Food Research International 224 (2026) 117951

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Inclusion of pulsed electric fields-treated broccoli by-products to improve pork frankfurters: evidence at the nutritional, metabolomic and functional levels

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ARTICLE INFO

Keywords:
Functional foods
Chemometrics
Bioactive compounds
Foodomics
Lipid oxidation
UHPLC-QTOF-HRMS

ABSTRACT

Broccoli by-products (BB), rich in polyphenols and glucosinolates, are emerging as sustainable ingredients for functional food reformulation. In this study, pulsed electric fields (PEF) and encapsulation (EN) were applied to improve the extraction and stability of broccoli by-products prior to incorporation in pork frankfurters, which were subsequently evaluated through metabolomic (UHPLC-QTOF-HRMS), nutritional, antioxidant and sensory analyses. Untargeted metabolomics, combined with chemometric analysis, revealed distinctive metabolic signatures in PEF-treated samples, which favored the extraction of lignans and phenolic acids. The incorporation of broccoli by-products into pork frankfurters led to an overall accumulation of glucosinolates and polyphenols, as well as an increase in antioxidant activity, especially after the addition of encapsulated by-products. In addition to modulating phenolic profiles, their incorporation also improved the nutritional profile of the frankfurters by increasing the content of dietary fiber and essential minerals. Their incorporation also exerted a protective effect during 14 days of refrigerated storage, enhancing oxidative and microbial stability while maintaining color integrity. Sensory analysis further indicated that encapsulation contributed to masking the characteristic flavor of broccoli while preserving texture-related attributes. These results highlight the potential of green technologies to add value to agro-industrial waste by developing nutritionally enhanced and functionally stable meat products that offer a natural alternative to conventional additives.

1. Introduction

Broccoli (*Brassica oleracea* var. *italica*) is a cruciferous vegetable cultivated worldwide and highly valued for its nutritional benefits and health-promoting properties. However, approximately 85 % of its biomass, including leaves, stems, and roots, is discarded as by-products (Quizhpe et al., 2024). Global broccoli and cauliflower production exceeded 26 million tons globally in 2023, emphasizing the large volume of potentially valuable by-products generated worldwide (FAOSTAT, 2023). These by-products, traditionally considered agricultural waste, have recently gained attention due to their composition rich in essential minerals (calcium, potassium, iron, and selenium), vitamins A, C, E, and K, and other bioactive compounds (Gudiño et al., 2024). Among the most studied bioactive compounds are glucosinolates (GSLs), particularly glucoraphanin and glucoerucin. These sulfur-containing metabolites undergo enzymatic hydrolysis to produce isothiocyanates, which exhibit anticancer and anti-inflammatory properties (Olayanju et al., 2024). Additionally, phenolic compounds, especially flavonoids and phenolic acids, are predominant in leaves and florets (edible flower heads) and play a key role in health-related mechanisms, such as the regulation of oxidative stress, inflammation, and metabolic processes (Duan et al., 2021).

In this context, the valorization of plant by-products through emerging green technologies is increasingly being considered as a promising strategy to improve the sustainability of food systems. The application of Pulsed Electric Fields (PEF) is a non-thermal method that

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https://doi.org/10.1016/j.foodres.2025.117951
Received 12 September 2025; Received in revised form 4 November 2025; Accepted 25 November 2025
Available online 29 November 2025

enhances the extraction of bioactive compounds from solid plant matrices by inducing electroporation. This process involves the application of short, high-voltage electrical pulses (1–10 $kV \cdot cm^{-1}$), which temporarily alter the integrity of cell membranes, forming nanopores that facilitate the diffusion of intracellular components, such as phenolic compounds, into the surrounding medium (Brito & Silva, 2024). PEF has shown success in enhancing polyphenol release from food by-products like tomato peels and apple pomace (Andreou et al., 2020; Pollini et al., 2021). In parallel, encapsulation has also emerged as a sustainable alternative to protect and stabilize plant extracts rich in bioactive compounds. Using food-grade biopolymers, i.e., maltodextrin or inulin, this technique creates protective matrices that reduce the degradation of compounds during storage and allow their controlled release in food or agricultural applications (Peixoto et al., 2022).

Meat products are increasingly questioned due to their limited content of dietary fiber and bioactive compounds, as well as the prevalent use of synthetic additives in their formulation. However, meat products have been shown to play a fundamental role in human diets, providing a significant source of high-quality protein, heme iron, and vitamin B_{12} (Leroy et al., 2023). In addition, meat naturally contains endogenous antioxidant peptides such as carnosine, anserine, and glutathione, which contribute to its oxidative stability (López-Pedrouso et al., 2023). Despite their nutritional value, the regular consumption of processed meat has raised concerns among health-conscious and environmentally aware consumers (Bouvard et al., 2015). This has led to a growing preference for clean-label natural products, perceived as healthier and more sustainable foods (Ciobanu et al., 2024). In response, the meat industry is increasingly exploring reformulation strategies that involve partial or total replacement of synthetic additives with plant-based natural extracts rich in antioxidants and antimicrobial compounds. In particular, the use of plant-derived by-products as functional ingredients has emerged as a promising approach to improve the nutritional profile of meat products while contributing to waste reduction and the implementation of sustainable food processing practices (Ronie et al., 2024). Different plant by-products, such as guarana seeds (Pateiro et al., 2018), olive leaves (Martínez-Zamora et al., 2020), or citrus peels (Nieto et al., 2021), have proven effective as natural antioxidants in meat products. These natural extracts derived from by-products not only serve as effective substitutes for synthetic additives but also contribute to the valorization of agro-industrial waste, aligning with sustainability goals and promoting a more circular and resource-efficient food system.

Nevertheless, the functional incorporation of broccoli by-products into meat products has not been thoroughly explored, despite the recognized nutritional and bioactive potential of these materials. Moreover, the combined application of PEF and encapsulation technologies in plant matrices remains scarcely investigated. Their integration could be particularly valuable, as encapsulation can protect PEF-extracted compounds from degradation during processing and storage. In this context, pork frankfurters represent an attractive model system for reformulation, given their standardized composition, wide industrial relevance and the high processing temperatures typically involved in their manufacture, which can promote the loss of thermolabile compounds. In addition, sausages are among the most consumed meat products worldwide, with a projected global market revenue of US $116.5 billion in, 2025 (Statista, 2025), making them particularly relevant targets for nutritional improvement. Therefore, assessing the performance of these green technologies in this matrix provides a relevant framework for evaluating both compound stability and functional efficacy under realistic food-processing conditions.

In this study, untargeted metabolomics was applied to analyze the chemical large-scale impact of PEF and encapsulation techniques on the polyphenol and glucosinolate profiles of broccoli by-products (BB), as well as their performance after their incorporation into pork frankfurters. In parallel, the potential of these by-products as natural substitutes for conventional additives was evaluated, assessing their effects on nutritional quality, oxidative stability and sensory attributes of frankfurters. This approach aims to improve the functionality of the products while advancing in the sustainable management of agro-industrial waste.

2. Materials and methods

2.1. Chemicals and reagents

Ultrapure water (UHPLC grade), methanol (HPLC gradient grade, ≥99.9 %), acetonitrile (HPLC gradient grade, ≥99.9 %) and formic acid (≥ 95 %) were obtained from Sigma-Aldrich (St. Louis, MO, USA). 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were also obtained from Sigma-Aldrich. Folin–Ciocalteu reagent was obtained from PanReac AppliChem (Barcelona, Spain). The pure standard compounds employed for semi-quantitative analyses were of the highest purity available (HPLC grade, ≥95 % or 99 % purity) and were purchased from Extrasynthese (Lyon, France). All culture media and reagents used were supplied by Bio-Rad Laboratories (Hercules, CA, USA).

2.2. Broccoli by-products preparation

Broccoli (*Brassica oleracea* var. *italica)* by-products were supplied by Cricket Campo de Lorca S.L. (Lorca, Spain). These plant materials, consisting mainly of stems and some leaves, were cut and immersed in a 0.5 % (*v/v*) citric acid solution.

Fresh broccoli by-products were treated with pulsed electric fields (PEF) using a Vitave system (Vitave, Prague, Czech Republic). PEF treatment was applied following the conditions described by Berzosa et al. (2025), with slight modifications. Treatments were carried out in batch mode in a parallel plate electrode chamber (8 × 15 × 10 cm; height × width × gap), filled with a treatment medium consisting of tap water (conductivity: 1.05 $mS \cdot cm^{-1}$). Monopolar square pulses were applied at 15 kV, corresponding to an electric field strength of 1.5 $kV \cdot cm^{-1}$. A total of 220 pulses with a width of 10 μs were delivered, resulting in a cumulative treatment time of 2200 μs. The specific energy input was 5.20 $kJ \cdot kg^{-1}$.

After PEF treatment, both untreated broccoli by-products (BB) and PEF-treated broccoli by-products (BBP) were subjected to a two-stage drying process using a forced-air oven (ArgoLab TCF 50, ArgoLab, Italy): 70 °C for 1 h, followed by 55 °C for 40 h, to achieve complete sample desiccation. The BB and BBP samples were ground and sieved to obtain a fine flour with a particle size of 250 μm, following Ayuso, Peñalver, et al. (2024).

Encapsulation (EN) of the broccoli by-products was performed according to the protocol described by Saavedra-Leos et al. (2021) with slight modifications. Briefly, 10 g of dried broccoli by-product (BB or BBP) was mixed with 200 mL of water and 2.5 g of maltodextrin. EN was performed in a Mini Spray Dryer B290 (BÜCHI, Labortechnik AG, Flawil, Switzerland) under the following operating conditions: feed temperature 40 °C, feed flow of 7 $mL \cdot min^{-1}$, hot air flow of 28 $m^3 \cdot h^{-1}$, suction of 70 % and pressure of 1.5 bar. The inlet temperature was set at 150 °C (Saavedra-Leos et al., 2021). The final encapsulated powders were designated as BBEN (encapsulated BB) and BBPEN (encapsulated BBP). The encapsulation efficiency (EE) was 62 %, slightly lower than that reported by Saavedra-Leos et al. (2021), probably due to differences in the composition of the by-product. However, microscopy analysis revealed predominantly spherical and homogeneous particles with smooth surfaces, indicating good encapsulation morphology.

2.3. Frankfurter elaboration

Lean meat and pork backfat were obtained on the day of preparation from a local butchery (Murcia, Spain). The process described by Nieto et al. (2009) was followed for the preparation of frankfurters and ingredients were mixed according to the formulation described in Table 1.

Table 1
Formulation of pork frankfurters.

