Supplementary Material

Coffee Silverskin Extract-Functionalised Pectin: A Sustainable Substrate to Obtain Chicken Meatballs with Antioxidant and Improved Sensory Properties

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The following section provides a comprehensive description of the methodologies employed for the functionalisation of the biopolymer pectin, the extraction of polyphenolic compounds from chicken meatballs and the spectrophotometric assays performed.

S1 Synthesis of antioxidant pectin conjugates

S1.1 Pectin functionalisation *via* grafting

The functionalisation procedure was performed according to the literature.[1] After complete solubilisation of pectin (High Methoxyl, 500 mg) in purified water, 250 mg of L-ascorbic acid and 12.5 mL of hydrogen peroxide (120v) were introduced into a suitable reaction flask. Under continuous magnetic stirring, after two hours, an amount of extract equivalent to 75 mg of gallic acid was added (calculated considering the availability of phenolic groups, as determined by the Folin-Ciocâlteau assay), previously solubilised in 12.5 mL of purified water. After twelve hours, the purification process was carried out, using dialysis membranes (MWCO: 12-14000 Da) in purified water, for 72 h. The resulting polymer **g**ROB1B was subjected to freeze-drying process (Micro Modulyo, Edwards, Italy), obtaining a dry, vaporous solid. It was stored at +4°C, waiting for characterisation and evaluation of antioxidant properties.

S1.2 Pectin functionalisation *via* enzymatic reaction

The procedure was carried out as reported in the literature,[2] with minor modifications. High methoxyl pectin (500 mg) and an extract amount equivalent to 75 mg gallic acid (calculated considering the availability of phenolic groups, as determined by the Folin-Ciocâlteau assay) were solubilised in 25 mL of distilled water, respectively, and then mixed in a reaction flask. Subsequently 1 g of porcine pancreatic lipase was added, and the mixture was maintained under magnetic stirring at 50°C, overnight. The purification process required precipitation of the conjugated polymer *via* addition of 50 mL of EtOH, followed by centrifugation at 4000 rpm, for fifteen minutes. After removal of the supernatant, the pellet was re-dispersed in 50 mL of purified water under magnetic stirring for ten minutes. The resulting suspension was centrifuged at 4000 rpm for five minutes, to recover the pellet (containing the enzyme), while the supernatant was collected and lyophilised to obtain a dry vaporous solid. The polymer **e**ROB1B was stored at +4°C for subsequent analysis.

S2 Extraction of polyphenols from chicken meatballs

S2.1 Extraction of polyphenols from raw chicken meatballs

Polyphenols from raw chicken patties were extracted following a modified version of the method reported by Al-Juhaimi et al. (2018).[3] After storage at +4°C for predetermined time (0, 5 and 10 days), each sample was freeze-dried. The resulting product was ground using a mortar and pestle and sieved through a 1mm mesh. 2.5 g of each powder was stirred in distilled water (20 mL) at 4°C (for 10h). The suspension was then centrifuged at 9000 rpm for 15 minutes, followed by vacuum filtration. The supernatant was stored at +4°C, until analysis.

S2.2 Extraction of polyphenols from cooked chicken meatballs

Extraction of polyphenols from cooked chicken meatballs was performed, as reported by Devatkal et al. (2010),[4] with some modifications. Each cooked patty was homogenised and placed in a reaction flask with the addition of 25mL of 70% aqueous acetone. The resulting suspension was stirred magnetically at +4°C (for 10h), after which it was filtered under vacuum and the solvent was removed using a rotary evaporator. The concentrated extract was stored at +4°C until further analysis**.**

S3 Antioxidant properties of extracts, conjugates and chicken meatball

S3.1 Determination of the total polyphenol content

With the aim of determining the total polyphenol content of the samples, the Folin-Ciocâlteau assay was carried out according to the methods reported in the literature.[5] To 6 mL of each aqueous sample at a known concentration, 1 mL of Folin-Ciocâlteau reagent was added. After three minutes, 3 mL of a 7,5% (w/w) aqueous Na_2CO_3 was added. The mixture was incubated in the dark for two hours, after which absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Thermo Scientific, Italy). A calibration curve was established using gallic acid solutions of known concentrations, and the regression line was calculated via the least squares method, with assessment of the coefficient of determination (R^2). The total polyphenol content was expressed as milligram gallic acid equivalents per gram of extract (mg GAE/g extract).

