

Supplementary information

Functional potential of crackers formulated with an innovative fermented cooked chickpea flour

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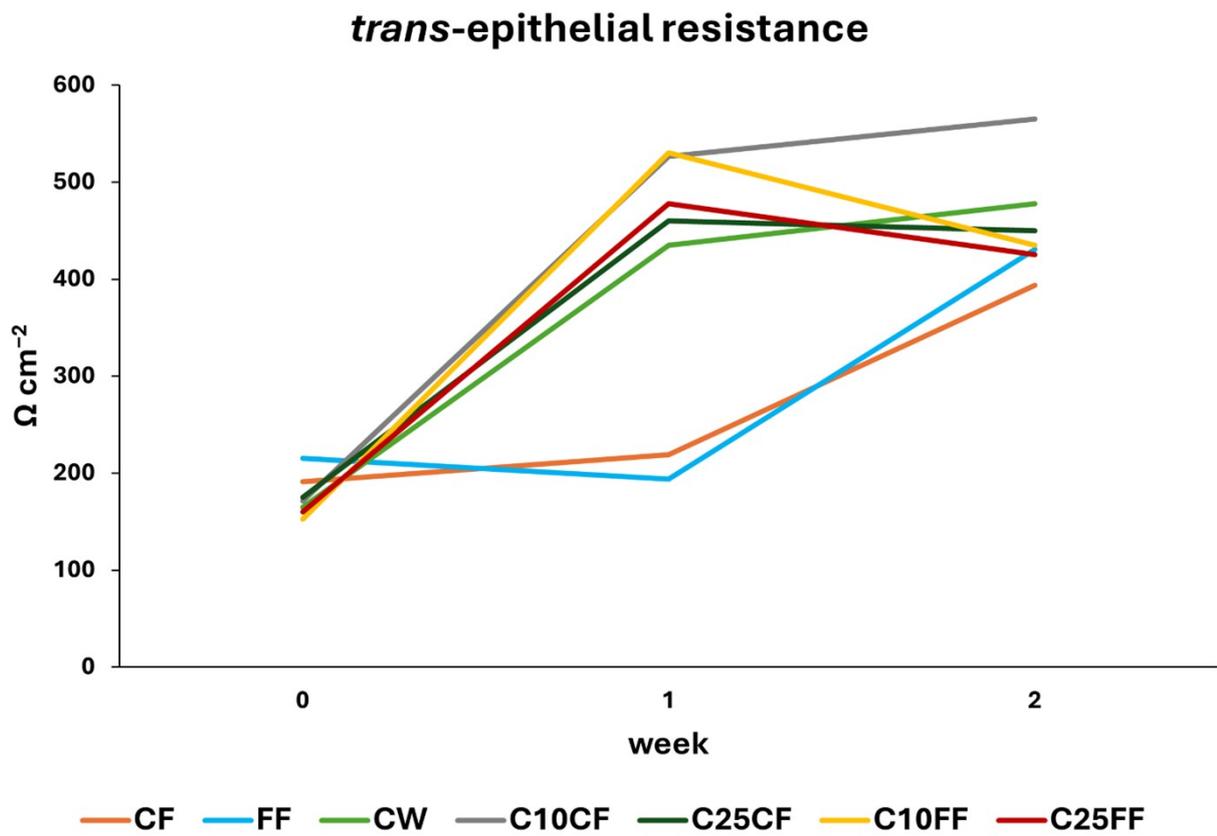
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g. S1. Trans-epithelial electrical resistance measured over two weeks on the Caco-2 cellular line of cooked chickpea flour (CF) (orange line), cooked fermented chickpea flour (FF) (blue line), 100% wheat-based cracker (CW) (green line), 10% cooked chickpea flour-enriched cracker (C10CF) (grey line), 25% cooked chickpea flour-enriched cracker (C25CF) (dark green line), 10% cooked fermented chickpea flour-enriched cracker (C10FF) (yellow line), and 25% cooked fermented chickpea flour-enriched cracker (C25FF) (dark red line). Data were expressed as $\Omega \text{ cm}^{-2}$ per two well (mean value).