Ingredients	FC−	FC+	FB	FBEN	FBP	FBPEN	(%)
Lean pork (g)	698.2	698.2	698.2	698.2	698.2	698.2	58.2
Pork backfat (g)	149.8	149.8	149.8	149.8	149.8	149.8	12.4
Ice (g)	323.5	323.5	323.5	323.5	323.5	323.5	27.0
Salt (g)	17.8	17.8	17.8	17.8	17.8	17.8	1.5
Commercial seasoning mix (g)	10.2		10.2	10.2	10.2	10.2	0.85
Commercial seasoning mix + additives (g)		10.2					0.85
BB (g)			24				2
BBEN (g)				30			2.5
BBP (g)					24		2
BBPEN (g)						30	2.5
Total (g)	1199.5	1199.5	1223.5	1229.5	1223.5	1229.5	100

Abbreviations: BB: broccoli by-products; BBEN: encapsulated broccoli by-products; BBP: broccoli by-products treated with pulsed electric fields; BBPEN: encapsulated broccoli by-products treated with pulsed electric fields; FB: frankfurter with 2 % BB; FBEN: frankfurter with 2 % BBEN; FBP: frankfurter with 2 % BBP; FBPEN: frankfurter with 2 % encapsulated BBPEN; FC−: control frankfurter without additives; FC+: control frankfurter with additives.

Seasonings from two commercial mixes were used (Fratelli Pagani Iberica S.L., Fortuna, Spain), both containing potato starch, salt, vegetable fiber, and spices, but one included additional food additives (E-250, E-316, E-331, E-450, E-451). Raw materials were chopped and mixed until an emulsion temperature of 15 °C was reached. Then, the frankfurters were manually stuffed into 22-mm diameter cellulose casings, resulting in 50-g frankfurters. Subsequently, they were steamed to an internal temperature of 71 °C for 60 min. After cooking, the frankfurters were immediately cooled with cold water for 2 min, vacuum-packed (SilverCrest®, Lidl Stiftung & Co. KG, Germany) and stored at 4 °C. Six different formulations were processed: control frankfurter without additives (FC−); control frankfurter with food additives (FC+); frankfurter with 2 % dried broccoli by-product (FB); frankfurter with 2 % encapsulated broccoli by-product (FBEN); frankfurter with 2 % PEF-treated broccoli by-product (FBP); and frankfurter with 2 % encapsulated and PEF-treated broccoli by-product (FBPEN). The visual appearance of the pork frankfurters is shown in Fig. 1. BBEN and BBPEN were added in adjusted amounts to ensure a 2 % by-products content in the formulation, considering that the encapsulated by-products contained 20 % maltodextrin. The inclusion level of 2 % was selected based on preliminary tests to ensure an appropriate balance between sensory acceptability and functional efficacy, in line with previous reports using vegetable powders in meat matrices (Ayuso, Quizhpe et al., 2024). FC− was included only in the metabolomic analysis as a reference formulation without additives, while FC+ served as a control to evaluate nutritional composition, oxidative stability and sensory properties to assess the potential of broccoli by-products as natural additive substitutes. A total of 26 frankfurters per treatment were prepared, of which 12 were used for shelf-life evaluation. The remaining samples were used for nutritional, sensory, antioxidant and metabolomic analyses. In addition, the pork frankfurters and broccoli by-products underwent freeze-drying prior to metabolomic analysis using a LyoEpic-85 freeze-dryer (Coolvacuum Technologies S.L., Barcelona, Spain).

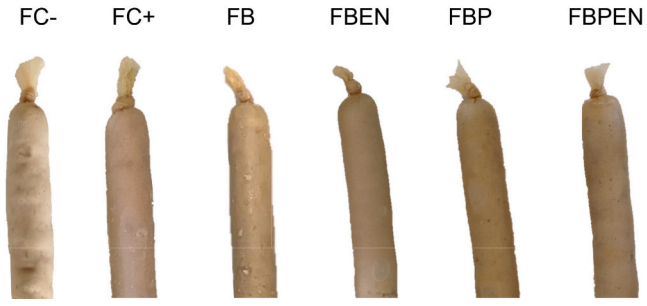

Fig. 1. Visual appearance of the different frankfurter formulations. Abbreviations: FB: frankfurter with 2 % BB; FBEN: frankfurter with 2 % BBEN; FBP: frankfurter with 2 % BBP; FBPEN: frankfurter with 2 % encapsulated BBPEN; FC−: control frankfurter without additives; FC+: control frankfurter with additives.

2.4. Phenolic and glucosinolate profiling by UHPLC-QTOF-HRMS

For untargeted metabolomic analysis, 0.5 g of freeze-dried broccoli by-products or freeze-dried frankfurters were extracted with 5 mL of an 80 % aqueous methanol solution acidified with 0.1 % (*v/v*) formic acid. The samples were subjected to ultrasound-assisted extraction for 10 min and then centrifuged at 8000×*g* at 4 °C for 10 min (OHAUS FC5718R, OHAUS Corp., USA). Finally, the supernatant was filtered using 0.22-μm pore-size syringe filters. The extraction process was performed twice to three independent replicates, making a total of six replicates per experimental group ($n = 6$). The glucosinolate and phenolic compounds profiling of by-products and frankfurters was determined by ultra-high performance liquid chromatography coupled to quadrupole time-of-flight high-resolution mass spectrometry (UHPLC-QTOF-HRMS). Chromatographic separation was conducted by a 1290 UHPLC system (Agilent Technologies®, Santa Clara, USA) with an Agilent® PFP reversed-phase column (2.1 × 100 mm, particle size 1.9 μm). The binary mobile phase included H_2O (solvent A) and acetonitrile (solvent B), both acidified with 0.1 % (*v/v*) formic acid. A constant gradient elution from 96 % to 6 % of solvent A was set for 32 min, with a flow rate of 200 μL min^{-1}. A volume of 6 μL was injected. Mass spectrometry was performed by a G6550 QTOF analyzer equipped with a JetStream electrospray ionization source (Agilent®). The equipment operated in positive ionization (ESI+) and SCAN mode (100–1200 *m/z*), to achieve simultaneous determination of polyphenols and glucosinolates (García-Pérez et al., 2025). Operating conditions included the use of nitrogen as sheath gas (12 L min^{-1}, 315 °C) and drying gas (14 L min^{-1}, 250 °C), nebulizer pressure set to 310.3 kPa, as well as applied voltages of 350 V at the nozzle and 4000 V at the capillary.

The UHPLC-QTOF-HRMS data acquisition of the samples was further processed by MassHunter Profinder (Agilent®), applying the find-by-formula algorithm for metabolite annotation. For this purpose, chemical features were aligned according to both retention time (range 1–32 min, tolerance ±0.05 min) and molecular mass (100–1200 m/z, ±5 ppm). Then, only those compounds that appeared in at least five of the six replicates of each experimental group were filtered out. Annotation of the resulting entities was carried out in compliance with the level 2 established by COSMOS Standard Initiative in Metabolomics (putatively annotated compounds), using an in-house curated database for phenolic compounds and glucosinolates (García-Pérez, Tomas, et al., 2024). Once annotated, phenolic compounds and glucosinolates were subjected to a semi-quantification approach, grouping compounds of the same family and quantifying against a representative standard of each family. Cyanidin was used as a reference standard for anthocyanins ($y = 13{,}657x$; $R^2 = 0.9869$), (+) catechin for flavanols ($y = 129{,}927x$; $R^2 = 0.9896$), quercetin for flavonols ($y = 363{,}099x$; $R^2 = 0.9858$), luteolin for other

flavonoids (y = 712,694x; R^2 = 0.9976), sesamin for lignans (y = 131,835x; R^2 = 0.9918), ferulic acid for phenolic acids (y = 13,070x; R^2 = 0.9782), oleuropein for low-molecular-weight (LMW) and other polyphenols (y = 82,615x; R^2 = 0.9923), trans-resveratrol for stilbenes (y = 46,669x; R^2 = 0.9844) and gluconapin for glucosinolates (y = 13,215x; R^2 = 0.9877). Semi-quantification results were expressed in mg of equivalents of each reference standard per 100 g of dry product.

2.5. Proximate composition

Determination of moisture (964.22), protein (955.04), fat (920.39), ash (923.03), soluble, insoluble and total dietary fiber (991.43) of the broccoli by-products and frankfurters was carried out following the Association of Official Analytical Chemists procedures (AOAC, 2012). Carbohydrate content and energetic values were determined following guidelines set by the Food and Agriculture Organization of the United Nations (FAO) (Charrondière et al., 2012).

The composition of macrominerals and trace elements in both broccoli by-products and pork frankfurters was determined by inductively coupled plasma optical emission spectrometry (ICP-OES) using a Thermo iCAP 6500 Duo (Thermo Fisher Scientific, Waltham, MA, USA) (Mroczek et al., 2023). Data were processed with iTEVA software and results were expressed in mg·100 g^{-1} of fresh weight (FW) for both macrominerals and trace elements.

2.6. Antioxidant activity and total phenolic content (TPC) analysis

Before analysis, two grams of fresh frankfurters and broccoli by-products were homogenized in 8 mL of a methanol/water mixture (80:20, *v/v*), extracted at 4 °C for 24 h, centrifuged at 4500 rpm for 25 min, and filtered through 0.45-μm membranes. Ferric Reducing Antioxidant Power (FRAP) assay (Benzie & Strain, 1996) was assessed by mixing 100 μL of extracted sample with 1 mL of FRAP reagent and measuring absorbance at 593 nm. The 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) cation radical (ABTS) assay (Re et al., 1999) was assessed by reacting the extracted sample ABTS reagent and measuring absorbance at 734 nm after 2 min. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was evaluated following the protocol described by Brand-Williams et al. (1995). Absorbance was measured after 30 min in the dark at 515 nm (Brand-Williams et al., 1995). All antioxidant activity results were expressed as μmol Trolox equivalents (TE)·100g^{-1} of FW sample. Finally, total phenolic content (TPC) was quantified following the method described by Singleton et al. (1999). Briefly, 100 μL of the extracted samples was mixed with 500 μL of Folin-Ciocalteu reagent and absorbance was measured at 750 nm after 1h incubation (Singleton et al., 1999). The results were expressed as mg gallic acid equivalents (GAE)·100g^{-1} of FW sample, using a gallic acid standard curve prepared in methanol. All Measurements were performed in triplicate using an Evolution 300 spectrophotometer (Thermo Scientific, Waltham, MA, USA).

2.7. Shelf-life assessment of pork frankfurters

The pH values of the frankfurters were measured using a sensION+ PH31 pH meter (Hach-Lange, Spain), following ISO guidelines (ISO 2917., 1999).