S3.2 Determination of total flavonoid content

Quantification of total flavonoids was performed using a colorimetric assay based on the method of Aiello et al. (2023)[6] To 0.5 mL of each aqueous extract, 2 mL of distilled water and 0.15 mL of 15% (w/w) aqueous $NaNO_2$ were added. Following six minutes of incubation in the dark, 0.15 mL of 10% (w/w) aqueous $AlCl_3$ was introduced, and the mixture was kept in the dark for an additional six minutes. Subsequently, 2 mL of 4% (w/w) aqueous NaOH and 0.2 mL of distilled water were added to achieve a final volume of 5 mL. After 15 minutes of incubation in the dark, absorbance was measured at 510 nm using a UV-Vis spectrophotometer. Calibration was performed using anhydrous catechin standards, and results were expressed as milligram catechin equivalents per gram of extract (mg CT/g extract).

S3.3 Scavenger activity against ABTS radical

The determination of the inhibition of the blue/green ABTS·+ radical, conducted in an aqueous medium, was carried out according to the literature, with minor modifications.[7] Aliquots of 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL and 0.5 mL of each sample at known concentration were diluted to a total volume of 0.5 mL with distilled water. Subsequently, 2 mL of an aqueous solution containing the ABTS·+ radical was added. The latter was previously prepared by solubilising 38 mg ABTS·+ and 5.6 mg ammonium persulphate in distilled water (10 mL), followed by sonication and overnight incubation. One millilitre of this stock solution was diluted with 35 mL distilled water for use in the assay. After six minutes of incubation in the dark, the absorbance was measured at 734 nm. The percentages of inhibition of radical activity were calculated using the formula:

$$Inhibition\ (\%) = \frac{A0 - A1}{A1} x\ 100$$

where A_0 represents the absorbance of the control (without extract), and A_1 represents the absorbance in the presence of the extract. Inhibition percentages were plotted against sample concentrations, and IC_{50} values were determined.

S3.4 Scavenger activity against DPPH radical

The evaluation of DPPH radical scavenging activity was conducted in organic medium, following the protocol of Carullo et al. (2022),[8] with modifications. Aliquots of 1 mL, 2 mL, 3 mL, 4 mL and 5 mL of an aqueous extracts at known concentration were adjusted to a final volume of 5 mL with distilled water. Subsequently, 5 mL of an ethanolic solution containing the DPPH radical (7.8 mg in 100mL of EtOH) was added. After twenty minutes in the dark, absorbance was measured at 517 nm. The percentages of inhibition of radical activity were calculated using the formula:

$$Inhibition\ (\%) = \frac{A0 - A1}{A1} x\ 100$$

where A_0 represents the absorbance of the control (without extract), and A_1 represents the absorbance in the presence of the extract. Inhibition percentages were plotted against sample concentrations, and IC_{50} values were determined.

