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Supporting Information for:

Kefir enriched with a carob (*Ceratonia siliqua* L.) leaves extract as a new ingredient during a gluten-free bread making process

By Umile Gianfranco Spizzirri,[1] Avazbek Abduvakhidov,[2] Paolino Caputo,[3] Pasquale Crupi,[4] Marilena Muraglia,[5] Cesare Oliviero Rossi,[3] Maria Lisa Clodoveo,[4] Francesca Aiello[1,*] Donatella Restuccia,[1]

[1]Dipartimento di Farmacia e Scienze della Salute e della Nutrizione, Dipartimento di Eccellenza 2018-2022, Università della Calabria, Rende (CS), Italy
[2]Dipartimento di Farmacia, Università degli Studi Federico II di Napoli, Napoli, Italy
[3]Dipartimento di Chimica e Tecnologie Chimiche, Università della Calabria & UdR INSTM della Calabria, Rende (CS), Italy
[4]Dipartimento Interdisciplinare di Medicina, Università degli Studi Bari, Bari, Italy
[5]Dipartimento di Farmacia-Scienze del Farmaco Università degli Studi di Bari, Bari, Italy
* Correspondence: author: Francesca Aiello, francesca.aiello@unical.it

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S1. Disposable phenolic groups by Folin-Ciocalteu procedure.

Briefly, 6.0 mL of aqueous solution of each carob leave extract or enriched kefir was placed in a volumetric flask (10 mL) and then Folin-Ciocalteu reagent (1.0 mL) was added and the contents of flask were mixed thoroughly. After 3 min, 3.0 mL of Na_2CO_3 (2% v/v) were added, and then the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm against a control prepared in the same conditions but without any extract. The value of total phenolic content (TPC) in the extracts was expressed as equivalents of gallic acid (GA) *per* gram of extract, by using the equation obtained from the calibration curve of the antioxidant molecule. This one was recorded by employing five different GA standard solutions. 0.5 mL of each solution were added to the Folin-Ciocalteu system to raise the final concentration of 8.0, 16.0, 24.0, 32.0, and 40.0 μM, respectively. After 2 h, the absorbance of the solutions was measured to record the calibration curve and the correlation coefficient (R^2), slope and intercept of the regression equation obtained were calculatedby the method of least square. For enriched kefirs and gluten-free breads, TPC values were expressed as CT equivalents per gram of sample. Each measure was performed in triplicate and data expressed as means (±SD). UV-Vis absorption spectra were recorded with a Jasco V-530 UV/Vis spectrometer (Jasco, Tokio, Japan).

S2. Phenolic Acid Content.

In a volumetric flask (10.0 mL) 1.0 mL of an aqueous solution of carob leave extract, 1.0 mL of HCl (0.5 mol L^{-1}), 1.0 mL of Arnov's reagent (sodium molybdate and sodium nitrite 0.1 mg mL^{-1}), 1.0 mL of NaOH (1.0 mol L^{-1}) and purified water were mixed. The absorbance of the solutions was measured by a spectrophotometer at 490 nm. The value of total phenolic content (TPC) in the extracts was expressed as equivalents of GA per gram of extract, by using the equation obtained from the calibration curve of the antioxidant molecule. This one was recorded by employing five different GA standard solutions. 0.5 mL of each solution were added to the Folin-Ciocalteu system to raise the final concentration of 20.0, 30.0, 40.0, 50.0, 60.0 and 70.0 μM, respectively. Each measurement was performed in triplicate and data expressed as means (±SD).

S3. Flavonoid Content.

In a volumetric flask (5.0 mL), 0.5 mL of an aqueous solution of carob leave extract and 0.150 mL of $NaNO_2$ (5.0 % w/v) solution were mixed. After 6 min, 0.300 mL of a 6.0 % (w/v) $AlCl_3$ was added and after 5 min, 1.0 mL of NaOH (1.0 mol L^{-1}) was also added to the mixture by immediately measuring the absorbance (510 nm) against a control solution. FC in each extract was expressed as milligrams of GA per gram of sample, by using a calibration curve of CT (Standard solutions concentration of GA equal to 10.0, 25.0, 50.0, 75.0, 100.0 μM). Each measurement was performed in triplicate and data expressed as means (±SD).