Color measurements were taken with a Konica Minolta CR 400 chromameter (Minolta, Japan), calibrated using a white calibration plate provided by the manufacturer. Lightness (L*), redness (a*), and yellowness (b*) parameters were recorded according to the CIEL*a*b* system (Ayuso, Peñalver, et al., 2024). Total color differences (ΔE) were determined using the following formula: $\Delta E = \sqrt{(L_s^* - L_c^*)^2 + (a_s^* - a_c^*)^2 + (b_s^* - b_c^*)^2}$, where L*, a* and b* correspond to lightness, redness and yellowness, respectively; the subscript s indicates the value in the sample and c for the control.

Thiobarbituric acid reactive substances (TBARS) content, directly related to lipid oxidation, was measured following the method described by Ayuso, Quizhpe, et al. (2024). TBARS values were expressed as mg malonaldehyde (MDA) per 100 g of sample. Metmyoglobin (MetMb) was measured following the method described by Liu et al. (2015), using the formula: MetMb (g·100g^{-1}) = −2.514 (A_{572}/A_{525}) + 0.777 (A_{565}/A_{525}) + 0.8 (A_{545}/A_{525}) + 1.098–100 (Liu et al., 2015). Color, pH, MetMb and TBARS analyses were performed in triplicate for days 0, 5, 9, and 14 of refrigerated storage (4 °C).

Total vial count (TVC), total coliform count (TCC) and *Escherichia coli* were measured by homogenizing 10 g of frankfurter sample with 90 mL of sterile 0.1 % (*v/v*) peptone water using a stomacher blender (Bag-Mixer, Interscience International, France), following the protocol described by Martínez-Zamora et al. (2021). Serial dilutions were seeded on PCA agar (TVC, 30 °C for 48 h) and Rapid *E. coli* agar (TCC, 37 °C for 24 h; *E. coli*, 45 °C for 48 h). The microbiological parameters were expressed as CFU·g^{-1}. For the detection of *Salmonella* spp., 25 g of sample were mixed with 225 mL of peptone water, incubated at 37 °C for 18 h and seeded on Rapid *Salmonella* agar. For the detection of *Listeria monocytogenes*, 25 g of sample were mixed with 225 mL of Fraser broth and incubated at 37 °C for 22 h, after which they were seeded on Rapid *L. monocytogenes* agar. All analyses were performed on days 0, 5, 9 and 14 of refrigerated storage (4 °C).

2.8. Sensory analysis of pork frankfurters

Sensory analysis was carried out exclusively in pork frankfurters, involving 13 panelists previously trained according to ISO guidelines (ISO 8586, 2012). The panelists, who ranged in age from 22 to 45, participated in two training sessions. These sessions were dedicated to refining their sensory descriptions of frankfurters and broccoli recognition, ensuring prior experience in sensory analysis. Frankfurters were cooked for 4 min at 180 °C and coded with three-digit numbers. The sensory evaluations were conducted in individual booths under controlled conditions and panelists rinsed their mouths with water between samples. Panelists evaluated the flavor, odor, and color of the frankfurters, including attributes related to the incorporated by-products, as well as the hardness, adhesiveness, chewiness, cohesiveness, and fatty flavor of the frankfurters. A 5-point scale was used for scoring. The study was approved by the Bioethics Committee of the University of Murcia with the code M10/2024/365.

2.9. Statistical and chemometrics analysis

One-way analysis of variance (ANOVA) was applied to evaluate differences among pork frankfurter formulations for all measured parameters, including nutritional and sensory analyses. Differences observed during the shelf-life study were analyzed using two-way ANOVA, considering time and treatment as fixed factors. Statistical significance was established at $p < 0.05$, and post-hoc comparisons were performed using the Tukey test. Differences between by-products were assessed using Student's *t*-test. Results were presented as mean ± standard deviation (SD). Statistical analyses were conducted using SPSS Statistics v28.0 software (IBM Corporation, Armonk, NY, USA).

Chemometric multivariate analysis of metabolomics results was performed using the online platform MetaboAnalyst 6.0 (https://www.metaboanalyst.ca). Abundance values were log-transformed, normalized by the median and auto-scaled (mean-centered and divided by the SD of each variable) before analysis. An unsupervised hierarchical cluster analysis (HCA) was applied using a heat map based on the normalized fold-change values obtained after the abundance and standardized by auto-scaled features, using Euclidean distances and Ward's linkage rule, to determine metabolome-wide similarities among experimental groups and discard outliers (García-Pérez, Becchi, et al., 2024). In addition, a principal component analysis (PCA) was performed to explore the distribution and dimensionality of the data. The quality of

PCA was evaluated through the R^2 fitting parameter to determine the intraspecific variability of data, and statistical assessment was performed by permutational multivariate analysis of variance (PERMANOVA), setting a significance threshold of $\alpha = 0.05$. A Volcano analysis combining an unpaired *t*-test ($\alpha = 0.05$) with a fold change analysis (FC; cut-off = | ± 2|) was performed to identify the metabolites showing a significantly different accumulation between by-products and frankfurters. False discovery rate (FDR) adjustment was applied on t-test statistical analysis to ensure correction of multiple testing effects. In parallel, a supervised orthogonal projection to latent structures discriminant analysis (OPLS-DA) was performed to predict the differential outcome of samples according to their metabolic profile. The quality of OPLS models was assessed by the goodness-of-fit (represented by the R^2Y parameter) and goodness-of-prediction (represented by the Q^2 parameter). Overfitting was excluded by permutation test, considering 100 permutations (Fig. S1). In addition to each OPLS model, a Variable importance in projection (VIP) analysis was performed to determine the features showing the highest contribution to the discriminant model, the so-called VIP markers. VIP markers were selected according to their VIP score, setting a threshold of 1.0 for critically discriminant metabolites. Differentially accumulated metabolites (DAMs) were filtered by Volcano analysis and VIP score thresholds, and they were further subjected to a chemical enrichment (ChemRICH) approach (Barupal & Fiehn, 2017). In parallel, DAMs were involved in a pathway analysis enrichment, based on the cumulative fold change (log_2(FC)) values of each subclass of phenolic compounds and glucosinolates. In addition, sensory data were normalized prior to analysis, and PCA biplot was performed using R software with the factoextra package to visualize the distribution of the samples and the contributions of the sensory attributes.

3. Results and discussion

3.1. Untargeted phenolic and glucosinolate profiling of broccoli by-products and frankfurters

Untargeted metabolomics is a powerful tool for evaluating the processing and reformulation of food products (Rocchetti et al., 2022; Wang et al., 2024). In this study, untargeted metabolomics was applied to profile phenolic and glucosinolate compounds in broccoli by-products subjected to different procedures and their incorporation into pork frankfurters. A total of 516 chemical entities were reported in all samples (Table S1). Flavonoids were the most represented polyphenol family, accounting for 151 metabolites, where anthocyanins (42) were the most abundant. This group was mainly formed by glucosides belonging to cyanidin, delphinidin, and petunidin. On the other hand, other subfamilies of flavonoids, such as flavanols (19), flavanones (11), isoflavones (31) and flavonols (25) were also detected in abundance. This last one showed the presence of many compounds derived from kaempferol, quercetin, and myricetin. Phenolic acids were also widely represented, with up to 72 compounds reported, of which 44 were hydroxycinnamic acids and 16 hydroxybenzoic acids. Finally, other phenolic subfamilies were identified in a minor way as stilbenes (11), lignans (24), tyrosols (12) and alkyphenols (9). All the above-mentioned phenolic subfamilies had been previously reported in *Brassicaceae* (García-Pérez et al., 2025). On the other hand, up to 196 different glucosinolates were reported in the dataset, indicating a large contribution of this class of compounds in the samples. Given the complexity of the data set, multivariate statistics were used for interpretation.

3.1.1. Metabolomic profiling of broccoli by-products and chemometric analysis

The results of the metabolomic analysis of broccoli by-products subjected to PEF and encapsulation treatments were analyzed by means of multivariate chemometric analysis, which are summarized in Fig. 2. Unsupervised multivariate analysis was applied to investigate metabolomic differences between broccoli by-products. Hierarchical cluster analysis (HCA) (Fig. 2A) revealed clear separation among the four samples, reflecting distinct metabolomic profiles. However, two main clusters were observed, as BB and BBEN clustered together, while both PEF-treated samples formed a separate cluster. This suggests a clear effect of PEF treatment on the composition of phenolic compounds and glucosinolates. These results were confirmed through principal component analysis (PCA), where the four broccoli by-products were separately distributed on the score plot (Fig. 2B). Notably, PC1 (24.4 % of the total variance) was mainly associated with the effect of PEF, confirming its significant role in leading to metabolic differences. The discrimination shown by the PCA was statistically supported by PERMANOVA ($R^2 = 0.989$; $p = 0.001$), confirming significant differences between groups.

To further distinguish the treatment effects, supervised modeling on the metabolic profile of broccoli by-products was performed using Volcano analysis, setting the following threshold for feature selection: $log_2(FC) \geq | \pm 2|$; and *t*-test $p < 0.05$. BB was established as the reference treatment to conduct pairwise comparisons. As a result, the impact of treatments on the metabolome of broccoli by-products was represented by metabolites showing significantly statistical differences in terms of abundance (Fig. S1). In parallel, an orthogonal projection to latent structures discriminant analysis (OPLS-DA) was performed for each pairwise comparison (Fig. 2C-D). All models presented robust performance metrics ($R^2Y \geq 0.998$ and $Q^2 \geq 0.916$) and allowed the identification of Variable Importance in Projection (VIP) markers (Table S2). Only those markers meeting the discrimination threshold (VIP scores >1) were considered in subsequent analyses. In order to provide insight into the significant impact of each treatment on the metabolome of broccoli by-products, those metabolites meeting the threshold requirements established by the Volcano and OPLS analyses were considered as differentially accumulated metabolites (DAMs). A total of 61 DAMs were detected after encapsulation of the by-products, while 96 and 95 DAMs were reported for BBP and BBPEN, respectively (Table S3). These results indicate a greater modulating effect on the metabolic profile of PEF treatment, as was confirmed through unsupervised analysis. DAMs were subsequently subjected to chemical enrichment analysis (ChemRICH) to identify the chemical classes mainly affected by the treatments (Table S4). The enrichment plot confirmed the minor effect of encapsulation on the metabolic profile (Fig. 2F).

