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Comprehensive analysis of soybean (*Glycine max* L.) by-products: Nutritional value, phenolic composition, and bioactive properties

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ABSTRACT

Soybeans are key components in vegetable beverage production, generating two by-products: soybean hulls and okara. For every ton of soybeans, 50–80 kg of hulls and 120 kg of okara are produced, being often discarded or used in low-value applications like fertilizers or feed. This study aims at characterizing their biochemical and nutritional profiles to assess their potential reintroduction into the food chain. Both by-products have high levels of protein and dietary fiber, mainly insoluble. Okara and hulls predominantly contain oleic acid and linoleic acid, respectively. Seventeen phenolic compounds, mainly isoflavones, were identified, with genistein as the main compound. Hulls exhibit superior antioxidant activity compared to okara. Neither extract showed cytotoxicity or anti-inflammatory effects and exhibited limited antimicrobial activity. However, both demonstrate prebiotic potential, promoting beneficial gut bacteria growth. The results suggest that these by-products have significant potential as new ingredients for their protein, isoflavone, and fiber content, alongside prebiotic properties.

1. Introduction

Soybean (*Glycine* max L.), a plant native to Asia, has significantly influenced human nutrition in the last decades (Grassini et al., 2021). Soybean production is mainly directed in the production of vegetable oil and also serves as input for food industry, as ingredient for tofu, soy sauce, and vegetable beverages (Colletti et al., 2020). According to recent reports from the Food and Agriculture Organization (FAO) of the United Nations (FAOSTAT, 2021), global soybean production reached 353 million tons in 2020, with the United States of America (USA) and Brazil being the main producers.

In recent times, consumer interest in vegetable products, such as plant-based milk alternatives, has been on the rise. Soybeans are considered an abundant source of polyphenols, proteins, and fibers and therefore suggested as a potential ingredient to produce functional foods and beverages (Takagi et al., 2015; Wang et al., 2021). Among various soy-based foods, soybeans are very popular for producing vegetable beverages, commonly referred to as “soy milk". The manufacturing of these beverages generally starts with the husking of the beans, their soaking in water followed by grinding to obtain a paste. Heat is applied

Abbreviations: AOAC, (Association of Official Analytical Chemists); AGS, (Human Gastric Epithelial Cell Line); ANOVA, (Analysis of Variance); CAA, (Cellular Antioxidant Activity); CaCo2, Human Colorectal Adenocarcinoma); CFU, (Colony-Forming Units); CLSI, (Clinical and Laboratory Standards Institute); DAD, (Diode Array Detector); DPPH, (2,2-Diphenyl-1-picrylhydrazyl); d.w, (dry weight); EC_{50}, (Effective Concentration 50%); ESI/MS, (Electrospray Ionization Mass Spectrometry); FAMEs, (Fatty Acid Methyl Esters); FOS, (Fructooligosaccharides); FRAP, (Ferric Reducing Antioxidant Power); Fw, (Fresh Weight); HPLC, (High-Performance Liquid Chromatography); HPLC MS2, (High-Performance Liquid Chromatography Mass Spectrometry); IDF, (Insoluble Dietary Fiber); MIC, (Minimum Inhibitory Concentration); m/z, (Mass-to-Charge Ratio); MUFA, (Monounsaturated Fatty Acids); f.w, (fresh weight); NO, (Nitric Oxide); ORAC, (Oxygen Radical Absorbance Capacity); PC, (Phenolic Compounds); PLP2, (Pig Liver Primary Cell Line); PUFA, (Polyunsaturated Fatty Acids); RP, (Reducing Power); SDF, (Soluble Dietary Fiber); SFA, (Saturated Fatty Acids); SPSS, (Statistical Package for the Social Sciences); TDF, (Total Dietary Fiber); TBARS, (Thiobarbituric Acid Reactive Substances); TNF, (Tumor Necrosis Factor); USA, (United States of America); UFLC, (Ultra-Fast Liquid Chromatography); λ_{max}, (Maximum Wavelength).

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at different stages of the production process depending on the producing method used (Chinese or Japanese). After filtration of the mixture, the liquid beverage and a solid leftover, known as okara, are obtained (Asghar et al., 2023). During this process, it is estimated that 50–80 kg of hulls are generated for every ton of hulled soybean (Bittencourt et al., 2021) while between 1.1 and 1.2 kg of okara is generated per kg of processed soybeans into soy milk or tofu production (Asghar et al., 2023; Feng et al., 2021).

While a substantial amount of by-products is generated during the production of soy-based beverages, currently, much of this material is either discarded or utilized in low-value applications, such as soil fertilization, animal feed or more recently, for biofuel production (Feng et al., 2021). Despite the interesting nutritional value of okara, rich in protein and fibers, one of the primary reasons for its limited reutilization in the food industry concerns the high moisture content of okara, making this by-product highly perishable. Additionally, challenges to the use of okara for direct supplementation into other food products arise from its content of trypsin inhibitors, which hinder digestion, the presence of oligo-saccharides known to cause flatulence, the occurrence of polyunsaturated fatty acids that may cause undesirable fishy and beany off-flavors, together with the high content of insoluble dietary fibers, that may lead to a gritty mouthfeel if used in high amounts (Feng et al., 2021). Therefore, despite some few recent works evaluating okara use as an ingredient in baked products or pasta (Kang et al., 2018; Mbaeyi-Nwaoha & Uchendu, 2016; Ostermann-Porcel et al., 2017; Park et al., 2015; Radočaj & Dimić, 2013), most works focused on its fermentation as an approach to overcome these limitations and produce a variety of new products and functional ingredients (de Moraes Filho et al., 2018; Feng et al., 2021; Mbaeyi-Nwaoha & Uchendu, 2016; Vong & Liu, 2016). Nevertheless, according to the review of Vong and Liu (2016), the current research on okara biovalorisation by bacterial or fungal fermentation remains largely at the bench scale, with challenges in scaling up and yield.

Another option would be to utilize the generated by-products to recover compounds that can serve as ingredients in the creation of new food products or the development of functional foods. Comprehensive studies encompassing the chemical characterization of these by-products, along with assessing their potential bioactivities, are essential for evaluating their suitability for these purposes. Despite the potential of soybean hulls for developing new ingredients as recently demonstrated by Fan et al. (2024) who incorporated soybean hull polysaccharides into plant-based yogurt improving its physicochemical properties, flavor and lactic acid bacteria growth, limited information is still available on soybean hull composition and biological activities (Liu et al., 2021; Tabibloghmany et al., 2020). Liu et al. (2021) evaluated the impact of particle size fractions in the protein, fiber, ash, soluble sugars, amino acids, and total phenolic compounds while the proximate analysis, fiber and total phenolics has been reported by Tabibloghmany et al. (2020) when optimizing the extrusion process for maximum soluble fiber content. Although Cabezudo et al. (2021) identified eight phenolic compounds, including two isoflavones, when optimizing polyphenol extraction from soybean hulls through green alternative methods, a comprehensive analysis of the isoflavone composition of soybean hulls is still needed. Similarly, information on the biological activity of soybean hull extracts remains scarce, since only limited data are available on their antimicrobial properties (Abutheraa et al., 2017) and antioxidant activity assessed through the 2,2-diphenyl-1-picrylhydrazy (DPPH) method (Liu et al., 2021).

Regarding okara, literature has primarily focused on the extraction and characterization of polysaccharides, particularly fiber, and to the recovery of proteins as recently reviewed by Privatti and Rodrigues (2023). The extraction of isoflavones from okara is also a subject of interest as they have been attributed with diverse health benefits such as their activity against hormone-derived cancers, osteoporosis and in menopausal disorders (Colletti et al., 2020), antioxidant potential (Kamble & Rani, 2020; Queiroz Santos et al., 2018) and functional properties (Swallah et al., 2021). Therefore, the application of different solvents and techniques has been considered for the extraction of phenolic compounds from okara (Aussanasuwannakul et al., 2023; Ferreira et al., 2023; Guimarães et al., 2018; Jankowiak, Trifunovic, et al., 2014; Shao et al., 2011; Vedovatto et al., 2021). However, a comprehensive characterization of this by-product encompassing a wide range of compounds, including nutrients and bioactive compounds, complemented with the assessment of associated bioactivities, is still missing in the literature. Although Nile et al. (2020) recently investigated the antioxidant activity, enzymes' inhibition, and cytotoxic effects of okara extracts against three cell lines, limited information is still available on the bioactivity of soybean by-products. In particular, key biological properties, such as anti-inflammatory and prebiotic activities, remain underexplored.

To address these gaps, the present study conducts a comprehensive assessment of the by-products produced by the soy-based vegetable drinks industry, namely okara and soybean hulls. In addition to the parameters most commonly evaluated for okara, namely protein, fiber, and phenolic compounds, several other groups of compounds of nutritional relevance were evaluated, together with a wide array of bioactivities, including antioxidant (using several different assays), cytotoxic, anti-inflammatory, antimicrobial and prebiotic properties. The same comprehensive assessment was extended to soybean hulls, for which data on chemical composition and bioactivities is still very scarce.

2. Materials and methods

2.1. Plant material and extract preparation

Okara and soybeans hulls were procured from White and green, Natural S.A, Vagos – Portugal, an industry that produces soy-based vegetable beverages in Portugal. The by-products were transported to the research center in refrigerated conditions and subsequently subjected to freeze-drying. The lyophilizates were ground to powder (≈20 mesh) and used to prepare hydroalcoholic extracts (80:20, ethanol: water, *v/v*) by maceration at room temperature, with magnetic stirring, using a 1 h extraction setting, filtering, and re-extracting for 1 h as previously detailed by Sprea et al. (2020).

2.2. Chemical parameters

Chemical analyses included the nutritional characterization, and additionally soluble sugars, organic acids, individual fatty acids, tocopherols, and phenolic compounds.

