavier, France). DiOC6(3) stains mitochondria depending on their $\Delta\Psi_m$. Cells were incubated in culture medium with 25 nM DiOC6(3) for 40 min at 28°C. Results were expressed as relative fluorescent units. Values are means \pm SD of 6 independent replicates. Different letters represent significant differences between the different conditions ($p < 0.05$).

Table S1. Absolute quantification of chlorogenic acid (and other phenolic acids) contents in US almond skin extract ²(in mg/g DW) and final concentration (in μM) applied on yeast.

	TPC ¹	Protocatechuic Acid	<i>p</i> -hydroxybenzoic Acid	Chlorogenic Acid	<i>p</i> -coumaric Acid
US extract (mg/g DW)	13.86 \pm 0.91	2.03 \pm 0.07	1.13 \pm 0.02	8.14 \pm 0.10	0.26 \pm 0.20
Concentration applied on yeast (in μM)	88.00 \pm 5.78	14.15 \pm 0.49	8.83 \pm 0.16	25.00 \pm 0.30	1.58 \pm 1.31

US: ultrasound; ¹TPC: total phenolic content expressed in gallic acid equivalent; other phenolic acids from the extract are: protocatechuic acid, *p*-hydroxybenzoic acid and *p*-coumaric acid contents. ² *Beldi* genotype grown in the Ain Sfa (34°46'42.4"N, 002°09'28.9"W) pilot location in the eastern Morocco. Values are means \pm SD of 3 independent replicates.

Table S2. Growth index and viability of yeast cells under the different treatment conditions determined 48h after treatment.

Conditions	Growth index	Viability
CTL	34.25 \pm 1.23 ^{ab}	95.37 \pm 2.61 ^a
RES (10 μM)	34.04 \pm 1.72 ^{ab}	94.27 \pm 2.01 ^a
AE	36.12 \pm 0.67 ^a	94.45 \pm 1.85 ^a
CHL (5 μM)	33.47 \pm 0.32 ^b	94.24 \pm 1.78 ^a
CHL (10 μM)	33.88 \pm 0.19 ^b	96.58 \pm 2.92 ^a
CHL (25 μM)	33.38 \pm 1.22 ^{ab}	96.30 \pm 3.04 ^a

Almond extract (AE, 1 mg/mL); Chlorogenic acid at 3 concentrations (CHL5, CHL10 and CHL25 corresponding to chlorogenic acid addition at 5, 10 and 25 μM , respectively). *E*-Resveratrol (RES, 10 μM) used as control antiaging drug. Values are means \pm standard deviations (SD) of 4 independent experiments. Different letters represent significant differences between the different conditions ($p < 0.05$).

Table S3. Estimation of chlorogenic acid and *E*-resveratrol uptake by yeast cell determined 6h after their additions in culture medium.

Compound	Concentrations/ Conditions	Relative Content in Culture Medium	Relative content in Yeast Cells	Total
<i>E</i> -Resveratrol	10 μM	9.43 \pm 2.59	80.40 \pm 1.81	89.83 \pm 4.40
Chlorogenic acid	AE 1	19.20 \pm 1.97	70.77 \pm 3.56	89.97 \pm 5.53
Chlorogenic acid	5 μM	21.10 \pm 2.41	70.70 \pm 0.53	91.80 \pm 2.94
Chlorogenic acid	10 μM	23.47 \pm 1.01	71.73 \pm 1.47	95.20 \pm 2.48
Chlorogenic acid	25 μM	16.60 \pm 1.11	74.67 \pm 3.90	91.27 \pm 5.01

Values are means \pm standard deviations (SD) of 4 independent experiments.

Table S4. Intracellular concentrations of NAD and NADH.

Conditions	NAD	NADH	NAD/NADH
CTL	1.56 ± 0.12 ^a	0.93 ± 0.13 ^a	1.70 ± 0.28 ^a
RES (10 µM)	1.59 ± 0.17 ^a	0.98 ± 0.14 ^a	1.63 ± 0.16 ^a
AE	1.46 ± 0.18 ^a	0.84 ± 0.16 ^a	1.77 ± 0.32 ^a
CHL (5 µM)	1.48 ± 0.17 ^a	0.91 ± 0.06 ^a	1.62 ± 0.10 ^a
CHL (10 µM)	1.59 ± 0.15 ^a	0.90 ± 0.19 ^a	1.84 ± 0.48 ^a
CHL (25 µM)	1.62 ± 0.16 ^{ab}	1.00 ± 0.12 ^a	1.62 ± 0.13 ^a

Values are means ± standard deviations (SD) of 4 independent experiments; expressed in mM for 10⁷ cells.



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