A slight accumulation of glucosinolates and anthocyanins was observed, while flavonols markedly decreased. These trends suggest that encapsulation mainly preserves the most labile compounds rather than promoting a generalized enrichment The thermal and mechanical conditions of spray-drying can degrade or partially transform some polyphenols, whereas others may become physically trapped within the maltodextrin matrix, reducing their apparent extractability (Shahidi et al., 2022). Consequently, encapsulation acts primarily as a protective mechanism, stabilizing heat and oxidation-sensitive compounds such as glucosinolate and anthocyanins, while less stable flavonoids are partially lost during processing.

The ChemRICH for PEF treatment (Fig. 2G) revealed a more distinct metabolic pattern, as confirmed through unsupervised analysis. Although the total semi-quantification results showed no significant differences in glucosinolate content (Fig. 2I), PEF caused a redistribution within this class, decreasing certain aliphatic glucosinolates while increasing their hydrolysis products, particularly isothiocyanates. This behavior may result from the electroporation effect, which promotes limited cell disruption and partial activation of myrosinase, as previously suggested by Frandsen et al. (2014). In parallel, PEF favored the accumulation of phenolic acids, especially hydroxycinnamic and hydroxyphenylpropanoic acids; and lignans, which were the most upregulated phenolic subfamilies. These findings were confirmed by semi-quantification results (Fig. 2I), showing a clear rise in lignans (7-fold increase in relation to BB) and simple phenolic structures such as flavanols and low-molecular-weight (LMW) polyphenols, while more complex flavonoids (flavonols, isoflavones) decreased. Overall, this

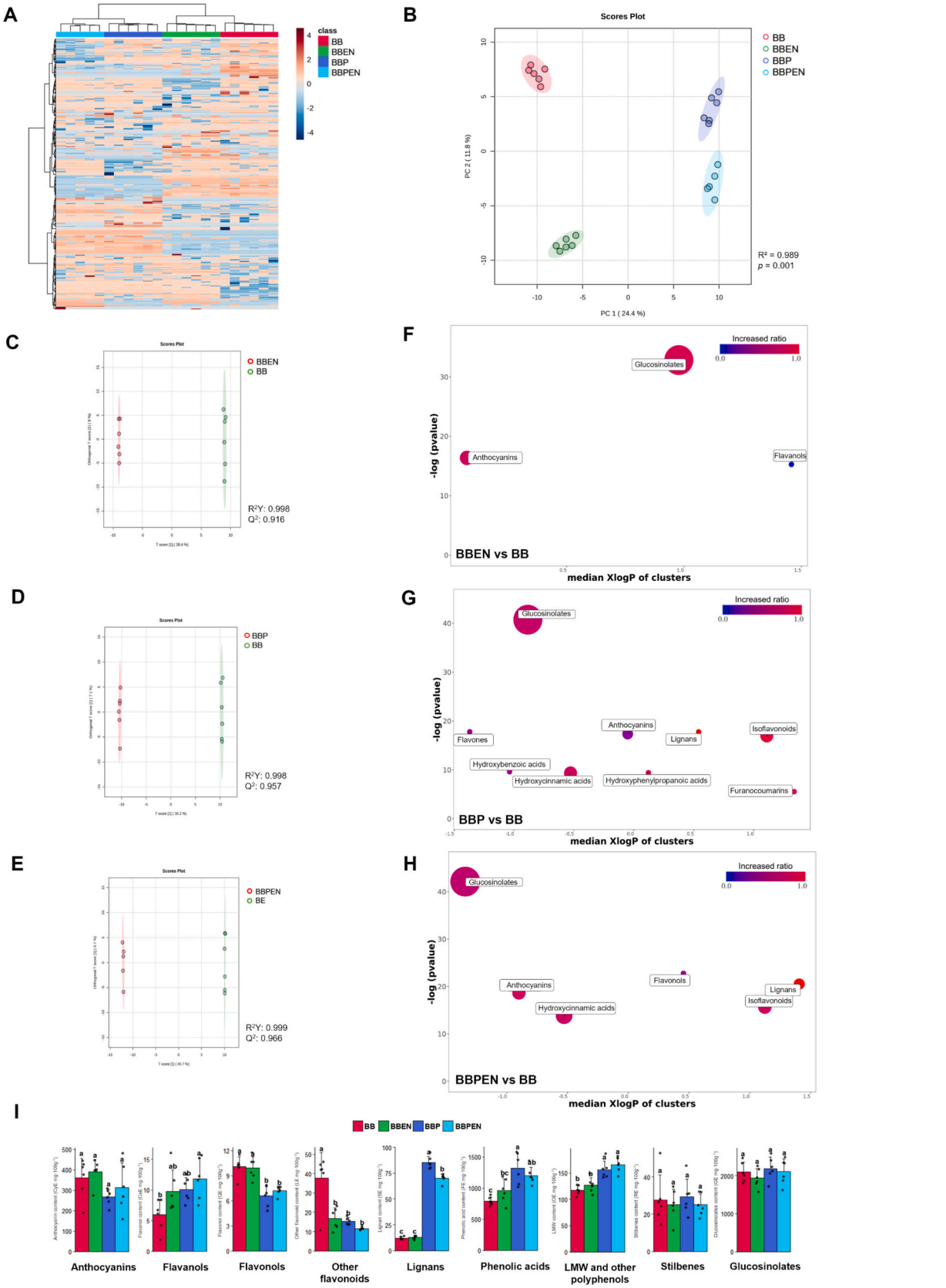

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Fig. 2. Chemometrics analysis on the untargeted UHPLC-QTOF-HRMS metabolomic profiling of broccoli by-products. A. Unsupervised hierarchical cluster analysis (HCA). The dendrogram was built according to the FC−based heatmap (Euclidean distances, Ward's clustering algorithm). The heat scale represents the FC values of each feature with respect to the median value involving all treatments. B. Principal component analysis (PCA), $R^2 = 0.989$, PERMANOVA p-value = 0.001. C-E. OPLS-DA models of BBEN (D); BBP (E) and BBPEN (E) in relation to BB. F– H. Chemical similarity enrichment analysis (ChemRICH) derived from DAMs of BBEN (F), BBP (G) and BBPEN (H) compared to BB. A $\log_2$ (FC) score $\geq | \pm 2|$ (p-value <0.05) and VIP score > 1 were used as criteria for identification of DAMs. Each node represents a group of significantly altered compounds. The size of the node reflects the number of metabolites, and the color indicates the trend: increase (red), decrease (blue) or mixing (purple) compared to the control. Enrichment p-values were calculated using the Kolmogorov-Smirnov test. The y-axis ranks the groups according to significance, and the x-axis shows increasing polarity. I. Semi-quantification of each subfamily of phenolic compounds and glucosinolates. All results (expressed as the average values and standard deviation of six replicates) are expressed in mg·100g^{-1} dry weight of equivalents for each reference compound. Abbreviations: BB: broccoli by-products; BBEN: encapsulated broccoli by-products; BBP: broccoli by-products treated with pulsed electric fields; BBPEN: encapsulated broccoli by-products treated with pulsed electric fields; CaE: catechin equivalents; CyE: cyanidin equivalents; FE: ferulic acid equivalents; GE: gluconapin equivalents; LE: luteolin equivalents; OE: oleuropein equivalents; PC1: first principal component; PC2: second principal component; QE: quercetin equivalents; RE: resveratrol equivalents; SE: sesamin equivalents. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

pattern suggests that PEF promotes the release of cell wall-bound and conjugated phenolics, improving extractability of compounds with higher antioxidant potential. The increase in lignans and hydroxycinnamic acids is consistent with electroporation-induced plasmolysis and the activation of hydrolytic enzymes that liberate bound phenolics (Marín-Sánchez et al., 2024), supporting the role of PEF as a selective enhancer of bioactive phenolic subclasses rather than a general intensifier of all metabolites. This modulatory effect of PEF has also been described in other plant matrices. López-Gámez et al. (2020) reported an increase in TPC (80.2 %) and individual compounds such as p-hydroxybenzoic acid (94.7 %) and chlorogenic acid (74.9 %) after PEF treatment in carrots (López-Gámez et al., 2020). However, this application also reduced the content of ferulic and p-coumaric acids. In addition, another study evaluated that PEF-treated strawberries showed a higher accumulation of phenolic compounds, such as epicatechin gallate, gallic acid, dihydrocaffeic acid and pseudobaptigenin (Zárate-Carbajal et al., 2024).

The broccoli by-products subjected to both PEF and encapsulation (BBPEN) displayed a similar metabolic profile to BBP, revealing 19 accumulated DAMs shared between both treatments (Fig. S2). Lignans, including secoisolariciresinol and arctigenin were consistently accumulated, confirming that the structural disruption caused by PEF was not reversed during encapsulation. However, the slightly lower levels phenolic acids and lignans in BBPEN compared to BBP suggest that part of these compounds became entrapped within the maltodextrin matrix, moderating their extractability. Interestingly, the modest rise in flavanols indicates that encapsulation may have stabilized specific phenolics that are otherwise sensitive to oxidation. Overall, the combined treatment balanced the extraction-enhancing effect of PEF with the protective role of encapsulation, maintaining a rich but more stable phenolic profile.

3.1.2. *Metabolic profiling of pork frankfurters and chemometric analysis*

The results of the untargeted multivariate analysis of the different pork frankfurter formulations are shown in Fig. 3. The unsupervised HCA plot (Fig. 3A) showed six distinct clusters, each corresponding to a specific formulation. However, these clusters originated from two major groups: one including the control samples (FC− and FC+), and another comprising all formulations with broccoli by-products, highlighting the influence of their incorporation on the metabolomic profile. Furthermore, these patterns were confirmed by PCA plot ($R^2 = 0.992$, PERMANOVA $p = 0.001$), with the samples being clearly separated into three groups: control formulations, formulations with untreated or encapsulated by-product (FB and FBEN), and those with PEF-treated by-products (FBP and FBPEN). Furthermore, PC1 explained 30 % of the total variance, mainly attributed to the separation between control and broccoli-containing samples (Fig. 3B). Additionally, control formulations with or without synthetic additives showed very similar profiles in both HCA and PCA, suggesting that these additives had a small impact compared to the effect of broccoli incorporation.