2.2.1. Proximal composition

Okara and soybeans hulls were evaluated with respect to macronutrients and moisture according to the Association of Official Analytical Chemists (AOAC, 2016). Briefly, crude proteins were determined by macro-Kjeldahl, using a correction factor of 6.25 (AOAC 978.04). Crude fat was determined by Soxhlet system, petroleum ether was used as solvent of extraction (AOAC 920.85). Ash (total mineral content) was determined by muffle incineration at 550 °C according to AOAC 923.03 and an enzymatic-gravimetric method was used to determine soluble, insoluble, and total dietary fiber (AOAC 993.43). Furthermore, disponible carbohydrates was calculate by difference of total carbohydrates and fiber (evaluated through enzymatic–gravimetric method). Energy was calculated according to following formula: Energy (kcal/100 g) = 4 × (g proteins + g disponible carbohydrates) + 9 × (g fat) + 2 × (Total dietary fibers), being disponible carbohydrates = Total carbohydrates – Total Dietary Fiber, according to Regulation (EU) N° 1169/2011.

2.2.2. Soluble sugars

Individual soluble sugars in soybean by-products were quantified using the method described by Sprea et al. (2020) with some modifications. Briefly, 1 g of the degreased sample (obtained from the

cartridges utilized in the crude fat analysis) was dissolved in 80% ethanol and heated at 80 °C in a bath for 90 min. The resulting solution was filtered, evaporated, and the obtained residue redissolved to a volume of 5 mL. The analysis equipment consisted of an integrated system comprising a pump (Knauer, Smartline system 1000, Berlin, Germany), degasser system (Smartline manager 5000), auto-sampler (AS-2057 Jasco, Easton, MD, USA), and a RI detector (Knauer Smartline 2300). Data were analyzed using Clarity 2.4 Software (DataApex). Chromatographic separation was achieved with a Eurospher 100-5 NH2 column (4.6 × 250 mm, 5 mm, Knauer) operating at 30 °C (7971 R Grace oven). The mobile phase consisted of acetonitrile/deionized water, 70:30 (*v/v*), at a flow rate of 1 mL/min. The injected sample volume corresponded to 10 μL. Melezitose, an uncommon sugar in plants, was used as internal standard, in a concentration of 25 mg/mL (1 mL being added to the sample). The obtained data was analyzed with Clarity 2.4 software (DataApex, Prague, Czech Republic). The identification of soluble sugars was performed by comparing the retention time of each peak with a mix of commercial standards. Results were expressed in g/100 g fresh weight (f.w.).

2.2.3. Organic acids

Organic acids were analyzed according to Spréa et al. (2020). Succinctly, 1 g of lyophilized soybean by-products was subjected to cold maceration for 20 min with 25 mL of metaphosphoric acid (4.5%), stirred at 150 rpm using magnetic agitation and protected from light. The mixture was submitted to centrifugation (5 min, 4000×*g*), and the resulting solution was filtered through a Ø 22 μm disposable filter. The filtered solution was subsequently analyzed by UFLC (Ultra-Fast Liquid Chromatography, Shimadzu 20A series, Kyoto, Japan), equipped with a C18 SphereClone reverse phase column (Phenomenex, 5 μm, 250 × 4.6 mm), thermostated at 35 °C. Elution was carried out with 3.6 mM sulfuric acid at a flow rate of 0.8 mL/min. Detection was performed using a diode array detector (DAD) at wavelengths of 215 nm and 245 nm (for ascorbic acid).

Organic acids were identified and quantified by comparison of the retention time with those of commercial standards, namely ascorbic, oxalic, quinic, malic, shikimic, citric, succinic and fumaric acids Sigma-Aldrich (St. Louis, MO, USA). Results were expressed in g/100 g (f.w.).

2.2.4. Fatty acids

Lipids were extracted by Soxhlet apparatus using petroleum ether added with butylated hydroxytoluene (BHT), and then submitted to derivatization to obtain FAMEs (fatty acid methyl esters). Derivatization was performed by adding 10 mL of Reagent A (2:1:1, methanol, sulfuric acid, and toluene) and incubating the samples at 50 °C under constant agitation overnight. After, 3 mL of distilled water was added to obtain the different phases. FAMEs were recovered with 3 mL of ethyl ether with vortex agitation. To eliminate any water residue, the supernatant was passed through a microcolumn of anhydrous sodium sulfate, then the sample was filtered using a 0.2 μm Millipore nylon filter and transferred to a vial with a Teflon-coated cap.

A gas chromatography system with flame ionization detection (YOUNG IN Chromass 6500 GC System) equipped with a split/splitless injector, a flame ionization detector (FID) and a Zebron-Fame column (ZB-FAME 30 m × 0.25 mm ID × 0.20 μm df, Phenomenex, Lisbon, Portugal) was used for the determination of individual fatty acids. The analysis was conducted using the following oven temperature profile: starting at 100 °C with a hold time of 2 min, followed by a temperature increase at a rate of 10 °C/min until reaching 140 °C. The temperature was then ramped at 3 °C/min to 190 °C, and then increasing 30 °C/min to 260 °C, where it was held for 2 min. Hydrogen served as the carrier gas, with a flow rate of 1.1 mL/min, recorded at 100 °C. The injection was performed with a split ratio of 1:50, while the injector and detector were maintained at 250 °C and 260 °C, respectively.

The compounds' identification and quantification were performed by comparison to a standard mixture containing 37 FAMEs (standard mixture 47885-U, Sigma, St. Louis, USA).

2.2.5. Tocopherols

After adding the internal standard (400 μL of Tocol, Matreya, Pleasant Gap, State College, PA, USA) to 500 mg of each sample, tocopherols (vitamin-E isoforms) were extracted as previously described bby Barros et al. (2010). Individual compounds were determined by using an HPLC system (Knauer, Smartline system 1000, Berlin, Germany) coupled to a fluorescence detector (FP-2020; Jasco, Easton, MD, USA) as previously described (Barros et al., 2010). Compounds were identified by comparison with the external standards (α-, β-, γ-, and δ-tocopherols, Sigma, St. Louis, MO, USA) and results reported in mg/100 g (f.w.).

2.3. HPLC-DAD-ESI/MS analysis of phenolic compounds

The phenolic compounds was determined on the hydroalcoholic extract obtained through cold maceration (as described in section 2.1). The freeze-dried material was re-dissolved in an ethanol:water solution (20% *v/v*) up to a final concentration of 10 mg/mL, and subsequently filtered through disposable filters (Ø 0.22 μm). The analysis of the phenolic composition was performed by ultra-performance liquid chromatography (HPLC Dionex Ultimate™ 3000, Thermo Scientific, San Jose, CA, USA) with an Orbitrap mass spectrometer (MS, Orbitrap Exploris™ 120, Thermo Fisher Scientific—San Jose, CA, USA) and a diode array detector (HPLC-DAD-ESI/MS). A Waters Spherisorb S3 ODS-2 C18 column (3 μm, 150 × 4.6 mm, Waters, Milford, MA, USA), operating at 35 °C was used for compounds' separation. The mobile phase consisted of 0.1% formic acid in water (A) and acetonitrile (B). The elution gradient was as follows: 15% B (5 min), 15% B to 20% B (5 min), 20%–25% B (10 min), 25%–35% B (10 min), 35%–50% B (10 min), followed by re-equilibration (10 min) at a flow rate of 0.5 mL/min. The mass spectrometry detector used nitrogen (N) at 50 psi as carrier gas. The system operated with a spray voltage of 5 kV at 325 °C with a capillary voltage of −20 V and the tube lens offset voltage was set at −66 V. Spectra were acquired in negative ion mode within the range of 100–1500 *m/z*. Collision energy used was 35 (arbitrary units). Data were acquired and processed using the Xcalibur® program (Thermo Finnigan, San Jose, CA, USA). When available, the compounds' identification was performed by comparison with standards, which were also used to construct the corresponding calibration curves. For those compounds whose standards were not available, analysis of UV spectra recorded for each peak and retention times, together with MS-MS data, led to their tentative identification from comparison with literature data. Results are expressed in mg/g of extract.

2.4. Bioactive properties

2.4.1. Antioxidant potential

In vitro antioxidant properties of the soybean by-products were determined by four different protocols, namely, the inhibition of lipid peroxidation (formation of thiobarbituric acid reactive substances, TBARS), free radical scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH), reducing power (RP) and the cellular antioxidant activity (CAA).

In the TBARS assay, the hydroethanolic extracts were diluted in water to obtain a concentration of 30 mg/mL and serially diluted to a final concentration of 0.12 mg/mL. Then, 200 μL of each dilution was added in a 96-well microplate, following the addition of 100 μL of ascorbic acid (0.1 M) and 100 μL of iron (II) sulfate heptahydrate (0.1 M) to each well. Porcine brain tissues, sourced from animals slaughtered in accordance with official regulations, were utilized in this study as a lipid source. The brain tissues were homogenized in a Tris-HCl buffer solution (20 mM, pH 7.4) to achieve a 1:2 (*w/v*) ratio of brain homogenate following centrifugation at 4000×*g* for 10 min. The supernatant was collected post-centrifugation, and 100 μL of this supernatant was added to the sample solutions before incubation at 37 °C for 1 h. After

incubation, the reaction was terminated by adding 500 μL of trichloroacetic acid (28%, *w/v*), followed by thiobarbituric acid (TBA, 2%, *w/v*, 380 μL). The mixture was then heated at 80 °C for 20 min and centrifuged at 3000×*g* for 10 min to remove the protein precipitate. The color intensity of the malondialdehyde (MDA) - TBA complex in the supernatant was measured at 532 nm, and the inhibition of lipid peroxidation (%) was calculated.