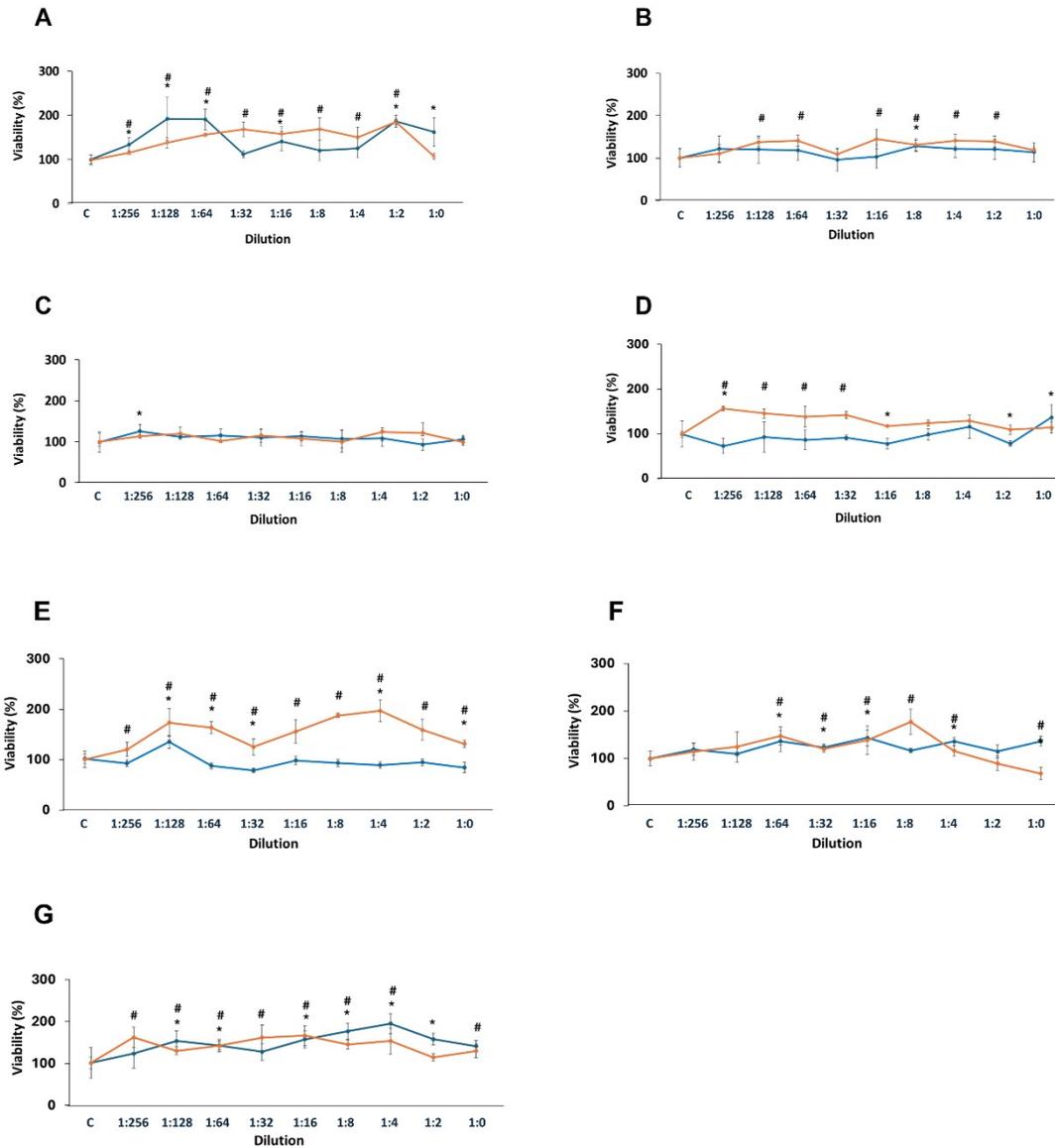


Fig. S2. Dilution–effect curves of cooked chickpea flour (A), cooked fermented chickpea flour (B), 100% wheat-based cracker (C), 10% cooked chickpea flour-enriched cracker (D), 25% cooked chickpea flour-enriched cracker (E), 10% cooked fermented chickpea flour-enriched cracker (F), 25% cooked fermented chickpea flour-enriched cracker (G) intestinal digests in Caco-2 cells after 24 h (blue lines) and 48 h (orange line) of exposure. The results are expressed as viability (%) of 8 replicates (mean \pm SD). * Indicated significant difference as compared to positive control (C) values after 24 h whereas # indicated significant difference as compared to control values after 48 h assessed by t-Test ($p < 0.05$).