As remarked previously, to further differentiate the incorporation of broccoli by-products regarding the FC− formulation, Volcano analysis was applied, considering ($\log_2$(FC) $\geq | \pm 2|$; $p < 0.05$) (Fig. S3) and subsequently corroborated by OPLS-DA supervised modeling, to identify DAMs. (Table S5–6). The OPLS-DA models (Fig. 3C-F) presented robust performance metrics ($R^2Y \geq 0.997$ and $Q^2 \geq 0.978$). Chemical enrichment analysis showed a clear impact of different subclasses of phenols as well as glucosinolates after incorporation of broccoli by-products. Notably, the isoflavones and hydroxybenzoic acids subclasses were increased in all treatments, indicating an inherent impact of the broccoli regardless of its processing (Fig. 3G-J; Table S7). Additionally, up to 27 accumulated DAMs were detected in all formulations, belonging to different families such as flavonoids, phenolic acids, and glucosinolates. This reinforces that broccoli itself, irrespective to processing, introduces phenolic families absent or scarce in conventional frankfurters. Cumulative $\log_2$(FC) analysis (Fig. 3K) and semi-quantification analyses (Fig. S5) both revealed a generalized enrichment in phenolic acids and isoflavones after broccoli incorporation, consistent with the ChemRICH analysis. In addition, the accumulation of aliphatic glucosinolates, including glucoerucin and its degradation product 4-methylthiobutyl-glucosinolate (erucin), was also pronounced in all formulations. These compounds are highly abundant in broccoli, especially in stem tissues, with concentrations up to 10 and 20 times higher than those in florets and leaves, respectively (Liu et al., 2018).

On the other hand, the cumulative fold change showed differences according to the processing of the by-products. PEF treatment increased the content of lignan, phenolic acid and flavanone subfamilies, and led to a decrease of anthocyanins. Importantly, lignans such as schisandrin and todolactol A were enriched only in PEF-treated formulations (FBP, FBPEN). These results are consistent with the behavior of the treated by-products (Fig. 2), indicating the maintained effect of the PEF treatment after incorporation in a meat emulsion. On the other hand, the cumulative $\log_2$(FC) showed a decrease in glucosinolates after PEF treatment. However, these results contrast with the semi-quantification results (Fig. S3), where PEF presented the highest values (2284 mg·100g^{-1}). This discrepancy could be due to a possible selective transformation of glucosinolates induced by the treatment. Encapsulation, in contrast, exhibited a stabilizing rather than enhancing effect, slightly moderating phenolic acid losses and increasing the relative abundance of flavonols, tyrosols and glucosinolates. This protective effect is particularly relevant given the thermal sensitivity of glucosinolates during frankfurter cooking (up to 75 °C), suggesting that encapsulation mitigated their degradation and improved metabolite retention, as also observed by Rocchetti et al. (2023) and Tolve et al. (2021). In summary, metabolomic analysis demonstrated that the biochemical fingerprint of broccoli by-products was successfully transferred to the meat matrix, modulated by the type of processing applied.

3.2. *Nutritional composition and in vitro antioxidant activity*

The chemical composition, trace element content, antioxidant capacity and TPC of the different frankfurters, as well as the by-products

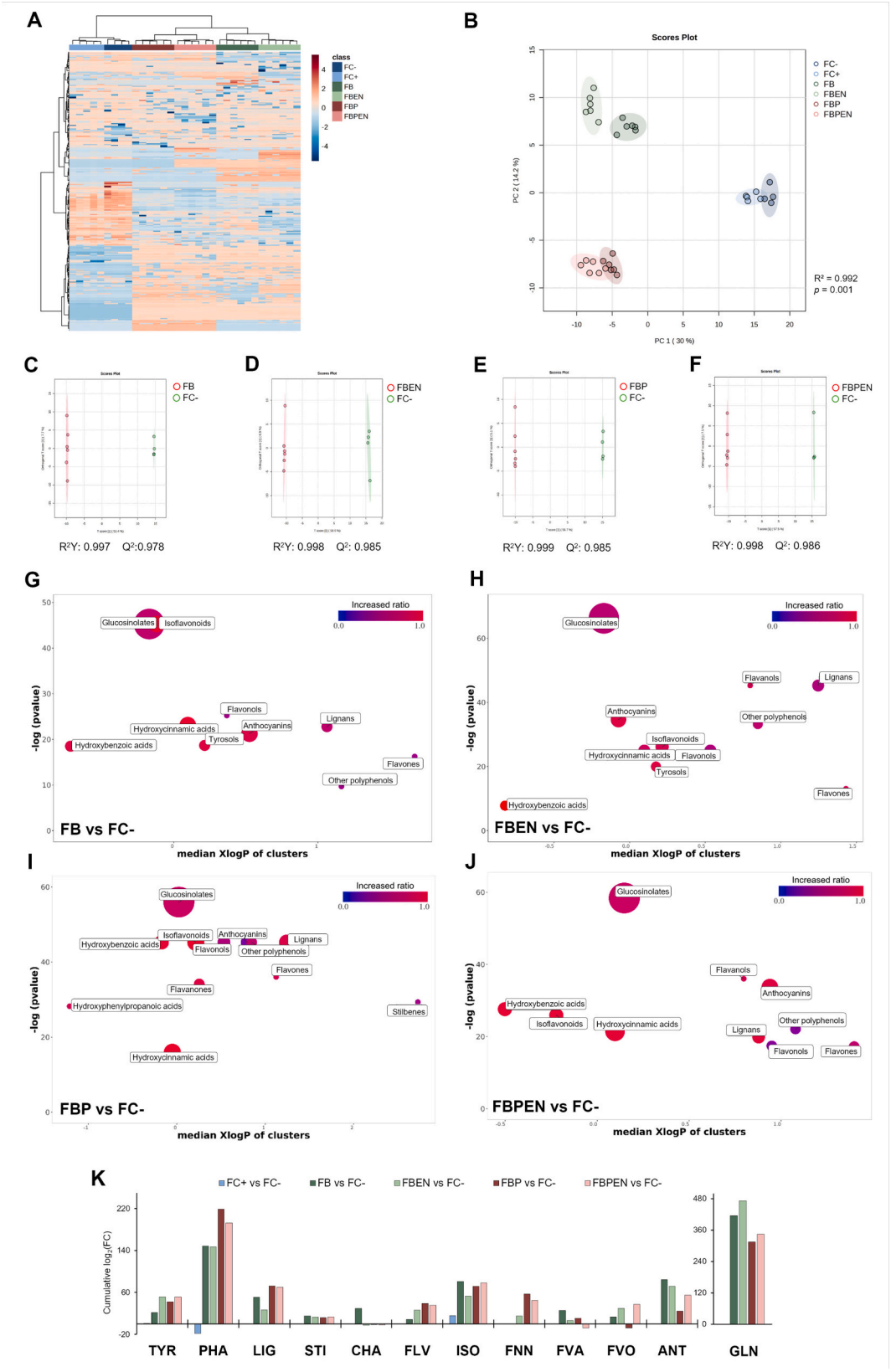

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Fig. 3. Chemometrics analysis on the untargeted UHPLC-QTOF-HRMS metabolomic profiling of pork frankfurters. A. Unsupervised hierarchical cluster analysis (HCA). The dendrogram was built according to the fold-change-based heatmap (Euclidean distances, Ward's clustering algorithm). The heat scale represents the fold-change values of each feature with respect to the median value involving all treatments. B. Principal component analysis (PCA), $R^2 = 0.992$, PERMANOVA p-value = 0.001. C–F. OPLS-DA models of FB (D); FBEN (E), FBP (E) and FBPEN (F) in relation to FC−. G–J. Chemical similarity enrichment analysis (ChemRICH) derived from DAMs of FB (G); FBEN (H), FBP (I) and FBPEN (J) compared to FC−. A $\log_2$ (FC) score $\geq | \pm 2|$ (p-value <0.05) and VIP score > 1 were used as criteria for the identification of DAMs. Each node represents a group of significantly altered compounds. The size of the node reflects the number of metabolites, and the color indicates the trend: increase (red), decrease (blue) or mixing (purple) compared to the control. Enrichment p-values were calculated using the Kolmogorov-Smirnov test. The y-axis ranks the groups according to significance, and the x-axis shows increasing polarity. K. Fold change (FC) analysis of DAMs, resulting from the cumulative $\log_2$(FC) of each chemical subclass. Abbreviations: ANT: anthocyanins; CHA: chalcones; FB: frankfurter with 2 % BB; FBEN: frankfurter with 2 % BBEN; FBP: frankfurter with 2 % BBP; FBPEN: frankfurter with 2 % encapsulated BBPEN; FC−: control frankfurter without additives; FC+: control frankfurter with additives; FLV: flavones; FNN: flavanones; FVA: flavanols; FVO: flavonols; GLN: glucosinolates; ISO: isoflavones; LIG: lignans; PC1: first principal component; PC2: second principal component; PHA: phenolic acids; STI: stilbenes; TYR: tyrosol and other phenolics. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

used, are detailed in Table 2. Comparative analyses were performed using FC+ as a reference, to evaluate the potential of broccoli by-products as natural substitutes for synthetic additives. The reformulation of pork frankfurters with broccoli by-products significantly influenced their protein content and energy value ($p < 0.05$). This reduction can be attributed to the nutritional composition of the dried by-products used, which are characterized by a protein (14.9–18.0 g·100 g^{-1}) and fat (0.9–2.0 g·100 g^{-1}) content substantially lower than the meat matrix. Therefore, their incorporation probably diluted the overall content of these macronutrients in the final product, resulting in low-calorie frankfurters. The incorporation of broccoli by-products significantly increased both insoluble dietary fiber (IDF) and total dietary fiber (TDF) content in frankfurters. This effect can be attributed to its high natural fiber content, especially in its insoluble fraction. In addition, PEF treatment further increased the soluble dietary fiber (SDF) content, and this change was coupled with a reduction in the IDF fraction, suggesting that PEF contributed to the solubilization of insoluble components. These molecular effects have been observed in other food matrices; Qin et al. (2025) observed an increase in the total soluble fraction of wheat bran (7.69 to 12.02 g·100 g^{-1}) after application of the induced electric fields (Qin et al., 2025). Fan et al. (2022) reported that PEF pretreatment (electric field intensity of 6.0 kV·cm^{-1} and 20 pulses) of orange peel was an effective method to improve SDF yield (Fan et al., 2022). PEF treatment reduces the stiffness and crystallinity of cellular components, especially cellulose, which weakens the interactions between cellulose molecules, facilitating their disintegration and release in the form of soluble fiber.

Additionally, Table 2 also describes different trace elements present in the frankfurters and broccoli by-products. The remaining macroelements and trace elements can be found in Table S8. Concentrations of copper (Cu), nickel (Ni), manganese (Mn), strontium (Sr), and titanium (Ti) were significantly increased compared to the control formulation ($p < 0.05$), especially in FB. This enrichment is mainly due to the high natural content of these microelements in BB. Among the increased elements, Cu is an essential minor element involved in enzymatic antioxidant defense and immune response (Mandarano & McGargill, 2023).

Table 2
Proximate composition, trace elements and antioxidant activity of pork frankfurters and broccoli by-products.