The same serial dilutions for each sample were used in the DPPH assay. Briefly, in a 96-well microplate, 30 μL of the sample in different concentrations was mixed with 270 μL of DPPH solution (0.15 mM), followed by incubation in the dark for 1 h. After the incubation process, the radical scavenging activity (RSA) was calculated based on the percentage of discoloration of the DPPH solution at 515 nm according to following equation: $\%ACR = [A_{DPPH}\text{-}A_A)/(A_{DPPH})]$ x 100. Results were obtained as EC_{50} (50% of maximal Effective Concentration).

Regarding to reducing power (RP) assay, the same serial dilutions of the samples were used to evaluate the capacity of reduction of Fe^{+3} to Fe^{+2}. Briefly, the different concentrations (500 μL) were mixed with 0.5 mL sodium sulfate (200 mmol/L; pH 6.6) and 0.5 mL potassium ferricyanide (1% *w/v*), and the mixture was then incubated at 50 °C for 20 min, followed by the addition of 0.5 mL trichloroacetic acid (10% *w/v*). Subsequently, the mixture (0.8 mL) was dispensed into 48-well microplates, along with deionized water (0.8 mL) and 0.16 mL iron chloride (0.1% *w/v*). Absorption was subsequently measured at 690 nm. The results were presented as EC_{50} values, which were calculated from the graphical representation obtained by absorption plotted against the corresponding extract concentration.

The CAA assay was performed as previously described (Wolfe & Liu, 2007), with modifications as proposed by de la Fuente et al. (2022). Briefly, the method is based on the determination of intracellular reactive oxygen and nitrogen species (ROS/RNS), measuring the ability of compounds to prevent the oxidation of intracellular dihydrodichlorofluorescein (DCFH2), which is readily oxidized to fluorescent dichlorofluorescein (DCF) by peroxyl radicals (ROO•). Murine macrophage cells (RAW 246.7) were seeded at a density of 2×10^4 cells/well and incubated for 48 h at 37 °C, with a humidified atmosphere and 5% CO_2. After this period, the culture medium was discarded, and the cells were washed twice with 100 μL of Hanks' Balanced Salt Solution (HBSS) in a concentration of 100 mM and pH 7.4. Subsequently, 200 μL of the sample at different concentrations and 100 μL 2′,7′-dichlorofluorescein diacetate (DCFH-DA) in a concentration of 50 μM were added to each well and re-incubate at 37 %C for 1 h. Then, the mixtures were removed, and the cells were washed twice with 100 μL of HBSS. After, 100 μL of 2, 2′-azobis(2-amidinopropane) dihydrochloride (AAPH) in a concentration of 600 μM, were added to each well. The reaction was carried out at 37 °C in a plate reader (Biotek FLX800, Bio-Tek Instruments, Inc., Winooski, VT, USA) with fluorescence filters for excitation and emission wavelengths of 485 nm and 535 nm, respectively. Fluorescence was recorded every 5 min for 1 h, and differences in area under the curve (AUC) were considered for calculation of CAA values using the following equation: CAA unit = 100 – (∫AUC/∫AUCc) x 100 where ∫AUCs is the integrated area under the sample fluorescence curve versus time, and ∫AUCc is the integrated area of the control curve.

Results were expressed as inhibition percentage of the oxidation reaction, with quercetin being used as a positive control.

2.4.2. Cytotoxic and anti-inflammatory activity

The assessment of cytotoxic activity was determined using the sulforhodamine B method. The cytotoxic activity was evaluated in 5 different cell lines, 3 tumoral, namely human gastric epithelial cell line (AGS), human colorectal adenocarcinoma (CaCo2), breast carcinoma (MCF-7), and 2 non-tumoral, pig liver primary cell line (PLP2) and renal epithelial cells extracted from an African green monkey (VERO). All cells were obtained from Leibniz-Institute (DSMZ - German Collection of Microorganisms and Cell Cultures GmbH) except for the PLP2 line, which was established in the laboratory and maintained (Mandim et al., 2021). Briefly, the extracts were diluted in water in order to obtain a concentration of 400 μg/mL and serially diluted in water to a final concentration of 0.78 μg/mL, and cells were cultured in 25 cm^2 tissue culture flasks in a humidified atmosphere with 5% CO_2. RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM non-essential amino acids and glutamine, 100 U/mL penicillin, and 100 mg/mL streptomycin served as the culture medium. Continuous monitoring of cells was conducted using a phase-contrast microscope (Icon Eclipse Ts 100).

In a next step, culture medium was removed from the flasks, and cells were washed with 2 mL of HBSS. After washing, cells were treated with 1.5 mL of trypsin for 3 min to disaggregate. Afterwards, 5 mL of culture medium was added to neutralize trypsin, the cell suspension was centrifuged for 5 min, the trypsin-containing medium was discarded, and the cell suspension (75 μL) was transferred to an Eppendorf tube, where an equal volume (75 μL) of trypan blue was added for cell counting using a Neubauer chamber. Moreover, 10 μL aliquot of the cell suspension was transferred to each well of a 96-well microplate along with the calculated volume of cell suspension for the desired density, and the well volume was completed with culture medium. The microplates were sealed and incubated at 37 °C with 5% CO_2 and humidity for 72 h. After cell adhesion, the microplate content was discarded, and the sulforhodamine B (SRB) assay was initiated by adding 100 μL of cold trichloroacetic acid (10%) to each well, followed by incubation at 4 °C for 60 min. Subsequently, the microplates were washed with distilled water and allowed to dry. SRB solution (0.1 in 1% acetic acid; 100 μL) was added and incubated for 30 min at room temperature. The microplates were washed again, this time with acetic acid (1%), to carefully remove the excess SRB, and allowed to dry. SRB was solubilized with 200 μL of Tris (pH 7.4) using a microplate shaker (Stat Fax-2100), and absorbance was read at 540 nm on a microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). Results were expressed as the concentration inhibiting 50% of cell growth (GI_{50}). All procedures were performed under aseptic conditions in a vertical laminar flow safety cabinet (TLStar, AV-30/70).

Furthermore, the same sample dilutions and murine macrophage cells (RAW 264.7, European Collection of Authenticated Cell Cultures) were used to evaluate the anti-inflammatory potential as described by Mandim et al. (2019). The cells were cultivated in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% heat-inactivated fetal bovine serum and glutamine at 37 °C with 5% CO_2 in a humidified environment. Cells were seeded in 96-well plates at 150,000 cells per well and allowed to attach to the plate for 18 h. After the incubation period, cells were exposed to the extract in different concentrations for 1 h. The effect of the tested samples in the absence of lipopolysaccharide (LPS) was also evaluated, to observe if they induced changes in NO basal levels, which were determined using a Griess Reagent System kit (Promega), consisting of sulfanilamide, NED, and nitrite solutions. 100 μL of each cell culture supernatant were added to the plate in duplicate and mixed with sulfanilamide and NED solutions for 5–10 min each at room temperature. The nitrite content was quantified by measuring the optical density at 515 nm using a microplate reader and compared against a standard calibration curve.

Dexamethasone was used as positive control. In negative controls, no LPS was added.

2.4.3. Antimicrobial potential

Antimicrobial assay was conducted through adaptation of the broth microdilution assay as per guidelines from the Clinical and Laboratory Standards Institute (CLSI, 2018), including the use of *p*-iodonitrotetrazolium chloride colorimetric test described by (Kuete et al., 2011). A total 5 Gram-negative and 3 Gram-positive bacteria, namely *Enterobacter cloacae (ATCC 49741), Escherichia coli* (ATCC 25922)*, Pseudomonas aeruginosa (ATCC 9027), Salmonella enterica* (ATCC 13076)*, Yersinia enterocolitica* (ATCC 8610)*, Bacillus cereus* (ATCC 11778)*, Listeria monocytogenes* (ATCC 19111) *and Staphylococcus aureus* (ATCC 11632), were evaluated. Streptomicin, methicilin and ampicillin were used as positive

control.

To evaluate the antifungal efficacy of soybean by-products, two micromycetes, namely *Aspergillus brasiliensis (ATCC 16404)* and *Aspergillus fumigatus* (ATCC 1022) were used to evaluate the fungicidal capacity. Antifungal activity was evaluated using a microdilution technique (de la Fuente et al., 2022). Ketoconazole was used as positive control.

2.4.4. Prebiotic activity

Okara and soybean hull were tested for potential prebiotic activity using an *in vitro* method, based on the evaluation of optical density and growth curves of probiotic bacteria. For that purpose, three *Lactobacillus* and one *Bifidobacterium* strains were used, namely *Lactobacillus casei* NCTC 6375, *Lactobacillus plantarum* DSM 12028, *Lactobacillus acidophilus* LA-5 (Probio-Tec, Denmark) and *Bifidobacterium animalis* spp. *lactis* Bb12 (Probio-Tec, Denmark). Strains were stored at −80 °C in de Man–Rogosa–Sharpe (MRS) broth (Liofilchem, Italy). Initially, the inoculum was prepared by suspending the microorganism in MRS broth containing 20 g/L of glucose, in anaerobiosis. For the growth of *B. animalis* Bb12, 0.05% of L-cysteine was added to the broth. The microorganisms were allowed to multiply for at least 3 days and were replicated to a new broth 12 h before the assay to reach the log phase of growth.

The samples and the positive controls, inulin and fructooligosaccharides (FOS), were w/diluted in MRS broth without glucose at a concentration of 2% (*w/v*) and further pasteurized (72–75 °C for 1 min), followed by centrifugation (3 min, 8000×*g*).