S4 ^{1}H-NMR spectroscopy of CSS extracts

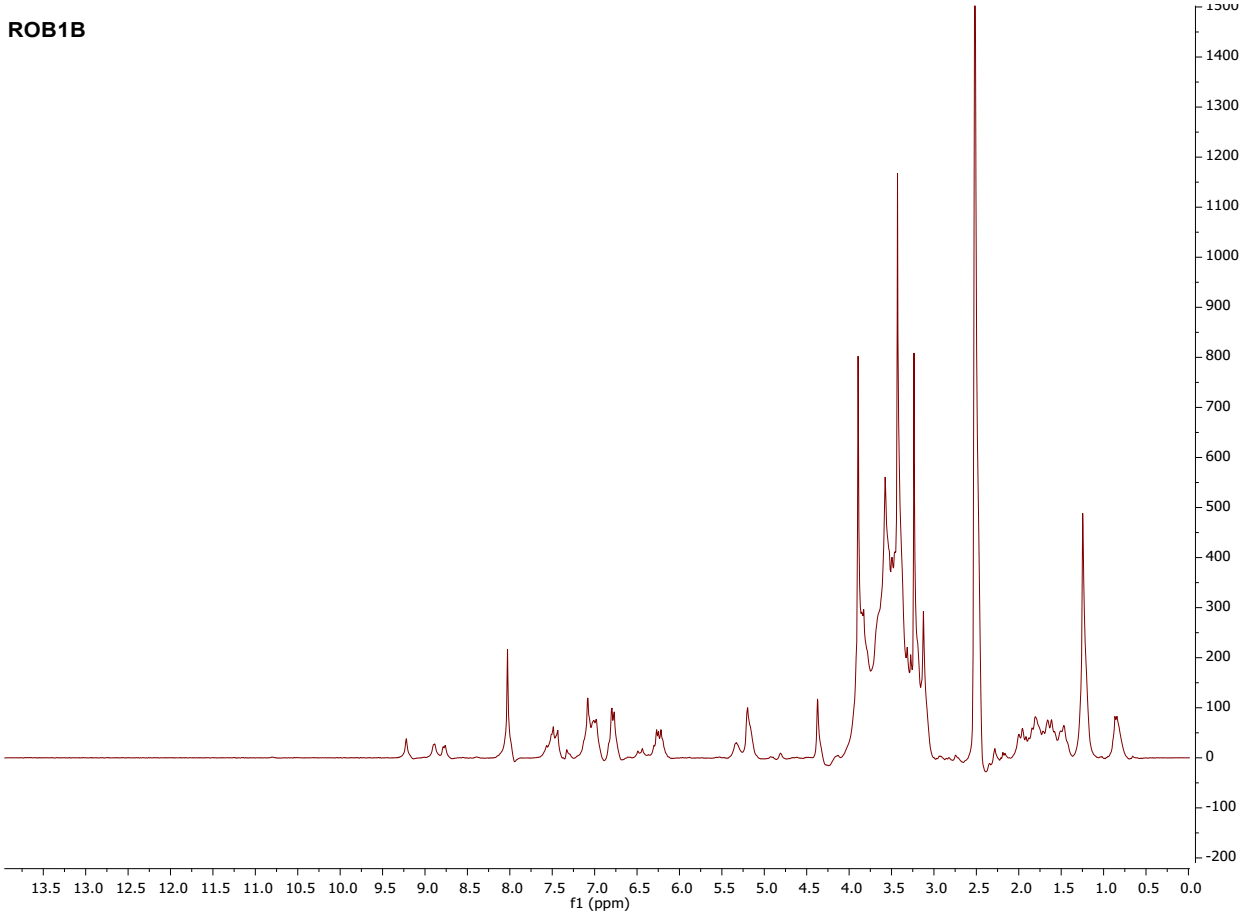

Figure S1. ^{1}H NMR spectrum of extract ROB1B

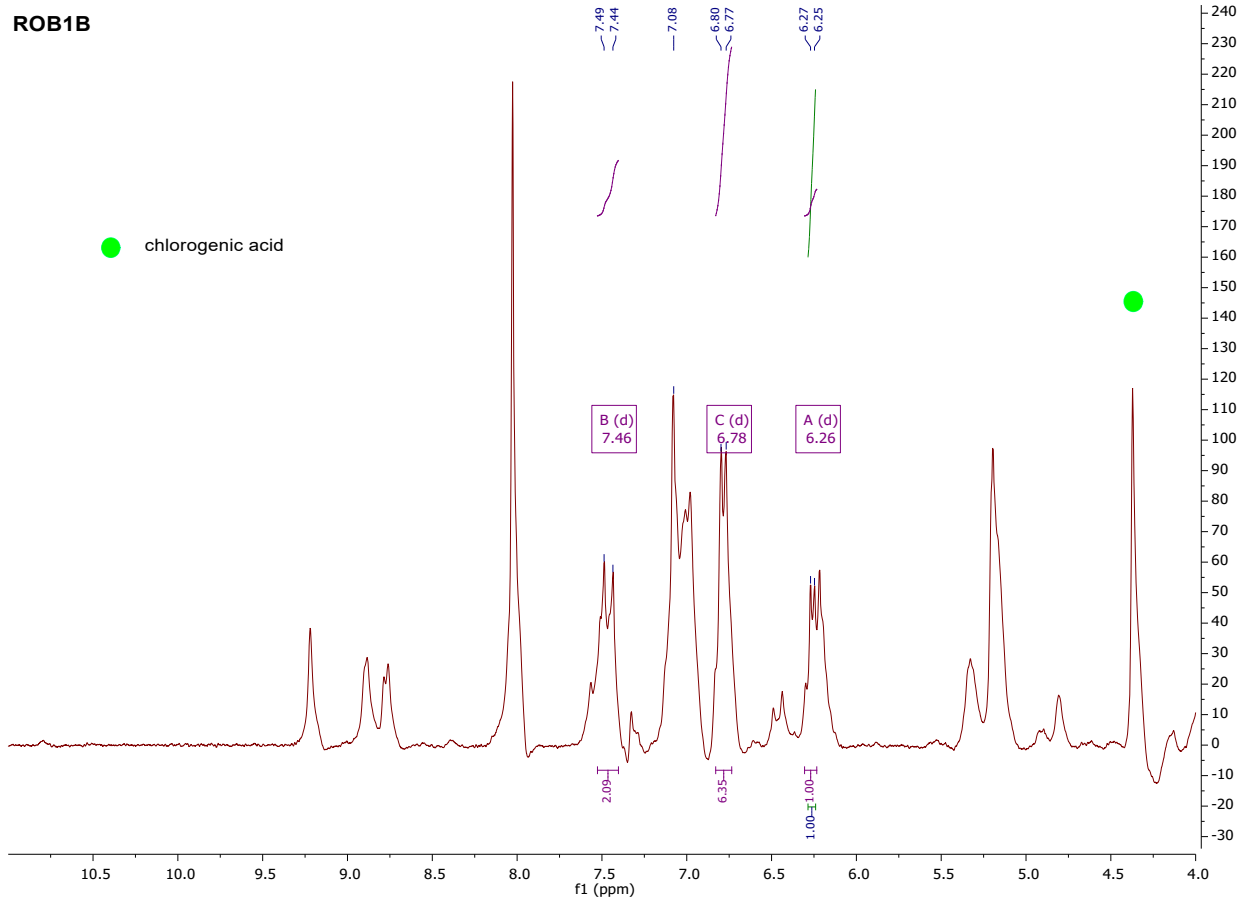

Figure S2. ^{1}H NMR spectral window highlighting