	Pork frankfurters					Broccoli by-products	
	FC+	FB	FBEN	FBP	FBPEN	BB	BBP
Proximate composition							
Energy content (kcal·100g^{-1} FW)	213.56 ± 5.71^{a}	179.75 ± 8.96^{b}	196.82 ± 1.26^{ab}	189.63 ± 1.90^{b}	196.52 ± 5.28^{ab}	113.18 ± 5.32^{a}	108.64 ± 0.20^{a}
Moisture (g·100g^{-1} FW)	62.28 ± 0.85^{c}	67.29 ± 1.09^{a}	65.53 ± 0.00^{ab}	67.74 ± 0.34^{a}	64.64 ± 0.02^{bc}	7.68 ± 0.39^{b}	13.39 ± 0.40^{a}
Ash (g·100g^{-1} FW)	1.99 ± 0.04^{a}	2.34 ± 0.34^{a}	2.01 ± 1.83^{a}	1.83 ± 0.11^{a}	2.18 ± 0.08^{a}	16.61 ± 0.17^{a}	15.73 ± 0.06^{a}
Fat (g·100g^{-1} FW)	14.58 ± 0.30^{a}	12.45 ± 0.90^{a}	13.86 ± 0.31^{a}	12.58 ± 0.48^{a}	13.08 ± 0.84^{a}	2.04 ± 0.65^{a}	0.92 ± 0.13^{a}
Protein (g·100g^{-1} FW)	21.26 ± 0.78^{a}	17.48 ± 0.22^{c}	18.22 ± 0.05^{bc}	19.74 ± 0.62^{ab}	19.85 ± 0.12^{a}	18.03 ± 0.03^{a}	14.91 ± 1.03^{a}
Carbohydrates (g·100g^{-1} FW)	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	7.16 ± 2.76^{a}	10.60 ± 1.34^{a}
IDF (g·100g^{-1} FW)	0.47 ± 0.22^{b}	1.82 ± 0.24^{a}	1.79 ± 0.10^{a}	1.80 ± 0.08^{a}	1.96 ± 0.04^{a}	47.20 ± 0.97^{a}	37.80 ± 0.66^{b}
SDF (g·100g^{-1} FW)	0.10 ± 0.13^{a}	0.24 ± 0.05^{a}	0.26 ± 0.09^{a}	0.26 ± 0.07^{a}	0.26 ± 0.05^{a}	1.28 ± 0.61^{b}	6.64 ± 0.38^{a}
TDF (g·100g^{-1} FW)	0.56 ± 0.36^{b}	2.06 ± 0.29^{a}	2.04 ± 0.01^{a}	2.06 ± 0.15^{a}	2.21 ± 0.00^{a}	48.48 ± 1.57^{a}	44.43 ± 0.28^{a}
Trace elements							
Al (mg·100g^{-1} FW)	0.06 ± 0.03^{a}	0.28 ± 0.17^{a}	0.13 ± 0.01^{a}	0.13 ± 0.04^{a}	0.21 ± 0.10^{a}	1.43 ± 0.02^{a}	1.13 ± 0.04^{b}
Cr (mg·100g^{-1} FW)	0.01 ± 0.00^{a}	0.06 ± 0.03^{a}	0.02 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.14 ± 0.01^{a}	0.06 ± 0.01^{b}
Cu (mg·100g^{-1} FW)	0.09 ± 0.02^{b}	0.17 ± 0.02^{a}	0.11 ± 0.01^{ab}	0.09 ± 0.02^{b}	0.08 ± 0.01^{b}	0.41 ± 0.00^{a}	0.40 ± 0.01^{a}
Mn (mg·100g^{-1} FW)	0.13 ± 0.01^{b}	0.19 ± 0.02^{ab}	0.22 ± 0.04^{a}	0.17 ± 0.02^{ab}	0.17 ± 0.01^{ab}	4.36 ± 0.02^{a}	4.24 ± 0.06^{a}
Ni (mg·100g^{-1} FW)	0.01 ± 0.01^{b}	0.02 ± 0.00^{a}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.14 ± 0.02^{a}	0.08 ± 0.00^{a}
Si (mg·100g^{-1} FW)	0.17 ± 0.02^{a}	0.66 ± 1.14^{a}	0.46 ± 0.06^{a}	0.57 ± 0.27^{a}	0.55 ± 1.03^{a}	4.57 ± 0.40^{a}	2.41 ± 0.09^{b}
Sr (mg·100g^{-1} FW)	0.07 ± 0.00^{b}	0.24 ± 0.04^{a}	0.13 ± 0.03^{b}	0.11 ± 0.01^{b}	0.11 ± 0.00^{b}	3.74 ± 0.00^{a}	3.35 ± 0.04^{b}
Antioxidant activity and TPC							
TPC (mg GAE·100g^{-1} FW)	15.17 ± 1.46^{bc}	19.22 ± 2.69^{ab}	22.51 ± 2.31^{a}	21.84 ± 2.67^{a}	23.95 ± 1.26^{a}	120.23 ± 10.25^{b}	277.16 ± 16.88^{a}
FRAP (μM TE·100g^{-1} FW)	11.75 ± 1.11^{d}	17.69 ± 1.38^{c}	28.76 ± 1.63^{b}	25.20 ± 2.02^{b}	44.45 ± 0.24^{a}	471.31 ± 50.69^{b}	604.90 ± 45.15^{a}
ABTS (μM TE·100g^{-1} FW)	29.41 ± 2.29^{c}	31.86 ± 0.46^{bc}	39.92 ± 1.41^{a}	35.92 ± 2.35^{ab}	39.12 ± 2.18^{a}	245.99 ± 73.25^{b}	420.45 ± 37.97^{a}
DPPH (μM TE·100g^{-1} FW)	19.12 ± 5.25^{a}	24.36 ± 3.02^{a}	27.60 ± 3.43^{a}	28.84 ± 3.02^{a}	24.86 ± 6.53^{a}	450.21 ± 73.07^{b}	625.79 ± 59.12^{a}

Results were expressed as the mean ± standard deviation. For each frankfurter formulation, different letters within the same row indicate statistically significant differences, according to Tukey HSD ($p < 0.05$). For broccoli by-products, different letters within the same row indicate statistically significant differences, as determined by Student's t-test ($p < 0.05$). **Abbreviations:** ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); BB: broccoli by-products; BBEN: encapsulated broccoli by-products; BBP: broccoli by-products treated with pulsed electric fields; BBPEN: encapsulated broccoli by-products treated with pulsed electric fields; DPPH, 2,2-diphenyl-1-pycrylhydrazyl; FB: frankfurter with 2 % BB; FBEN: frankfurter with 2 % BBEN; FBP: frankfurter with 2 % BBP; FBPEN: frankfurter with 2 % encapsulated BBPEN; FC+: control frankfurter with additives; FRAP, ferric reducing antioxidant potential; FW: fresh weight; GAE: gallic acid equivalents; IDF: insoluble dietary fiber; SDF: soluble dietary fiber; TDF: total dietary fiber; TE: trolox equivalents; TPC: total phenolic content.

Mn is also an essential trace element, which has a minor capacity as a cofactor for cancer-preventive catalysts, helping protect the body against oxygen-free radicals produced during oxidative stress (Islam et al., 2023). In contrast, PEF treatment did not increase the content of trace elements and even caused a decrease in certain elements, including aluminum (Al), chromium (Cr), silicon (Si), and strontium (Sr). This decrease could be explained by the electropermeabilization effect induced by PEF, which disrupts cell membranes and walls, facilitating

Table 3
Evolution of the oxidative and microbiological quality of pork frankfurters during 14 days of refrigerated storage.