To carry out the assay, the inoculum was suspended in MRS broth without glucose to attain a turbidity corresponding to 0.5 McFarland standard (1.5×10^8 CFU/ml). Upon reaching this turbidity level, 10 μL of the inoculum was added to 2990 μL of MRS broth containing 2% of the samples or controls to achieve a concentration of probiotics of 5×10^5 CFU/mL. Subsequently, 200 μL of the mixture (MRS + 2% sample/control and the inoculum at 5×10^5 CFU/mL) was added to each well of a 96-well plate with a U-shape bottom. To maintain anaerobic conditions, 50 μL of sterilized liquid paraffin was added to each well. In addition, also as positive control, the inoculum was added to MRS both containing 20 g/L glucose. Finally, the samples or positive controls at 2% (*w/v*) in MRS broth without inoculum were used as blank controls.

The microplate was incubated at 37 °C for 48 h, with absorbance measurements at 620 nm registered every hour. After 48 h, the data were analyzed, and growth curves were generated.

2.4.5. Statistical analysis

Each experiment was performed in triplicate, with the results expressed as mean values ± standard deviation. The statistical analysis was carried out using SPSS Statistics software (version 23) to evaluate differences between the samples. A Student's t-test was applied at a 95% confidence level ($p < 0.05$) to assess the statistical significance of the results.

3. Results and discussion

3.1. Proximal analysis of soybean by-products

Table 1 presents the proximal composition of soybean by-products expressed in fresh weight. As expected, okara presented a high moisture content which is related to the production process of the vegetable drink where the soy flour is mixed with water. On the contrary, the low moisture content of the hulls is related to the physiological conditions of the dried beans (Riaz, 2016). Total Dietary Fiber (TDF, including insoluble dietary fiber (IDF) and soluble dietary fiber (SDF))was the main component in soybean hulls and okara, followed by protein. Interestingly, the hulls revealed a high content of protein (17.12 g/100 g f.w.), which may explain its use in animal feed (Barbosa et al., 2008).

From a nutritional perspective, IDF can be defined as carbohydrates with complex structures resistant to digestion. IDF, act as promoters of intestinal digestion, reducing the risk of conditions such as diabetes, obesity, and hyperlipidemia. Moreover, IDF serves as valuable technological aids, possessing properties such as water and oil retention, emulsification, and gel formation. This makes them a natural alternative for supplementation (Tang et al., 2024). In the other hand, SDF are defined as nonstarch polysaccharides soluble in hot water and can not be digested and decomposed by human digestive enzyme (Shen et al., 2020). On the contrary, SDF are metabolized by gut bacteria, modulating the gut microbiota, and undergoing metabolic processes to produce beneficial substances, primarily short-chain fatty acids, offers numerous health benefits, including the reduction of various gastrointestinal disorders, such as Functional intestinal disorders and inflammatory and neoplastic conditions. SDF can also have a prebiotic impact, improving the quality of functional foods (Guan et al., 2021; Li, Liu, et al., 2023).

Table 1
Proximal composition, energy value, free sugars, and organic acids of soybean by-products (mean ± SD, n = 9).

Sample		
Nutritional Value (g/100 g f.w.)	Okara	Hull
Moisture	80.67 ± 0.58 [a]	7.96 ± 0.71 [b]
Ash	0.68 ± 0.01 [a]	3.95 ± 0.01 [b]
Protein	4.70 ± 0.10 [a]	17.12 ± 0.49 [b]
Fat	1.65 ± 0.09 [a]	1.56 ± 0.03 [a]
Insoluble Fibers	8.22 ± 0.53 [a]	63.17 ± 0.52 [b]
Soluble fibers	0.71 ± 0.23 [a]	2.72 ± 1.03 [b]
Total Dietary Fibers	8.93 ± 0.76 [a]	65.89 ± 1.55 [b]
Disponible Carbohydrates	2.77 ± 0.57 [a]	2.21 ± 1.05 [a]
Energy	64.53 ± 2.14 [a]	225.80 ± 1.14 [b]
Free Sugars (g/100 g f.w.)		
Fructose	0.004 ± 0.001 [a]	0.096 ± 0.004 [b]
Glucose	0.009 ± 0.001 [a]	nd
Sucrose	nd	0.089 ± 0.003
Total Sugars	0.013 ± 0.001 [a]	0.185 ± 0.007 [b]
Organic Acids (g/100 g f.w.)		
Oxalic	0.0245 ± 0.003 [a]	0.131 ± 0.001 [b]
Quinic	nd	0.940 ± 0.034
Malic	nd	0.586 ± 0.018
Shikimic	nd	tr
Citric	nd	0.99 ± 0.03
Fumaric	nd	0.028 ± 0.001
Total Organic Acids	0.0245 ± 0.003 [a]	2.675 ± 0.084 [b]

nd, not detected; tr, traces; f.w., fresh weight; Different letters in the same row indicate significant differences ($p < 0.05$) between samples.

The conversion of IDF to SDF has been explored, Tabibloghmany et al. (2020) reported an increasing in SDF of 7.01% of soybeans black hulls through extrusion, with screw speed being one of the most important response factors for extraction optimization. Li, Liu, et al. (2023) demonstrated that it is possible to increase the SDF content in okara by 16.85% through steam explosion, with 1.2 MPa steam pressure for 90 s. The method resulted in a positive impact on capacity for glucose adsorption, capacity for cholesterol adsorption, and antioxidative properties.

In light of this, the findings presented in the current study align with the literature, as both Okara and Soybean hulls exhibited total dietary fiber (TDF) and protein as the primary constituents. Specifically, soybean hulls and okara contained 67.1 and 9.4 g/100g of TDF, respectively, and 17 and 4.7 g/100g of total protein, respectively.

Results are also in agreement with the results reported by Park et al. (2015) who developed cookies from fresh okara and Ambawat and Khetarpaul (2018), concluding that incorporating fresh okara into cookies can increase protein and fiber content.

In this context, soy by-products demonstrate potential use and exploration of new ingredients and foods. It is evident that in the near future, soy by-products may be regarded as potential new ingredients to

enhance protein and fiber content (de Figueiredo et al., 2018; Vong & Liu, 2016).

3.2. Free sugars and organic acids

Free sugars composition is shown in Table 1 and Fig. S1 (supplementary material). Soybean by-products evidenced a low content of free sugars, which is most probably due to their loss to the liquid beverage during the processing with a high quantity of water.

In okara samples, only two free sugars have been identified: glucose, the most abundant (0.009 g/100g f.w.), and fructose (0.004 g/100 g f.w.). These results are inferior to those documented by Mateos-Aparicio et al. (2010a), who extract free sugars using a 85% ethanolic solution (50 °C, 60 min) and identified rhamnose (0.3% d.w.), fucose (0.5% d.w.), arabinose (5.7% d.w.), xylose (2.7% d.w.), mannose (1.5% d.w.), galactose (10.4% d.w.) and glucose (11.9% d.w.) in okara from Spain.

A higher amount of soluble sugars were identified in soybean hulls (0.185 g/100 g f.w.) as compared to okara, corresponding to sucrose (0.089 g/100 g f.w.) and fructose (0.096 g/100 g f.w.). Liu et al. (2021) concluded that the reduction in soybean hull mesh is directly proportional to the increase in soluble sugar content, which can vary from 3 to 6% according to the mesh reduction. This variation occurs because processing can break down the polysaccharide structures, making carbohydrates available in the form of free sugars.

Results of organic acids (OA) are present in Table 1 and Fig. S2 (supplementary material). OA are compounds of industrial interest, as they can be used as preservatives or acidifiers in foods. Regarding to soybean by-products, only oxalic acids was identified in okara samples, while five organic acids were identified in soy hulls, with citric acid being the predominant one (0.99 g/100 g f.w.), followed by quinic, malic, oxalic and fumaric. Citric acid has been widely used in recent years as an acidulant, preservative, emulsifier, flavoring agent, masking agent and buffer, which are widely used in many industries, especially in the food and beverage industry (Ciriminna et al., 2017).

3.3. Crude fat, fatty acids and tocopherols

Soybeans are also known by their high content of fat, being used for oil extraction, and biodiesel production. The literature reports that soybeans are abundant in crude fat, with contents being approximately 20% of its total mass (Gasparetto et al., 2022). However, the crude fat content, expressed as fresh weight, was determined to be 1.64 g/100 g for okara and 1.56 g/100 g for the hulls, with no significat differences being observed between the by-products. Nonetheless, the crude fat content of okara can vary due to differences in processing and origin (Redondo-Cuenca et al., 2008). In previously studies, Vong and Liu (2016) report that the crude fat content of okara can vary from 8.3% to 10.9% in dry mass. Similarly, Redondo-Cuenca et al. (2008), reported an okara fat content of 9.84% in dry weight. The results obtained in this study, when converted to dry mass, yield a crude fat content of 8.8% for okara, which are consistent with the literature (Redondo-Cuenca et al., 2008; Vong & Liu, 2016). The values for crude fat content in soybean hulls have been previously reported by Nino-Medina et al. (2017) and Zhong and Zhao (2015) but were found to vary considerably across the two studies. While Nino-Medina et al. (2017) reported a content of 0.63 ± 0.03 g/100 g (f.w., corresponding to 0.69 g/100 g dry matter), the study by Zhong and Zhao (2015) found a significantly higher value of 4.58 ± 1.20 g/100 g (dry matter). Additionally, Barbosa et al. (2008) evaluated the nutritive value of a total of 39 samples of soybean hulls to be used in swine diets. The obtained results also showed a high variation of crude fat, which ranged from 0.6% to 4.3% (f.w.). These variations and discrepancies can be attributed to several factors, including, the origin of the soybean hull samples, and specific processing and storage conditions.Okara presents a high fat content due to the high fat percentage (around 20%) that generally exists in soybean seeds. Mateos-Aparicio et al. (2010b) described the fatty acid profile of okara, identifying the presence of polyunsaturated fatty acids (PUFA). Linoleic acid (C18:2n6) and linolenic acid (C18:3n3) were the main PUFAs reported by Mateos-Aparicio et al. (2010b), with the former being predominant, representing approximately 54% of the total fatty acid content. Additionally, okara presented significant amounts of oleic acid (C18:1) and a moderate level of saturated fatty acids (SFA), such as palmitic acid (C16:0) and stearic acid (C18:0). Regarding the qualitative composition obtained in this study (Table 2 and Fig. S3, supplementary material), a total of 12 fatty acids were identified in the okara sample and 8 in the soybean hulls. Both by-products presented SFA as the major group, with palmitic acid (C16:0) being the major compound in both okara and soybean hulls. However, soy by-products exhibited an interesting profile of monounsaturated fatty acids (MUFA) and PUFA, with oleic acid (C18:1n9c) being the predominant MUFA and linoleic acid (C18:2n6c) the predominant PUFA for both by-products. It is noteworthy that consuming adequate levels of polyunsaturated fatty acids is linked to positive health outcomes such as prevention of cardiovascular disease (Oliveras et al., 2022; Wu et al., 2022).