Table S1. Nutritional composition and moisture content of conventional cracker 100% wheat-based cracker (CW), 10% cooked chickpea flour-enriched cracker (C10CF), 25% cooked chickpea flour-enriched cracker (C25CF), 10% cooked fermented chickpea flour-enriched cracker (C10FF), and 25% cooked fermented chickpea flour-enriched cracker (C25FF). The nutritional composition was calculated considering the composition of each ingredient, as reported on product labels and in the CREA Food and Nutrition database (CREA. Tabelle di Composizione degli Alimenti). The moisture content (mean±SD) was assessed experimentally by lyophilization. Data are expressed per 100 g of baked product.

	CW	C10CF	C25CF	C10FF	C25FF
Energy (kcal)	368.0 (1540.4 kJ)	357.0 (1494.4 kJ)	362.0 (1515.3 kJ)	359.0 (1502.8 kJ)	362.0 (1515.3 kJ)
Protein (g)	11.9	12.5	14.1	12.5	14.1
% En. from protein	12.9%	14.0%	15.6%	14.0%	15.6%
Fat (g)	0.8	1.3	2.2	1.3	2.2
of which saturated (g)	0.0	0.3	0.4	0.3	0.4
Carbohydrates (g)	77.3	72.2	68.9	72.5	69.0
of which sugars (g)	1.8	2.0	2.3	2.0	2.3
Dietary fiber (g)	2.4	3.5	5.2	3.5	5.2
Salt (g)	0.5	0.5	0.5	0.5	0.5
Moisture (g)	7.7±0.1	7.7±0.1	5.2±0.1	7.4±0.1	5.2±0.1
Sum nutrients + moisture (g)*	100	98	96	98	96

* In CW, the sum accounted for 100%, whereas it was 98% and 96% in crackers containing 10% and 25% chickpea flour, respectively. The slight discrepancies are attributable to rounding procedures, the use of food composition database values, and minor components not included in the calculation.

Table S2. Retention times and LOD-LOQ of polyphenols identified by HPLC UV/VIS.

Polyphenol	LOD (µg/mL)	LOQ (µg/mL)	Retention time (min)
Pyrogallol	0.02	0.06	5.3
Protocatechuic 4-O-glucoside	0.1	0.3	6.9
Sinapoyl D-glucoside	0.04	0.08	7.5
p-hydroxybenzoic acid	0.05	0.1	10.4
Vanillic acid	0.05	0.1	11.4
4- hydroxyphenyl acetic acid	0.01	0.03	11.5
Sinapic acid	0.04	0.08	15.5
Kampferol 3-O-glucoside	0.03	0.06	17.9
Ferulic acid	0.05	0.01	16.7

LOD: Limit of detection; **LOQ:** Limit of quantification

Table S3. Molecular formula, m/z, and retention time of polyphenols identified by High-Resolution Mass Spectrometry (LC-HMRS).

Compound	Molecular formula	Theoretical mass	Experimental mass	Retention time
p-hydroxybenzoic acid	C6H6O3	137.02442	137.02353	10.8
protocatechuic 4-O-glucoside	C13H16O9	315.07216	315.07242	6.1
4- hydroxyphenyl	C8H8O3	151.04007	151.03918	11.3

acetic acid sinapoyl D- glucoside	C17H22O10	385.11402	385.11376	7.4
Kampferol 3-O- glucoside	C21H20O11	447.09328	447.09372	18.0
Pyrogallol	C6H6O3	125.02442	125.02341	5.1

Table S4. Physicochemical and microbiological changes during 48h of fermentation of the chickpea puree.

	T0h	T48h
pH	6.2 ± 0.0	4.4 ± 0.04
LAB counts (log CFU/ml)	7.2 ± 0.1	9.0 ± 0.1
Glucose (g/L)	2.5 ± 0.4	2.1 ± 0.2
Fructose (g/L)	3.2 ± 0.2	1.3 ± 0.1
Lactic acid (g/L)	nd	6.2 ± 0.2
Acetic acid (g/L)	nd	0.8 ± 0.1
Ethanol (g/L)	nd	1.3 ± 0.1

nd: not detected