Formulation	Days of storage				Formulation	Days of storage			
	0	5	9	14		0	5	9	14
L*					**pH**				
FC+	70.58 ± 0.16^{Cc}	71.25 ± 0.39^{Cbc}	73.54 ± 0.42^{Aa}	71.89 ± 0.30^{Cb}	FC+	5.97 ± 0.02^{Ab}	6.10 ± 0.01^{Aa}	6.12 ± 0.02^{Aa}	6.08 ± 0.03^{Ba}
FB	71.00 ± 0.12^{Bc}	71.89 ± 0.45^{BCb}	72.42 ± 0.17^{Bab}	72.62 ± 0.03^{Ba}	FB	5.96 ± 0.02^{ABab}	5.91 ± 0.03^{Bb}	5.95 ± 0.01^{Bab}	6.00 ± 0.04^{Ba}
FBEN	71.97 ± 0.08^{Ac}	72.98 ± 0.14^{Ab}	72.25 ± 0.27^{BCc}	73.74 ± 0.14^{Aa}	FBEN	5.93 ± 0.01^{Bab}	5.90 ± 0.02^{Bb}	5.97 ± 0.02^{Ba}	5.84 ± 0.03^{Cc}
FBP	69.27 ± 0.09^{Db}	70.33 ± 0.03^{Da}	69.04 ± 0.17^{Db}	70.52 ± 0.17^{Da}	FBP	5.97 ± 0.02^{Aa}	5.94 ± 0.02^{Ba}	5.97 ± 0.02^{Ba}	5.83 ± 0.04^{Ca}
FBPEN	69.55 ± 0.14^{Dc}	72.00 ± 0.08^{Bb}	71.65 ± 0.16^{Cb}	73.22 ± 0.23^{Aa}	FBPEN	5.97 ± 0.01^{Ac}	5.95 ± 0.01^{Bc}	6.15 ± 0.08^{Ab}	6.33 ± 0.01^{Aa}
a*					**TCC (log UFC·g^{-1})**				
FC+	11.52 ± 0.03^{Aa}	10.30 ± 0.11^{Ab}	6.08 ± 0.74^{Ac}	5.72 ± 0.23^{Ac}	FC+	1.70 ± 0.08^{Bc}	1.78 ± 0.21^{Dc}	3.64 ± 0.08^{Cb}	8.16 ± 0.06^{Aa}
FB	4.40 ± 0.01^{Ca}	2.46 ± 0.04^{Cd}	2.72 ± 0.03^{Dc}	3.15 ± 0.02^{Db}	FB	1.95 ± 0.08^{ABc}	2.43 ± 0.02^{Cc}	5.41 ± 0.16^{Bb}	5.98 ± 0.17^{Ba}
FBEN	4.30 ± 0.02^{Da}	4.14 ± 0.03^{Bb}	2.81 ± 0.01^{CDc}	$1.82 \pm 0.02E^{d}$	FBEN	1.82 ± 0.10^{ABb}	3.52 ± 0.04^{Ba}	3.74 ± 0.17^{Ca}	3.90 ± 0.24^{Ca}
FBP	5.00 ± 0.05^{Ba}	4.02 ± 0.07^{Bc}	3.67 ± 0.06^{BCd}	4.60 ± 0.06^{Bb}	FBP	1.86 ± 0.06^{ABc}	4.40 ± 0.05^{Ab}	5.39 ± 0.14^{Ba}	5.40 ± 0.07^{Ba}
FBPEN	5.06 ± 0.01^{Ba}	4.17 ± 0.06^{Bb}	4.21 ± 0.08^{Bb}	3.94 ± 0.06^{Cc}	FBPEN	2.05 ± 0.06^{Ad}	3.57 ± 0.07^{Bc}	6.88 ± 0.04^{Ab}	7.58 ± 0.17^{Aa}
b*					**TVC (log UFC·g^{-1})**				
FC+	10.62 ± 0.05^{Dd}	11.66 ± 0.17^{Db}	13.41 ± 0.10^{Ca}	11.34 ± 0.03^{Dc}	FC+	2.84 ± 0.58^{Ac}	3.11 ± 0.10^{Bc}	8.56 ± 0.02^{Ab}	10.35 ± 0.01^{Ca}
FB	15.74 ± 0.03^{Cab}	15.85 ± 0.09^{Ba}	15.64 ± 0.06^{Bb}	15.88 ± 0.03^{Ca}	FB	2.70 ± 0.12^{Ad}	5.59 ± 0.06^{Ac}	8.57 ± 0.08^{Ab}	11.18 ± 0.04^{Ba}
FBEN	15.68 ± 0.02^{Cb}	15.12 ± 0.02^{Cc}	16.19 ± 0.08^{ABa}	16.34 ± 0.11^{Ba}	FBEN	2.48 ± 0.21^{Ad}	5.89 ± 0.62^{Ac}	8.42 ± 0.02^{Ab}	11.53 ± 0.27^{Aa}
FBP	16.75 ± 0.02^{Ba}	16.36 ± 0.46^{ABa}	16.46 ± 0.29^{Aa}	16.73 ± 0.12^{Aa}	FBP	2.40 ± 0.12^{Ad}	6.32 ± 0.10^{Ac}	8.39 ± 0.16^{Ab}	10.87 ± 0.11^{Ca}
FBPEN	16.89 ± 0.07^{Aa}	16.66 ± 0.08^{Aa}	16.69 ± 0.41^{Aa}	16.81 ± 0.11^{Aa}	FBPEN	2.88 ± 0.04^{Ad}	6.37 ± 0.01^{Ac}	9.05 ± 0.47^{Ab}	11.63 ± 0.01^{Aa}
ΔE					***E. coli***				
FC+	0.00 ± 0.00^{Ac}	1.78 ± 0.28^{BCb}	6.81 ± 0.80^{Aa}	5.99 ± 0.17^{Aa}	FC+		< 10		
FB	0.00 ± 0.00^{Ac}	2.15 ± 0.14^{ABb}	2.21 ± 0.06^{Bb}	2.05 ± 0.06^{Db}	FB		< 10		
FBEN	0.00 ± 0.00^{Ad}	1.17 ± 0.15^{Dc}	1.63 ± 0.01^{Bb}	3.12 ± 0.09^{Ca}	FBEN		< 10		
FBP	0.00 ± 0.00^{Ac}	1.53 ± 0.24^{CDa}	1.41 ± 0.10^{Ba}	1.32 ± 0.23^{Ea}	FBP		< 10		
FBPEN	0.00 ± 0.00^{Ac}	2.62 ± 0.18^{Ab}	2.30 ± 0.34^{Bb}	3.85 ± 0.32^{Ba}	FBPEN		< 10		
TBARS (mg MDA·100g^{-1})					***Salmonella* spp.**				
FC+	0.02 ± 0.00^{Bc}	0.04 ± 0.01^{Ab}	0.19 ± 0.00^{Aa}	0.20 ± 0.00^{Aa}	FC+	Absence	–	–	Absence
FB	0.06 ± 0.00^{Ad}	0.13 ± 0.04^{Ac}	0.14 ± 0.01^{Ab}	0.19 ± 0.00^{Aa}	FB	Absence	–	–	Absence
FBEN	0.06 ± 0.01^{Abc}	0.04 ± 0.00^{Ac}	0.18 ± 0.04^{Aa}	0.14 ± 0.02^{Bab}	FBEN	Absence	–	–	Absence
FBP	0.06 ± 0.00^{Aa}	0.13 ± 0.04^{Aa}	0.11 ± 0.00^{Aa}	0.12 ± 0.01^{Ba}	FBP	Absence	–	–	Absence
FBPEN	0.04 ± 0.00^{Ab}	0.12 ± 0.03^{Aa}	0.12 ± 0.01^{Aa}	0.15 ± 0.02^{Ba}	FBPEN	Absence	–	–	Absence
MetMb (g·100g^{-1})					***L. monocytogenes***				
FC+	29.91 ± 0.65^{Aa}	28.70 ± 2.30^{Aa}	29.41 ± 1.58^{ABa}	29.93 ± 0.30^{Ba}	FC+	Absence	–	–	Absence
FB	30.14 ± 0.98^{Aa}	29.61 ± 0.49^{Aab}	26.79 ± 0.84^{Bb}	30.86 ± 0.37^{BCa}	FB	Absence	–	–	Absence
FBEN	32.01 ± 0.35^{Aa}	28.04 ± 1.40^{Aa}	31.65 ± 1.53^{Aa}	32.05 ± 0.06^{Aa}	FBEN	Absence	–	–	Absence
FBP	29.93 ± 1.63^{Aa}	28.84 ± 1.04^{Aa}	30.88 ± 0.43^{ABa}	28.02 ± 0.31^{Ca}	FBP	Absence	–	–	Absence
FBPEN	32.14 ± 2.07^{Aa}	29.22 ± 0.76^{Aa}	28.03 ± 0.95^{ABa}	32.01 ± 0.46^{Aa}	FBPEN	Absence	–	–	Absence

A–E: different letters within the same column indicate significant differences between frankfurter formulations (Tukey HSD; $p < 0.05$); $^{a–d}$: different letters within the same row indicate significant differences between days of storage (Tukey HSD; $p < 0.05$). **Abbreviations:** FB: frankfurter with 2 % BB; FBEN: frankfurter with 2 % BBEN; FBP: frankfurter with 2 % BBP; FBPEN: frankfurter with 2 % encapsulated BBPEN; FC+: control frankfurter with additives; MetMb: metmyoglobin; TBARS: thiobarbituric acid reactive substances; TCC: total coliform count; TVC: total viable count.

the release of free ions and loosely bound minerals.

On the other hand, the impact of broccoli by-products reformulation on phenolic content and antioxidant activity was evaluated through different assays (FRAP, ABTS and DPPH). Reformulation led to a significant increase in TPC over control with synthetic additives. This increase was more pronounced in frankfurters containing PEF-treated by-products, with FBPEN showing the greatest enhancement. These findings are consistent with the results of the untargeted analysis, which revealed a general increase across all phenolic families (Fig. 3), particularly, isoflavones and hydroxybenzoic acids in all broccoli-enriched formulations. Among these subclasses, glycosylated isoflavones, sinapoylquinic acid, and dihydroxyphenyl valerolactone derivatives were found to have high antioxidant capacity due to its chemical structure, which includes several hydroxyl groups capable of donating electrons or hydrogen atoms to neutralize free radicals. In addition, PEF treatment increased the TPC content of the by-products by 130.8 %, which can be correlated with the reported increase in lignans and phenolic acids (Fig. 2I). This high TPC content resulted in a high antioxidant capacity of by-products and reformulated frankfurters. Lignans such as schisandrin and todolactol A, highly accumulated in FBP and FBPEN (log_2(FC) = 20.1–21.3), are known for their strong redox activity and capacity to scavenge reactive oxygen species (Herrera et al., 2020; Kopustinskiene & Bernatoniene, 2021), contributing to the high FRAP and ABTS values observed. Finally, frankfurters containing encapsulated by-products (BBEN) obtained the best results in FRAP and ABTS assays, probably due to the protective effect of maltodextrin on phenolic stability. Altogether, these results demonstrate that metabolite-level enrichment of phenolic compounds directly explains the improved antioxidant functionality observed in the PEF-treated and encapsulated formulations, indicating that broccoli by-products in the reformulation of pork frankfurters could serve as an effective natural alternative to synthetic antioxidants.

3.3. Shelf-life analysis of pork frankfurters

The oxidative stability of the pork frankfurters after 14 days of refrigerated storage was evaluated according to color (CIEL*a*b*), TBARS and MetMb parameters, which are shown in Table 3. The L* parameter (lightness) showed significant differences between formulations and throughout refrigerated storage ($p < 0.05$). In general, the FBP formulation showed the lowest L* values at all time points, indicating a darker appearance. PEF treatment altered the color of the broccoli by-products (Table S9), possibly due to the increased release of phenolic compounds and the partial inactivation of polyphenol oxidase (PPO) (Meneses et al., 2013), which may have promoted their enzymatic oxidation and subsequent browning. As for the redness values (a*), control frankfurter initially showed the highest values, followed by a significant decrease over time ($p < 0.05$), due to the higher oxidation of the iron (II) heme group (Domínguez et al., 2019). In contrast, FBP and FBPEN showed higher reddening stability, possibly due to the protective effects of PEF treatment and encapsulation, respectively. Regarding total color differences (ΔE), FC+ also showed the greatest chromatic changes from day 9 onwards, reaching values higher than 6, indicating visually perceptible color differences (Altmann et al., 2022). In contrast, the FBP and FBEN formulations showed the lowest ΔE values throughout storage, suggesting higher color stability, probably due to the antioxidant protection provided by the broccoli by-products.

Lipid oxidation values (TBARS) increased progressively during storage, reaching their highest level on day 14. At this point, FC+ showed the highest levels of lipid oxidation, exceeding the commonly accepted threshold of 0.2 mg MDA·100g^{-1}, above which sensory perceptions of rancidity may occur (Mojaddar Langroodi et al., 2021). In contrast, formulations containing broccoli by-products, in particular FBEN, FBP and FBPEN, showed significantly lower TBARS values after 14 days of storage, indicating that PEF treatment and the encapsulation of broccoli by-products positively influence the inhibition of lipid oxidation. This effect against oxidation may be due to the high in vitro antioxidant activity of broccoli by-products, as well as to their high polyphenol content, as exhibited by chemometric analysis. Specifically, PEF and encapsulation increased the presence and stability of these bioactive polyphenols, probably preserving their capacity against lipid oxidation more effectively than the synthetic antioxidants. Other authors have reported the capacity of various agricultural food by-products to inhibit lipid oxidation in meat products. Specifically, by-products from grapes (Carta et al., 2025), açai (Lima Campos et al., 2025), or artichoke (Ayuso, Quizhpe, et al., 2024) have been used as natural meat antioxidants. This higher oxidative stability observed in FBP and FBPEN aligns with the lower total color difference (ΔE) and better preservation of a* values, reflecting better retention of red color during storage. However, metmyoglobin (MetMb) formation did not show clear differences between treatments or consistent trends, suggesting that color deterioration is more related to lipid oxidation than protein oxidation.