A similar fatty acid profile was found by Zang et al. (2021), who studied the substitution of soybean meal with okara meal in animal feed. Similarly, SFA and PUFA was the primary categories of fatty acids found in okara across various thermal treatments and storage conditions were documented (Voss et al., 2018). Regarding to the fatty acids profile of soybean hulls, to the current understanding, this represents the first report in the literature.

Table 2 also reports the composition of tocopherols (mg/100 g f.w.), a cluster of compounds that have a vital function in safeguarding fatty acids against oxidation. Both soybean by-products had δ-tocopherol as the main isomer, with 0.12 and 0.21 mg/100 g Fw for okara and soybean hulls respectively. Ko et al. (2003) reported the tocopherol content in different anatomical parts (germ, endosperm, and hull) of soybean, describing the presence of all four isoforms of vitamin E. The results found by the authors showed that for soybean hulls, α-tocopherol was the most abundant form, with a concentration of 10.7 mg/kg of dry weight. γ-tocopherol was the second most abundant form, with a concentration of 2.7 mg/kg, followed by β-tocopherol (0.2 mg/kg) and

Table 2
Fatty acids and tocopherols content of soybean by-products (mean ± SD, n = 9).

Sample		
Fatty acids (%)	Okara	Hull
C8:0 (Caprylic acid)	1.2 ± 0.1 [a]	3.2 ± 0.1 [b]
C10:0 (Capric acid)	nd	1.3 ± 0.3
C14:0 (Myristic acid)	0.4 ± 0.1	nd
C15:0 (Pentadecanoic acid)	0.6 ± 0.1	nd
C16:0 (Palmitic acid)	30.7 ± 0.7 [a]	30.4 ± 1.7 [a]
C16:1 (Palmitoleic acid)	7.5 ± 0.1 [a]	13.8 ± 0.3 [b]
C17:0 (Margaric acid)	0.4 ± 0.1	Nd
C18:0 (Stearic acid)	14.9 ± 0.2 [a]	11.4 ± 0.3 [b]
C18:1n9c (Oleic acid)	29.2 ± 1.0 [a]	24.2 ± 0.7 [b]
C18:2n6c (Linoleic acid)	12.9 ± 0.2 [a]	4.8 ± 0.1 [b]
C18:3n3 (α-Linolenic acid)	0.4 ± 0.1 [a]	10.9 ± 0.6 [b]
C20:0 Arachidic acid)	1.1 ± 0.2	nd
C20:1 (Eicosenoic acid)	0.6 ± 0.1	nd
SFA	49.3 ± 0.7 [a]	46.3 ± 1.7 [b]
MUFA	37.4 ± 1.0 [a]	38.0 ± 1.0 [a]
PUFA	13.3 ± 0.3 [a]	15.7 ± 0.7 [b]
Tocopherols (mg/100 g f.w.)		
	Okara	**Hull**
α-Tocopherol	nd	0.11 ± 0.01
β-Tocopherol	nd	nd
γ-Tocopherol	0.11 ± 0.01	tr
δ-Tocopherol	0.12 ± 0.01 [a]	0.21 ± 0.01 [b]
Total	0.23 ± 0.02 [a]	0.32 ± 0.11 [b]

nd, not detected; tr, traces; f.w., fresh weight; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Different letters in the same row indicate significant differences ($p < 0.05$) between samples.

δ-tocopherol (0.1 mg/kg). Regarding okara, previous studies have reported the presence of alpha-tocopherol and gamma-tocopherol with concentrations of 0.04 g/100 g and 0.01 g/100 g, respectively (Echeverria et al., 2022). The observed variations as compared with this study can be attributed to several factors, such as the grain source and processing method.

3.4. Phenolic composition of soybeans by-products

The phenolic composition of soybean by-products (Table 3 and Fig. S4, supplementary material), reveals a diverse array of phenolic compounds (PCs). Specifically, 13 PCs were identified in okara and 17 PCs in soybean hulls, with flavonoids being the predominant phenolic compounds detected. Through retention time analysis, molecular ions $[M-H]^-$, and the examination of characteristic fragment ions for each peak, the existence of various isoflavone forms in soybean by-products extracts was confirmed. By comparing these retention times and mass spectra with corresponding standards, one aglycone (genistein), one glucoside (daidzin), and their acetylated and malonylated derivatives were identified. Soy isoflavone glycosides typically include genistin, daidzin, and glycitin, in addition to the aglycones genistein, daidzein, and glycitein. Generally, for all glucoside forms, a glucosyl group is substituted at the aglycones 7′ or 4′ position, while in acetyl/malonyl derivatives, an acetyl/malonyl group is coupled to the sugar moiety (6'' position) (Devi et al., 2018).

Peaks 1, 2, and 3, with $[M-H]^-$ at *m/z* 355, exhibit identical fragmentation patterns with the loss of a malonyl group ([M-H-86]), resulting in the genistein aglycone (269*u*). The presence of a fragment ion *m/z* 269 consistent with genistein, along with the characteristic λ_{max}, strongly suggests that these compounds are isomers of 6"-*O*-malonylgenistein, potentially differing in the position or configuration of the malonyl substituent.

Peaks 4 ($[M-H]^-$ at *m/z* 593), 5 and 9 ($[M-H]^-$ at *m/z* 431), 10 ($[M-H]^-$ at *m/z* 595), and 15 ($[M-H]^-$ at *m/z* 473) present an ion with fragment *m/z* 269 (genistein) with the addition of a glucoside group (genistin). For peak 4, the compound showed the loss of an additional glucose unit ([M-H-162]), thus was identified as Genistin-6-*O*-glucoside. Peak 10 revealed the loss of an acetyl group and the rutinoside sugar ([M-H-42-308]), was identified as Genistin-7-*O*-(6"-*O*-acetyl)rutinoside. Peak 15 underwent only acylation, was identified as 6"-*O*-Acetylgenistin. Peak 16, with $[M-H]^-$ at *m/z* 573, suggests the presence of additional modifications of acetylation ([M-H-42]), malonylation ([M-H-86]), and glucuronidation ([M-H-176]), was identified as Genistein-7-*O*-(6"-*O*-acetyl)malonylglucuronide. These compounds have previously been documented in seeds of various soybean cultivars (Lee et al., 2022).

Peaks 6 ($[M-H]^-$ at *m/z* 441) and 8 ($[M-H]^-$ at *m/z* 295), with ions presenting fragments at *m/z* 253 (daidzein), showed the loss of an acetyl group ([M-H-42]). However, the first also lost a rhamnoside sugar, being identified as daidzein-7-*O*-(6"-*O*-acetyl)rhamnoside and 6"-*O*-acetyldaidzein, respectively. Peaks 11 ($[M-H]^-$ at *m/z* 415) and 12 ($[M-H]^-$ at *m/z* 577), with the addition of a glucoside group (daidzin), were identified as daidzin and daidzin-6-*O*-hexoside, the latter with an additional sugar loss ([M-H-162]) at the 6"*O* position. Peak 13, with $[M-H]^-$ at *m/z* 607 and fragments at *m/z* 283 (glycitein), showed a glycosidic bond, plus a loss of a glucose unit ([M-H-162]), identified as glycitin-6-*O*-glucoside.

Peak 14, identified as daidzein, was confirmed through its UV–vis spectrum and fragments, with a molecular ion $[M-H]^-$ *m/z* 253 and significant fragments at *m/z* 225 and 197, reflecting its fundamental importance in soy matrices due to its biological activity and role as a precursor to other derivatives. Peak 17 was positively identified by its molecular ion $[M-H]^-$ *m/z* 269 and a key fragment at *m/z* 145 as

Table 3
Phenolic compounds identified in soybeans by-products hydroethanolic extracts. Results are expressed in mg/g of extract.