the chemical shifts of chlorogenic acid

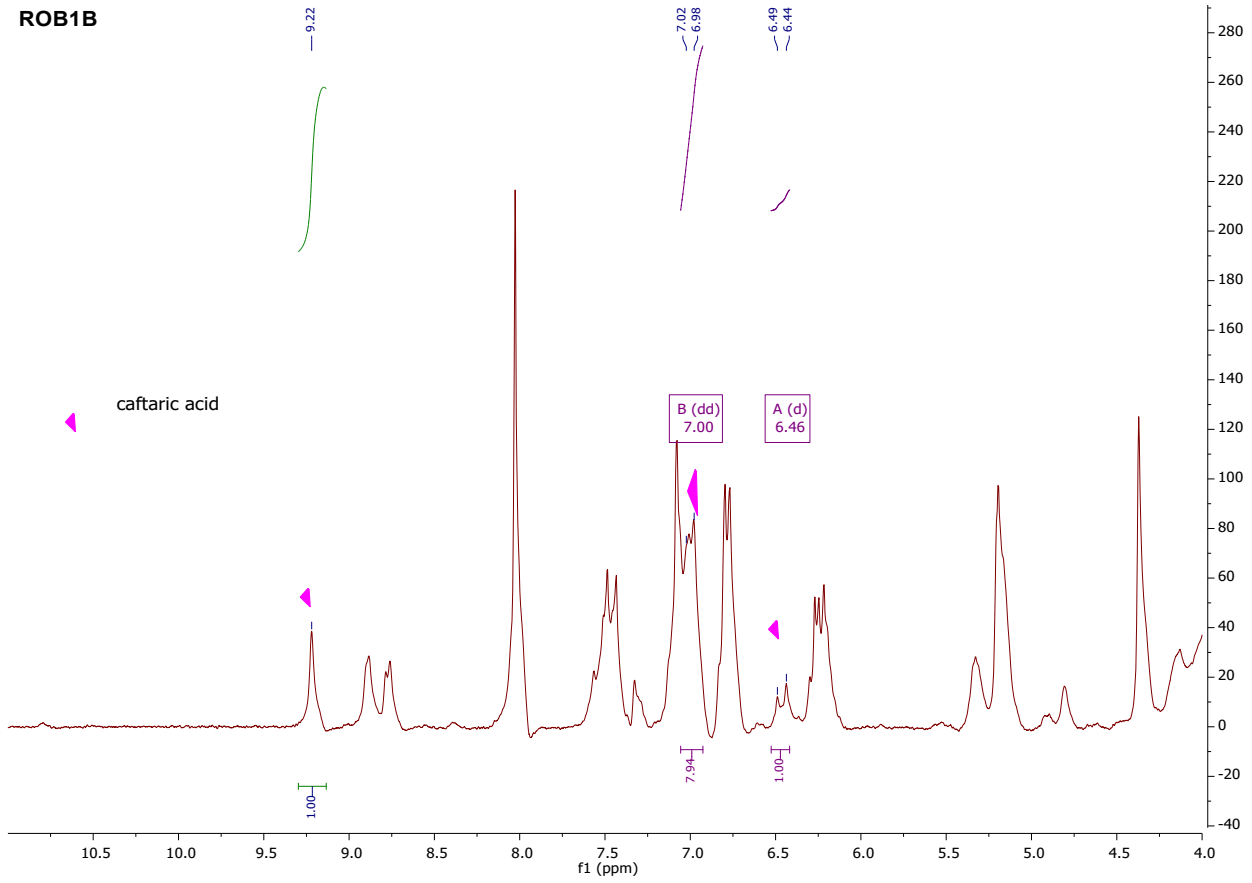

Figure S3. ^{1}H NMR spectral window highlighting the chemical shifts of caftaric acid

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