In addition, Table 3 also details the results related to microbiological stability during refrigerated storage. Throughout the refrigerated storage period, pH increased moderately in FC+, whereas it remained more stable in some samples with broccoli (FBEN and FBP), suggesting a modulating effect of the broccoli by-products on product stability. As for microbiological analysis, no *Salmonella* spp., *L. monocytogenes* or *E. coli* were detected in any of the samples, confirming the food safety of all formulations.

Regarding total coliform count (TCC), on day 14 of storage, formulations containing broccoli by-products exhibited superior microbial control compared to FC+, which included conventional preservatives such as sodium nitrite (E-250). Specifically, the three formulations with the lowest microbial counts at this time point were FBEN, FBP and FB. This potent antimicrobial effect may be mechanistically linked to the metabolomic profile of the reformulated frankfurters (Fig. 3). In particular, all formulations showed high levels of glucosinolates and isothiocyanates (3-methylthiopropyl-desulfoglucosinolate (log_2(FC) = 16.7–19.0) and 4-methylthiobutyl glucosinolate (log_2(FC) = 15.5–16.8). These compounds, especially isothiocyanates, can react with thiol (-SH) groups and amines in bacterial membrane proteins, as well as inhibiting essential enzymes (Bischoff, 2021). On the other hand, this generalized antimicrobial activity can be attributed to the accumulation of isoflavones or phenolic acids, which have also been shown to have a high inhibition of Gram-negative bacteria due to their membrane-active properties, which cause leakage of cell constituents (Lobiuc et al., 2023). Specifically, flavonoids such as sinapoylquinic acid have been shown to inhibit *Bacillus subtilis*, *E. coli*, and *Pseudomonas syringae* (Nićiforović & Abramovič, 2014). In addition, lignans such as schisandrin A, present in FBP samples, have been shown to be effective against *Pseudomonas aeruginosa* (Xiao et al., 2024). Moreover, formulation with encapsulated broccoli (FBEN) (3.9 log CFU·g^{-1}) showed the longest and most effective antimicrobial effect in maintaining the lowest TCC levels. Microencapsulation with maltodextrin may have prolonged the effect of these antimicrobial and antioxidant agents, improving shelf life. Other authors have also emphasized the potential antimicrobial effect of microencapsulation technology when applied to red onion (Sarvinehbaghi et al., 2021) and green tea (Özvural et al., 2016) in meat products. Nevertheless, no appreciable differences in TVC were observed between formulations on days 9 and 14, suggesting that broccoli by-products were comparable to the synthetic additives in controlling overall microbial growth, exerting a stronger antimicrobial effect on specific microbial groups rather than showing broad-spectrum activity against all pathogenic bacteria.

3.4. Sensory evaluation

Fig. 4 presents the sensory evaluation results of the frankfurter samples, assessed by a trained panel. No significant differences ($p < 0.05$) were observed between formulations in texture-related attributes such as hardness, fatty flavor, adhesiveness, and chewiness. These

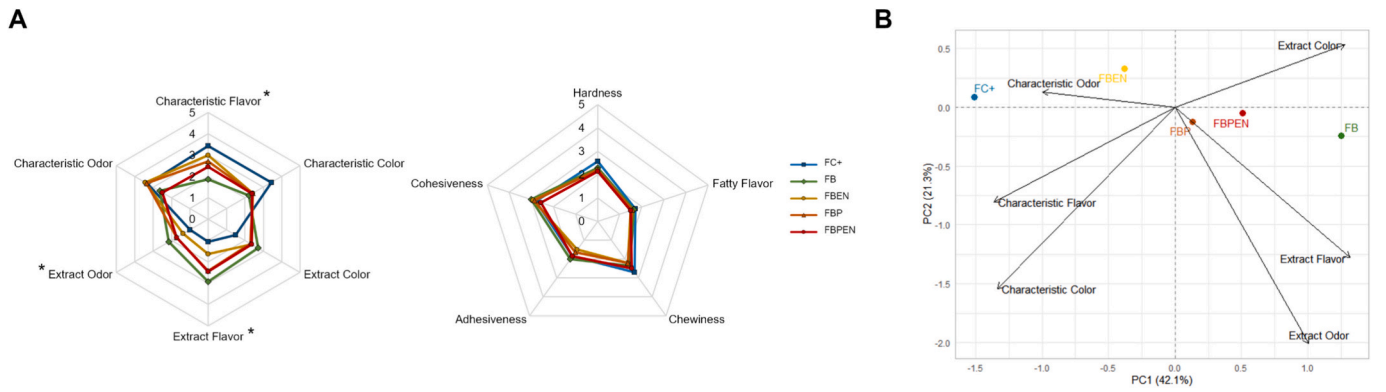

Fig. 4. Sensory analysis of frankfurter formulations. Radar plots showing the sensory attributes of the different formulations (A), grouped by (left) appearance/flavor and (right) texture-related descriptors. Principal component analysis (PCA) (B) biplot illustrating the distribution of frankfurter samples according to their sensory profiles and the contribution of each attribute to sample differentiation. (*$p < 0.05$). Abbreviations: FB: frankfurter with 2 % BB; FBEN: frankfurter with 2 % BBEN; FBP: frankfurter with 2 % BBP; FBPEN: frankfurter with 2 % encapsulated BBPEN; FC+: control frankfurter with additives.

results suggest that the inclusion of broccoli by-products, regardless of the treatment (untreated, PEF-treated or encapsulated), did not negatively impact the overall texture perception of the reformulated frankfurters, maintaining acceptable levels comparable to the control formulation. In contrast, the trained panel was able to discriminate the presence of broccoli by-products in the formulations, as significant differences were observed in extract-related sensory attributes, including characteristic flavor, extract odor and extract flavor. For an in-depth analysis of the sensory recognition of broccoli by-products among different formulations, a PCA plot of the extract-related sensory scores was performed, as shown in Fig. 4B. The PCA plot revealed that the FB formulation was the most distinct from the control (FC+), clustering with extract flavor and odor attributes, which are strongly associated with PC1. Formulations with PEF-treated and encapsulated by-products (FBEN, FBP, FBPEN) clustered closer to FC+ indicating that these treatments masked the sensory impact of the by-products. The application of electric pulses reduced the extract flavor and odor, resulting in a closer alignment to FC+. This trend aligns with the metabolic profiling results of frankfurters (Fig. 3), which showed that PEF treatment modulated the glucosinolate composition by partially converting certain aliphatic glucosinolates into isothiocyanates and reducing anthocyanins and flavonols associated with bitter and astringent sensations (Bell et al., 2018). In particular, the relative decrease of compounds such as cyanidin 3-O-(6″-acetyl-glycoside) and the transformation of sulfur-containing metabolites likely mitigated the vegetal and sulfurous notes characteristic of Brassicaceae. Moreover, FBEN scored similarly to the control in characteristic flavor and odor (Table S10). These results suggest that encapsulation played a key role in reducing the sensory perception of broccoli by-products by modulating the release of flavors and odors typically associated with broccoli. Similar improvements in sensory acceptability have been reported for meat products enriched with encapsulated plant extracts, such as *Satureja khuzestanica* (Pabast et al., 2018), *Artemisia dracunculus* L. (Zhang et al., 2020), or *Myrciaria cauliflora* (Baldin et al., 2016), where encapsulation effectively reduced the impact of strong herbal notes.

4. Conclusions

Broccoli by-products induced a favorable metabolomic modulation when incorporated into pork frankfurters, characterized by the accumulation of polyphenols and glucosinolates. Furthermore, PEF treatment may have enhanced the levels of cell wall-bound polyphenols, particularly phenolic acids and lignans, through enzyme-mediated hydrolysis triggered by vacuolar disruption, while modulating the degradation of glucosinolates into bioactive isothiocyanates. In parallel, encapsulation further preserved and improved glucosinolate stability. These changes resulted in a significant improvement in antioxidant activity, especially in the PEF-treated and encapsulated formulations.

From a nutritional and technological commercial perspective, broccoli by-products acted as effective substitutes for synthetic additives, conferring improved oxidative and microbial stability during refrigerated storage. Frankfurters with PEF-treated or encapsulated by-products showed reduced lipid oxidation, better color retention, and stronger inhibition of coliform growth, even outperforming preservative-containing controls. Sensory analysis supported these findings, especially for encapsulated by-products, which mitigated Brassicaceae-associated odors and flavors without compromising textural attributes. Overall, broccoli by-products demonstrated a dual role as functional enhancers, while meeting consumer demand for natural and clean-label solutions. This approach supports the sustainable valorization of agro-industrial waste and offers new opportunities for innovation in the development of healthier meat formulations.

CRediT authorship contribution statement

Pablo Ayuso: Writing – review & editing, Writing – original draft, Software, Investigation, Formal analysis, Data curation, Conceptualization. **Jhazmin Quizhpe:** Methodology, Formal analysis, Data curation. **Javier Marín-Sánchez:** Resources, Conceptualization. **Leilei Zhang:** Validation, Software, Data curation. **Pascual García-Pérez:** Writing – review & editing, Supervision, Software, Formal analysis, Data curation, Conceptualization, Funding acquisition, Investigation. **Luigi Lucini:** Supervision, Software, Conceptualization. **Gema Nieto:** Writing – original draft, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Funding

This study is part of the Agroalnext program and has been funded by the Ministry of Science, Innovation and Universities (MCIU) through the European Union Next GenerationEU (PRTR-C17.I1), by the Regional Government of Murcia-Fundación Séneca, and by the research project PID2021-123628OB-C44, funded by MCIN/AEI/10.13039/501100011033 and co-financed by the European Regional Development Fund (ERDF), "A way of making Europe". Pascual García-Pérez acknowledges financial support through the Ramón y Cajal program of the Ministry of Science, Innovation and Universities, the State Research Agency (reference: RYC2023-044123-I) and the European Social Fund Plus (FSE+), and through the ATTRACT-RYC program of the University of Murcia. Appreciation is extended to Ibero-American Program on Science and Technology (CYTED – IBEROCIRCULAR, 323RT0142).

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2025.117951.

Data availability

Data are included as Supplementary Material.

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