Peak	Rt	λ_{max}	$[M-H]^-$ m/z	MS^2	Tentative Identification	Okara	Hulls	References
1	4.71	312	355	269(100)	6″-*O*-malonylgenistein isomer I	0.5 ± 0.01 [a]	0.32 ± 0.01 [b]	(Pabich et al., 2021; Xu & Chang, 2008)
2	5.14	312	355	269(100)	6″-*O*-malonylgenistein isomer II	0.201 ± 0.001 [a]	0.11 ± 0.002 [b]	
3	5.35	312	355	269(100)	6″-*O*-malonylgenistein isomer III	0.28 ± 0.01 [a]	0.114 ± 0.001 [b]	
4	7.58	290	593	431(100),269 (25)	Genistin-6-*O*-glucoside	0.248 ± 0.001 [a]	0.164 ± 0.002 [b]	
5	8.18	267	431	269(100)	Genistein-6-*O*-glucoside	0.186 ± 0.001 [a]	0.12 ± 0.01 [b]	
6	9.09	290	441	253(100)	Daidzein-7-*O*-(6″-*O*-acetyl)rhamnoside	0.226 ± 0.001 [a]	0.203 ± 0.001 [b]	
7	10.48	330	609	285(100)	Kaempferol-3-*O*-(6″-*O*-glucosyl) galactoside	nd	0.42 ± 0.02	(Lee et al., 2018; Pabich et al., 2021)
8	11.54	321	295	253(100)	6″-*O*-Acetyldaidzein	nd	0.051 ± 0.001	
9	14.81	326	431	269(100)	Genistin (Genistein-7-*O*-glucoside)	nd	0.034 ± 0.001	
10	15.26	318	595	269(100)	Genistin-7-*O*-(6″-*O*-acetyl)rutinoside	0.38 ± 0.02 [a]	0.247 ± 0.002 [b]	
11	15.84	319	415	253(100)	Daidzin (Daidzein-7-*O*-glucoside)	0.047 ± 0.001 [a]	0.055 ± 0.001 [b]	
12	18.19	315	577	415(100),253 (11)	Daidzin-6-*O*-hexoside	0.036 ± 0.001 [a]	0.123 ± 0.001 [b]	
13	21.01	316	607	445(100),283 (23)	Glycitin-6-*O*-glucoside	1.48 ± 0.01 [a]	0.168 ± 0.001 [b]	
14	24.85	301	253	225(100), 197 (15)	Daidzein	0.118 ± 0.001 [a]	2.12 ± 0.02 [b]	
15	26.59	318	473	431(100),269 (10)	6″-*O*-Acetylgenistin	tr	0.187 ± 0.001 [b]	
16	28.76	323	573	269(100)	Genistein-7-*O*-(6″-*O*-acetyl)malonyl-glucoronide	0.053 ± 0.002 [a]	0.116 ± 0.002 [b]	
17	32.81	321	269	145(100)	Genistein	1.82 ± 0.01 [a]	3.42 ± 0.02 [b]	
					TF	5.58 ± 0.06 [a]	7.97 ± 0.09 [b]	

TF: total flavonoids; nd: not detected; tr: traces. Calibration curves: Daidzin (y = 27652x+29187, R^2 = 0.9998, LOD = 2.05 μg/mL; LOQ = 6.23 μg/mL); Genistein (y = 64642x + 187360, R^2 = 0.9997, LOD = 1.03 μg/mL; LOQ = 3.13 μg/mL); Luteolin-6-*C*-glucoside (y = 27772x-11351, R^2 = 0.9986, LOD = 0.86 μg/mL; LOQ = 1.67 μg/mL). Different letters in the same row indicate significant differences ($p < 0.05$) between samples.

genistein.

Finally, peak 7, with $[M-H]^-$ at *m/z* 609 and the main fragment at *m/z* 285, pointing to the kaempferol base after two glycosidic losses ([M-H-162-162]), was identified as kaempferol-3-*O*-(6″-*O*-glucosyl)galactoside (Xu & Chang, 2008).

Isoflavones have gained significant interest as a potential alternative to hormone therapy for various health conditions, including menopause (Andres et al., 2015), osteoporosis (Li, Yang, et al., 2023) and certain types of cancer (Messina et al., 2006; Messina & Hilakivi-Clarke, 2009). Isoflavones can be in the form of aglycones such as daidzein, genistein and glycitein, and their respective glycosidic conjugates of three types: β-glycosides, acetyl glycosides and malonyl glycosides, with the total flavonoid content of soybeans reaching up to 400 mg/g (Genovese et al., 2006; Jackson et al., 2002; Zaheer & Humayoun Akhtar, 2017). Numerous researchers have noted the occurrence of malonyl and acetyl derivatives of daidzein, genistein, and glycitein in raw soybean seeds at varying concentrations (Ribeiro et al., 2007). Moreover, daidzein, an isoflavone found abundantly in soybeans, can be used to produce Equol-A, a bioactive compound used in food supplement formulations (Setchell et al., 2009).

The amount of phytoestrogens produced by a plant is influenced by several factors, such as growing conditions, cultivar, and environmental stresses, including low humidity, pathogen attacks, and adverse climatic conditions. Studies have reported higher levels of isoflavones in crops that were subjected to stress, indicating that environmental conditions can influence the concentration of these compounds (Daems et al., 2016).

Genistein and daidzein stand out as the primary isoflavones in soybeans (Campos, 2020), commonly observed and documented as predominant in diverse studies investigating soybean by-products (Jankowiak, Trifunovic, et al., 2014; Liu et al., 2021; Nile et al., 2020).

In okara, genistein was identified as the predominant phenolic compound (1.82 mg/g), along with the identification of 12 other phenolic compounds. These flavonoids mainly exist in glycosylated forms, with aglycones derived from genistin and daidzein being predominant, followed by malonyl forms and β-glycosides. Aglycones are resistant to heat, while β-glycosides are heat-sensitive. Therefore, the phenolic profile reported for okara aligns with existing literature, as the thermal and mechanical processes conducted under high humidity conditions during okara production directly affect its phenolic composition (Privatti et al., 2022).

Isoflavones undergo chemical changes during processing, such as the decarboxylation of malonyl into acetyl-glycosides and the hydrolysis of acetyl or malonyl-glycoside esters into β-glycosides. Additionally, processing temperature and immersion time also impact the quantity of aglycones and glycosides in soy. For instance, cold milling during soy milk production can increase the quantity of aglycones due to prolonged β-glycosidase activity (Jankowiak, Trifunovic, et al., 2014).

This study represents the first exhaustive identification of phenolic compounds in okara, providing valuable insights into this by-product and its potential effects. Additionally, the presence of isoflavones in okara underscores its nutritional significance.

Aglycones present in soybean by-products are more readily absorbed than heterosides due to their low molecular weight, facilitating diffusion and being associated with health benefits (Davy & Vuong, 2022). Thus, aglycones from isoflavones are particularly relevant for absorption and functional attributes in the human body (Hsiao et al., 2020). These aglycones found in soybean by-products, primarily daidzein and genistein, are associated with beneficial health properties (Sharifi-Rad et al., 2021).

The presence of aglycones in okara are in agreement with Nile et al. (2020), who identified 6 isoflavones present in okara, being Daidzein the predominant one (6.2 mg/100g dry weight (dw)) followed by Daidzin (5.9 mg/100g dw) and Genistein (3.7 mg/100g dw). Results of the phenolic composition may be related to the type of okara extraction method and sample preparation for phenolic compound extraction. Nile et al. (2020) used a mesh of 80, while the present study used mesh 20, with an increase in mesh size (decrease in particle size) potentially associated with enhanced extraction of bioactive molecules (Liu et al., 2021).

Moreover, studies have shown that the recovery of isoflavones is directly related to the type of extraction. Jankowiak, Kantzas, et al. (2014) observed a total of 0.9 mg/mL in an extract using 50% ethanol (*v/v*), suggesting that varying solvent concentrations yield different concentrations of isoflavones, with glucosides being predominantly extracted, followed by malonyl-glucosides and aglycones. Unfortunately, the phenolic profile for the extracts obtained by Jankowiak, Kantzas, et al. (2014) is not available in the literature. Regarding to the present study, an extract of 80% ethanol was able to extract a total isoflavone content of 5.58 mg/g in okara. However, higher values were reported by Nkurunziza et al. (2019) which used the response surface methodology to discovery the optimal extraction point for recovery of isoflavones in okara through subcritical water. These higher values are naturally acceptable compared to this study, as it involves a different and optimized extraction methodology compared to the one reported.

In relation to soybean hulls, genistein emerges as the predominant flavonoid with 3.42 mg/g, followed by daidzein with 2.12 mg/g. Furthermore, the identification and quantification of 17 distinct phenolic compounds (PCs) represent a significant advancement in our understanding of soybean hull composition. Prior to this study, the characterization of PCs in soybean hulls was limited, the profile of PCs through HPLC MS^2 was only identified in the studies of Cabezudo et al. (2021), who reported 3 phenolic acids (gallic, syringic and ferulic acid), 3 flavonoids (catechin, epicatechin and quercetin), an only 2 isoflavones (daidzein and genistein).

With the exception of the conducted research realized by Cabezudo et al. (2021), the literature only quantifies total PCs in soybean hulls using spectrophotometric methods, as in the study of de Nino-Medina et al. (2018), who reported a flavonoid content of 0.73 mg expressed in catechin equivalents based on aluminum chloride ($AlCl_3$) reaction and 1.75 mg of phenolic acids expressed in chlorogenic acid equivalents based on the Folin-Ciocalteu reaction, with methanol as the extraction solvent. The Folin-Ciocalteu reagent was also used to determine the total phenolic content of soybean hulls extracted with 50% ethanol (Liu et al., 2021), who reported a range of 1.81–3.24 mg of phenolic acids expressed in Gallic acid equivalent, with the variation increasing with the rise in mesh size, from 20 to 140 mesh.

Other studies also report the quantitative phenolic profile of soybean by-products (Voss et al., 2018; Yu et al., 2021). Although spectrophotometric methods like the Folin-Ciocalteu and aluminum chloride reaction are commonly used for total determination of PCs and flavonoids. However, these methods lack selectivity, which can result in potential inaccuracies due to reactivity with other sample components. On the contrary, high-performance liquid chromatography (HPLC) offers a precise alternative by separating sample components based on physicochemical properties for detailed analysis.

The PCs found in soybean by-products serve as a valuable source of bioactive substances with promising utility in the food sector, including the development of dietary supplements (Campos, 2020). These by-products contain a variety of phenolics, including isoflavones such as genistein and daidzein, as well as other PCs such as phenolic acids and flavonoids. The extraction and purification of these PCs from soybean by-products offer opportunities for the production of functional ingredients with high nutritional value. These compounds can be incorporated into dietary supplements to promote health benefits, such as antioxidant potential, hormonal regulation, and support for cardiovascular health (Ahsan & Mallick, 2017; Yoon & Park, 2014). Therefore, exploring the potential of PCs present in soybean by-products may represent a promising strategy for the food industry in the search for natural and healthy ingredients for dietary supplement formulation (Mikulić et al., 2022).