S5. Determination of scavenging activity on DPPH radicals.

In a standard protocol, 1.0 mL of aqueous solution of each carob leave extract, enriched kefir or gluten-free bread extract was placed in a volumetric flask (10 mL) and then 4.0 ml of water and 5.0 mL of ethanol solution of DPPH (200 μM) were added, obtaining DPPH final concentration of 100 μM. The solutions were incubated in a water bath at 25°C and, after 30 min, the absorbance of the remaining DPPH was colorimetrically determined at 517 nm. The scavenging activity of the samples was measured as the decrease in absorbance of the DPPH and it was expressed as percent inhibition of DPPH radicals calculated according the following equation (S1):

$$inhibition\% = \frac{A_0 - A_1}{A_0} \times 100 \quad \text{(S1)}$$

where, A_0 is the absorbance of a standard that was prepared in the same conditions, but without any sample, and A_1 is the absorbance of tested samples. Each measurement was carried out in triplicate and data expressed as means (±SD).

S6. Determination of scavenging effect on the ABTS radical cation.

ABTS was dissolved in water to reach 7.0 mM concentration. ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. Because ABTS and potassium persulfate react at a ratio of 1:0.5, this leads to an incomplete oxidation of the ABTS. Oxidation of the ABTS started immediately, but the absorbance was not maximal and stable until more than 6 hours. Finally, the concentration of the resulting ABTS.+ solution was adjusted to an absorbance of 0.970±0.020 at 734 nm. The radical was stable in this form for more than two days when stored in the dark at room temperature. In order to evaluate the scavenging effect of the carob leave extracts, enriched kefir and gluten-free bread extracts, 500 μL of an aqueous solution of each sample were mixed with 2.0 mL of the ABTS radical solution. The mixture, was then incubated in a water bath at 37°C and protected from light for 5 min. The decrease of absorbance at 734 nm was measured at the endpoint of 5 min. The scavenging activity was expressed as a percentage of scavenging activity on the ABTS radical according to equation (1). All samples were assayed in triplicate and data expressed as mean (±SD).

S7. Antioxidant capacity as Trolox equivalents.

To a fixed concentration of an antioxidant, $ABTS^{\cdot+}$ in a variable concentration was added. The concentration of $ABTS^{\cdot+}$ was varied from 0.0 to approximately 45.0 μM in several different incubations. After a 6 min incubation at 37°C, the absorbance at 734 nm was determined. The concentration of $ABTS^{\cdot+}$ was calculated, using a molar extinction coefficient of $1.5\ 10^4\ M^{-1}$. The reduction in $ABTS^{\cdot+}$ concentration was derived from the absorbance at 734 nm of the reference (only containing $ABTS^{\cdot+}$) and the incubation containing the fixed concentration of antioxidant plus the same concentration of $ABTS^{\cdot+}$. The reduction in $ABTS^{\cdot+}$ concentration, was plotted against the initial concentration of ABTS.+. The curve was fitted according to the exponential function $y = C\ (1 - e^{-bx})$ where y is the reduction in $ABTS^{\cdot+}$ concentration, x is the initial $ABTS^{\cdot+}$ concentration and C is the maximal amount of $ABTS^{\cdot+}$ scavenged by the antioxidant at the tested concentration. The Trolox equivalents antioxidant capacity (TEAC) was determined by dividing C by the concentration of antioxidant and by 1.9. The latter factor (1.9) is the number of molecules that can be scavenged per Trolox. TEAC of a compound is a relative value and has no dimension. All samples were assayed in triplicate and data expressed as means (±SD).