3.5. Bioactivity properties of okara and soybean hulls

3.5.1. Antioxidant activity

The literature reports that isoflavones present in okara and soybean hulls are potential antioxidant molecules, being genistein and daidzein the most promising (Danciu et al., 2017; Nile et al., 2020). Phenolic characterization highlights these molecules as the primary ones in the hydroalcoholic extracts obtained, potentially correlating with their positive impact on antioxidant activity across various techniques (Castro-López et al., 2017). Furthermore, studies show that okara extracts obtained with ethanol are more effective in antioxidant assays compared to other solvents such as acetonitrile and methanol (Nile et al., 2022).

Table 4 presents the antioxidant results of the hydroalcoholic extracts of soybean by-products. Regarding to the inhibition of lipidic peroxidation (TBARS), okara shown a higher EC_{50} in comparison with soybean hulls, 1.6 and 0.45 mg/mL, respectively. This indicates that a larger quantity of the extract is needed to achieve a 50% inhibition response in antioxidant activity for okara when compared directly to soybean hulls. The comparative analysis between soybean and okara extracts revealed significant differences in DPPH and RP assay. Soybean extract demonstrated approximately 1.96 and 2.24 times higher potency as an antioxidant compared to okara extract in the DPPH and reducing power (RP) assays, respectively, based on EC_{50} values.

Similar results were obtained in the cellular antioxidant assay (CAA) as it was observed that soybeans hulls also presented a higher prevention of oxidation at the highest concentration examined (2000 μg/mL) as compared to okara. It is worthwhile to emphasize that, up to the present moment, these are the first reported results for cellular antioxidant activity (CAA) in the literature for soybean by-products, highlighting their potential not only as conventional antioxidants, but also as cell-protective agents against oxidative stress.

The antioxidant potential of soybean hulls may be associated with the particle size of the samples during extraction. The antioxidant potential increases in proportion to the decrease in particle size (Liu et al., 2021).

Regarding to okara, Nile et al. (2020) assessed the antioxidative capacity of an 80% hydroalcoholic extract from okara using DPPH radical scavenging, FRAP, and ORAC assays. The authors noted that flavonoids, such as genistein and daidzein, found in the tested extracts likely contributed to their antioxidant activity. However, comparison with the present study is limited due to their use of Trolox equivalents as a response measure. The comparative analysis of the phenolic composition between soybean by-products, such as hulls and okara, revealed significant differences that may influence their antioxidant activity. Notably, hulls exhibited the presence of a kaempferol derivative absent in okara, suggesting a potential impact on antioxidant activity due to the known antioxidant properties of this flavonoid (Shields, 2017). Additionally, hulls displayed a higher concentration of tocopherols, including alpha-tocopherol, compared to okara, indicating a possible additional contribution to the antioxidant activity of these by-products.

These findings underscore the importance of a critical analysis of the phenolic composition for a deeper understanding of the antioxidant potential of soybean by-products and their applicability in food products and supplements.

It's worth noting that while hydroalcoholic extracts hold promise, the observed antioxidant activity stems from the extract itself rather than direct exposure of the by-products (hulls and okara) to antioxidant activities. Therefore, carefully determining the proportion of incorporating these by-products into new foods is crucial for achieving satisfactory results.

3.5.2. Cytotoxicity and anti-inflammatory activity

Although different *in vitro*, animal and clinical trials have produced conflicting results regarding the anti-cancer activity of soybean, in epidemiological and population-based studies its intake has been linked to decreased risk of different cancers, such as breast cancer (Kucuk, 2017). Additionally, soybean extracts and/or its major compounds, particularly genistein and daidzein, have been demonstrated to have cytotoxic activity against different cell lines (Farina et al., 2006; Rasheed et al., 2022; Varinska et al., 2015), including on breast carcinoma MCF-7 cell line (Ali et al., 2016; Elhamid et al., 2022; Rasheed et al., 2022).

Therefore, the extracts prepared from soybean by-products (okara and hull) were evaluated regarding their activity against three tumoral cell lines, namely AGS, CaCo2 and MCF-7. The findings regarding cytotoxicity and anti-inflammatory effects are presented in Table 5, where it can be observed that both by-products did not show cytotoxic potential on the evaluated tumoral cell lines assayed at 400 μg/mL. Although studies on the cytotoxicity of soybean extracts can be found in the literature, data on the activity of its by-products is very scarce. Nile et al. (2020) evaluated the cytotoxicity of different fractions (acetonitrile, ethanol, and methanol) obtained from an 80% hydroethanolic okara extract as well as that of the most common aglycones and corresponding glycosides and reported significant effects against HeLa (human cervical carcinoma) and MCF-7 (breast carcinoma). Among the assayed fractions, the one obtained with acetonitrile was the most effective against both cell lines compared to ethanolic and methanolic fractions, however presenting significantly higher EC_{50} values (31 ± 3 to 58 ± 3 μg/mL) as compared with that of the positive control (berberine, 9.1 ± 0.6 and 11 ± 1 μg/mL for HeLa and MCF-7, respectively). The activity reported by Nile et al. (2020) might be related to the higher concentration of isoflavone aglycones found in the initial hydroethanolic extract prepared from okara, which could be further concentrated in the fractions obtained with different solvents. Nevertheless, the detailed composition of individual isoflavones in each fraction is not reported, which does not allow a thorough comparison.

Overall, despite the lack of observed cytotoxic effects against the three tumoral cell lines assayed, the soy by-product extracts herein evaluated can be considered safe, as no cytotoxic effects were reported for healthy cell lines (PLP2 and VERO) at the concentration of 400 μg/mL (maximum tested concentration).

Table 4
Antioxidant activity of the hydroethanolic extracts obtained from soybean by-products (mean ± SD, n = 9).

Sample	Okara	Hulls	Trolox (μg/mL)	Quercetin
TBARS (EC_{50} mg/mL)	1.6 ± 0.1 [a]	0.45 ± 0.02 [b]	13.6 ± 0.1 [a c]	–
DPPH (EC_{50} mg/mL)	4.9 ± 0.1 [a]	2.5 ± 0.2 [b]	39.2 ± 1.2 [a c]	–
PR (EC_{50} mg/mL)	2.24 ± 0.1 [a]	0.65 ± 0.01 [b]	30 ± 1 [a c]	–
[b]CAA (% inhibition)	50 ± 1% [a]	72 ± 3% [b]	–	95 ± 5 [c]

[a] Results expressed in μg/mL.
[b] Tested at a concentration of 2000 μg/mL. Different letters in the same row indicate significant differences ($p < 0.05$) between samples.

Table 5
Cytotoxicity activity and anti-inflammatory activity (GI_{50} μg/mL) of the hydroethanolic extract obtained from soybean by-products.

	Okara	Hulls	Ellipticine	Dexamethasone
Cytotoxicity activity				
AGS	>400	>400	1.23 ± 0.03	nt
CaCo2	>400	>400	1.21 ± 0.02	nt
MCF-7	>400	>400	1.02 ± 0.02	nt
PLP2	>400	>400	1.4 ± 0.1	nt
VERO	>400	>400	1.41 ± 0.6	nt
Antinflammatory activity				
RAW 264.7	>400	>400	nt	6.3 ± 0.4

nt: not tested.

Likewise, despite genistein (Ji et al., 2012) daidzein extracted from soybean (Fang et al., 2024) and extracts/fraction from fermented soybean products (Kwak et al., 2015) have demonstrated anti-inflammatory activity, only a few studies have been conducted so far on soybean by-products (hull and/or okara) anti-inflammatory potential. In the work of Fang et al. (2024), the anti-inflammatory activity of different fractions of an okara extract was assessed by 4 different assays namely conjugated dienes, β-glucuronidase, hyaluronidase, and lipoxidase. In general, the fractions showed a better capacity to inhibit these enzymes as compared with dexamethasone. In the present study, both the okara and hull extracts did not evidence anti-inflammatory activity for the maximum tested concentration (Table 5). Nevertheless, the conducted assay was different than that of Nile et al. (2020) since it was based on the capacity to inhibit nitric oxide (NO) production in murine macrophage cells RAW 264.7. Although no anti-inflammatory potential was demonstrated in the present work, it is important to note that NO is just one marker within a complex signaling network of inflammation. Anti-inflammatory effects may arise from other mediators, such as prostaglandins, which were not examined in this study. Therefore, further research is necessary to explore additional inflammatory pathways.

3.5.3. Antimicrobial activity

The results obtained in the evaluation of the antimicrobial activity against a panel of five Gram-negative bacteria and two Gram-positive bacteria are presented in Table 6. In general, the antibacterial activity evidenced by both extracts was low since they did not shown activity against Gram-positive bacteria and were only able to inhibit the growth of two Gram-negatives, with none of the extracts presenting bactericidal activity at the assayed concentrations. As can be observed in Table 6, both by-products were able to inhibit *Y. enterocolitica* at the maximum concentration tested of 10 mg/mL. Moreover, okara extract was also able to inhibit *S. enterica* (10 mg/mL). Anjum et al. (2022) reported that an extract of okara derived from black and yellow soybeans, obtained by extraction with 85% ethanol, demonstrated positive inhibitory effects against strains of *B. subtilis*, *B. megaterium*, *E. coli*, and *Serratia marcescens*. These effects were observed in concentrations ranging from 0.3 to 1 mg/mL using the disc diffusion technique. The discrepancies in antimicrobial activity results, particularly concerning *E. coli,* a microorganism also tested in this study, may be attributed to the different methodologies used.

Soybean hulls of different varieties (black, brown, dark brown and yellow) extracted with 70% ethanol have showed potential as antimicrobial agents against *Salmonella typhimurium*, *Escherichia coli* O157:H7, and *Campylobacter jejuni* in broth cultures and chicken skin, demonstrating reductions of approximately 1 to 3-log (10–1000 times) CFU/mL, highlighting the antimicrobial capacity of soybean hulls extracts (Abutheraa et al., 2017).

Better results were obtained for the antifungal activity, with inhibitory capacity against the two tested strains being observed for both extracts. Nevertheless, the okara extract presented a slightly better MIC value against *A. brasiliensis* and *A. fumigatus* (5 mg/mL). None of the extracts presented fungicidal activity at the highest tested concentration. The information regarding the antifungal potential of soy by-products is still scarce, with no data reported in the literature for soy hulls. Regarding okara (De Benedetti et al., 2021), conducted a study on the development of a rapid and economical procedure for isolating proteins from okara and producing an enzymatic proteolyzed product that is active against phytopathogenic fungi. The proteins recovered from in okara submitted to enzymatic digestion demonstrated a dose-response inhibitory activity against *Fusarium* sp. Moreover, no fungicidal activity was observed at the maximum concentration tested (10 mg/mL).

3.5.4. Prebiotic activity

Soybean by-products were evaluated for their prebiotic potential on four probiotic strains, at a concentration of 2%. Inulin and fructooligosaccharides (FOS), as well-known prebiotics, and glucose (Glu) were used as positive controls. Fig. 1 shows the growth curves of the strains, measured by spectrophotometry at 620 nm of optical density (OD). In all cases, the biomass concentration began to increase within the first 10 h of incubation. Glucose (Glu), as expected proved to be the most effective carbon source for both *Bifidobacteria* and *Lactobacillus* strains, exhibiting the highest maximum growth rates among all strains, followed by inulin, a known plant-derived prebiotic (Mendonça et al., 2023).

For all four probiotics, soybean hulls resulted in higher bacteria growth compared to okara, which is probably related to its higher content in soluble fiber (Table 1). Soybean hulls showed very good prebiotic activity as it yielded superior growth values to the positive control FOS for all tested microorganisms (Fig. 1). Moreover, the curves show that soybean hulls exhibited a maximum growth similar to the positive control inulin for *L. casei*, *L. plantarum*, and *B. animalis,* however, reaching the exponential phase later (Table 7). Its molecular structure may allow for faster utilization by probiotic microorganisms compared to components of soybean hulls. Thus, while inulin initially promoted a faster growth, soybean hulls still managed to sustain significant growth of these probiotic strains over time. These results are in line with those reported by Song et al. (2022), who extracted

Table 6
Antimicrobial activity of the hydroethanolic extracts obtained from soybean by-products (mean ± SD, n = 9).

	Okara		Hulls		Streptomicin		Methicilin		Ampicillin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria										
Enterobacter cloacae	>10	>10	>10	>10	0.007	0.007	n.t.	n.t	0.15	0.15
Escherichia coli	>10	>10	>10	>10	0.01	0.01	n.t.	n.t.	0.15	0.15
Pseudomonas aeruginosa	>10	>10	>10	>10	0.06	0.06	n.t.	n.t.	0.63	0.63
Salmonella enterica	10	>10	>10	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Yersinia enterocolitica	10	>10	10	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Gram-positive bacteria										
Bacillus cereus	>10	>10	>10	>10	0.007	0.007	n.t.	n.t.	n.t.	n.t.
Listeria monocytogenes	>10	>10	>10	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Staphylococcus aureus	>10	>10	>10	>10	0.007	0.007	0.007	0.007	0.15	0.15

Antifungal Acitivity	**Okara**		**Hulls**		**Ketoconazole**	
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Aspergillus brasiliensis	5	>10	10	>10	0.06	0.25
Aspergillus fumigatus	10	>10	10	>10	0.5	1

MIC: minimum inhibitory concentration (mg/mL); MBC: minimum bactericidal concentration (mg/mL); MFC: minimum fungicidal concentration (mg/mL).

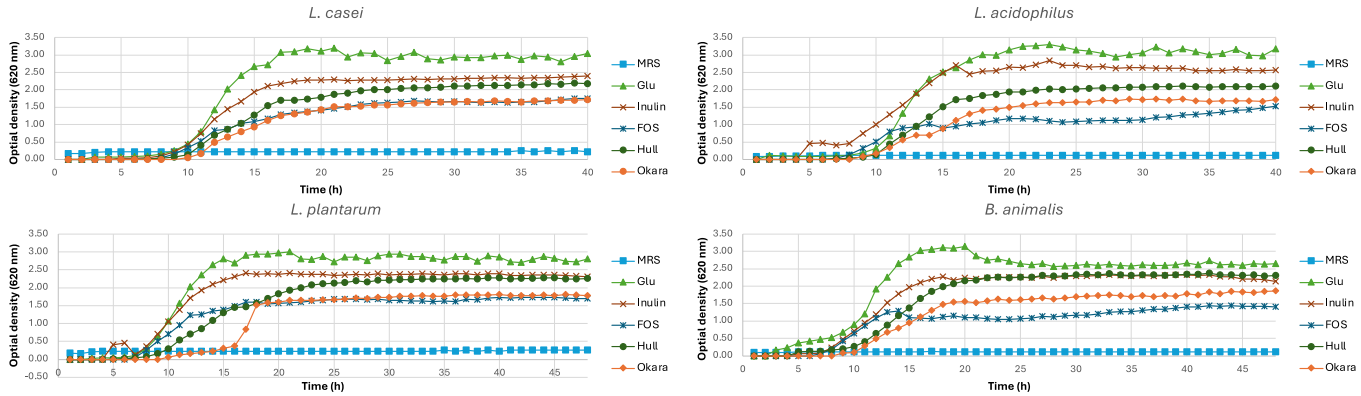

Fig. 1. Growth curves of *Bifidobacterium* and *Lactobacillus* probiotic strains obtained in the prebiotic activity assay.

Table 7
Maximum optical densities (OD at 620 nm) recorded at the respective incubation times during 48 h in experiments with *Bifidobacterium* and *Lactobacillus* probiotic strains.

Maximum optical densities (OD 620 nm)										
	Glu		FOS		Inulin		Hulls		Okara	
Prebiotic Strain	OD	Time	OD	Time	OD	Time	OD	Time	OD	Time
Bifidobacterium animalis spp. *lactis* Bb12	3.1	19 h	1.4	40 h	2.3	36 h	2.3	33 h	1.8	44 h
Lactobacillus casei	3.1	18 h	1.7	43 h	2.3	29 h	2.2	48 h	1.7	38 h
Lactobacillus acidophilus LA-5	3.3	23 h	1.6	45 h	2.8	23 h	2.1	33 h	1.7	33 h
Lactobacillus plantarum	3	21 h	1.7	43 h	2.3	29 h	2.2	37 h	1.8	36 h

OD – optical density, Glu – glucose 2%, FOS – fructooligosaccharides 2%.

polysaccharides from soybean hulls using different methods (hot water, single enzymes or enzymes mixture) and reported their potential as prebiotics since they were able to stimulate the growth of *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, and *L. acidophilus*.

The assessment of okara's prebiotic potential also yielded promising results. The maximum OD attained, ranging between 1.7 and 1.8, demonstrated okara's ability to support *in vitro* microbial growth of the assayed probiotic microorganisms. Although it presented lower probiotic growth as compared with inulin, Fig. 1 shows that okara surpassed the positive control FOS in terms of optical densities for *B. bifidum* and *L. acidophilus*, while reaching similar results for *L. casei* and *L. plantarum*, although for the last the exponential growth phase was reached latter. These findings suggest that okara may be a viable and effective source of prebiotics for promoting the growth of beneficial bacteria in the gut.

The prebiotic effects of okara have been evidenced in previous works, both *in vitro* and *in vivo*. Namely, Espinosa-Martos & Rupérez (2009) showed that okara can be effectively fermented by pure cultures of *L. acidophilus and B. bifidum* in batch cultures while Jiménez-Escrig et al. (2008) have confirmed its butyrogenic effects in the cecum of healthy female Wistar rats. Later, Villanueva-Suárez et al. (2013) evaluated the fermentability of okara submitted to an enzymatic treatment to increase its content of soluble dietary fiber, showing its *in vitro* bifidogenic capacity and significantly higher short chain fatty acids production as compared to untreated okara.

4. Conclusion

The present work provides a comprehensive analysis of the composition and bioactivity of soybean by-products, namely okara and hulls, underscoring their potential for application in functional foods. The obtained results confirmed that okara is particularly rich in protein and fiber suggesting it can be an excellent ingredient for enhancing the nutritional value of other food products. Both by-products revealed to be rich sources of dietary fiber and phenolic compounds, particularly isoflavones aglycones, such as genistein and daidzein, suggesting potential health benefits beyond the mitigation of oxidative stress, which was demonstrated by its antioxidant potential. The significant content of dietary fiber in both by-products underpins their utility in promoting digestive health with okara exhibiting notable prebiotic capacity, comparable to inulin and better than fructooligosaccharides. The hulls contain a high proportion of insoluble fibers, which may have applications in foods designed to enhance gut motility.

The results reinforce the potential for incorporating soybean by-products into food systems to promote health benefits while also contributing to sustainable food production practices by reducing waste and supporting a circular economy within the soybean industry.

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CRediT authorship contribution statement

Rafael Mascoloti Spréa: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Tiane C. Finimundy:** Writing – original draft, Methodology, Investigation, Formal analysis. **Ricardo C. Calhelha:** Methodology, Investigation. **Tânia C.S. P. Pires:** Methodology, Investigation. **Miguel A. Prieto:** Writing – review & editing, Supervision. **Joana S. Amaral:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Lillian Barros:** Writing – review & editing, Supervision, Funding acquisition,

Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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Data availability

Data will be made available on request.

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