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BIOACTIVITY OF BROCCOLI BY-PRODUCTS IN PORK SAUSAGES: EFFECT OF PULSED ELECTRIC FIELDS AND ENCAPSULATION IN A CACO-2/RAW 264.7 INFLAMMATORY MODEL

Pablo Ayuso, Luigi Lucini, Leilei Zhang, Jhazmin Quizhpe, Rocío Peñalver, Pascual García-Pérez, Gema Nieto*

Department of Food Technology, Nutrition and Food Science, Veterinary Faculty, University of Murcia, Regional Campus of International Excellence "Campus Mare Nostrum", 30100 Murcia, Spain.

*Corresponding author email: gnieto@um.es

I. INTRODUCTION

Consumers are increasingly demanding clean-label meat products with natural ingredients, prompting the search for alternatives to synthetic additives. Broccoli (*Brassica oleracea var. italica*) and its by-products (stems, florets and leaves) represent a valuable source of phenolic compounds with recognized antioxidant and anti-inflammatory properties [1], and their use in functional meat products has attracted the interest of the meat industry. However, the bioaccessibility and efficacy of these compounds may vary depending on processing strategies. This study aimed to evaluate the impact of different broccoli extract treatments (simple, encapsulated, treated with pulsed electric fields (PEF), and PEF-treated encapsulated) on the antioxidant and anti-inflammatory potential of pork sausages. It was hypothesized that pulsed electric fields and encapsulation would enhance the stability and anti-inflammatory bioactivity of broccoli-derived compounds during digestion and in a co-culture inflammation model.

II. MATERIALS AND METHODS

Six pork sausage formulations were developed (Table 1), including a control with synthetic additives (SC-A), a clean-label control (SC-WA), and formulations incorporating broccoli extract (SB), encapsulated broccoli extract (SBe), PEF-treated broccoli extract (SBP), and encapsulated PEF-treated extract (SBPe). Broccoli by-products (stems, leaves, and florets) were cut, immersed in 0.5% citric acid, and dried (70 °C for 1 h + 55 °C for 40 h). The dried material was ground, sieved (250 µm), and homogenized in water (1:20 w/v) with 2.5 g maltodextrin for encapsulation by spray-drying (BÜCHI B290). PEF treatment was applied to fresh cubes (3 cm^3) in a semicircular chamber (1.5 kV/cm, 220 pulses, 10 µs) using the EPULSUS®-PM1-10 system. All samples were subjected to *in vitro* gastrointestinal digestion following the INFOGEST 2.0 protocol [2].

Table 1 – Pork sausages formulation (g).

	Pork lean	Pork fat	Ice	Salt	Commercial blend **with** additives	Commercial blend **without** additives	Broccoli Extract	Encapsulated Broccoli Extract	Broccoli PEF Extract	Encapsulated Broccoli PEF Extract
SC-WA	698.2	149.8	323.5	17.8	-	10.2	-	-	-	-
SC-A	698.2	149.8	323.5	17.8	10.8	-	-	-	-	-
SB	698.2	149.8	323.5	17.8	-	10.2	24	-		-
SBe	698.2	149.8	323.5	17.8	-	10.2	-	32	-	-
SBP	698.2	149.8	323.5	17.8	-	10.2	-	-	24	-
SBPe	698.2	149.8	323.5	17.8	-	10.2	-	-	-	32

Antioxidant activity and total phenolic content (TPC) were measured in oral, gastric, and intestinal digests through FRAP [1], ABTS [2] and Folin–Ciocalteu methods [3]. To assess anti-inflammatory and anti-oxidative effects, intestinal digests were tested in a Caco-2/RAW 264.7 co-culture. Caco-2 cells were seeded on Transwell® inserts and differentiated for 21 days. RAW 264.7 macrophages were cultured in 12-well plates and co-incubated with the Caco-2 inserts. After exposure to the digested samples, inflammation was induced with LPS (1 µg/mL). Nitric oxide (NO) production was quantified using a colorimetric kit (Thermo Scientific, Rockford, IL, USA) based on enzymatic conversion of nitrate to nitrite and detection with Griess reagent. Intracellular ROS were measured using DCFH-DA staining and flow cytometry (λ_{ex} = 488 nm, λ_{em} = 530 nm).

III. RESULTS AND DISCUSSION

Figure 1 shows that intestinal digests of sausages with broccoli extracts exhibited higher antioxidant activity and TPC than both controls. The SBPe formulation demonstrated the highest FRAP values and TPC in the intestinal phase ($p < 0.05$), indicating a synergistic effect of PEF and encapsulation on compound stability and release. Oral and gastric phases showed lower values, emphasizing the importance of intestinal release for functional efficacy.

In the inflammation model (Figure 2), digests from all broccoli-treated sausages reduced ROS and NO production in LPS-stimulated RAW264.7 cells compared to the SC-WA control ($p < 0.05$). SBPe and SBP displayed the most significant reductions, suggesting enhanced anti-inflammatory potential. These results support the hypothesis that processing technologies such as PEF and encapsulation can improve the functionality of natural extracts in meat matrices.

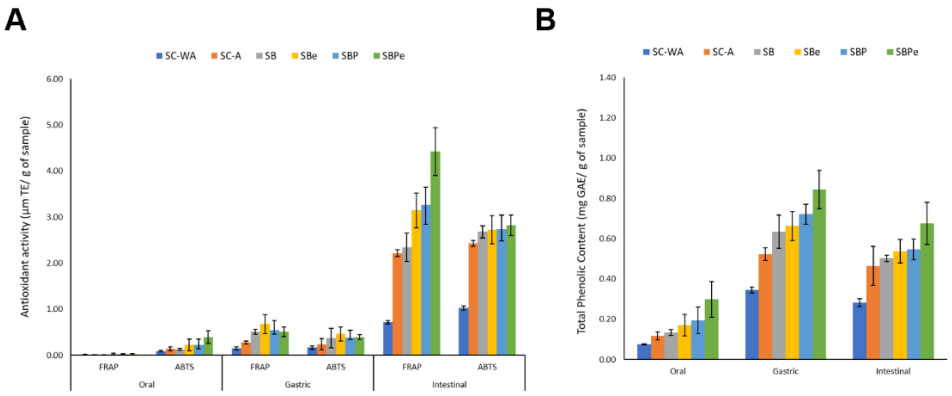


Figure 1. Antioxidant activity **(A)** and Total Phenolic Content (TPC) **(B)** after Oral, Gastric and Intestinal digestion of pork sausages.

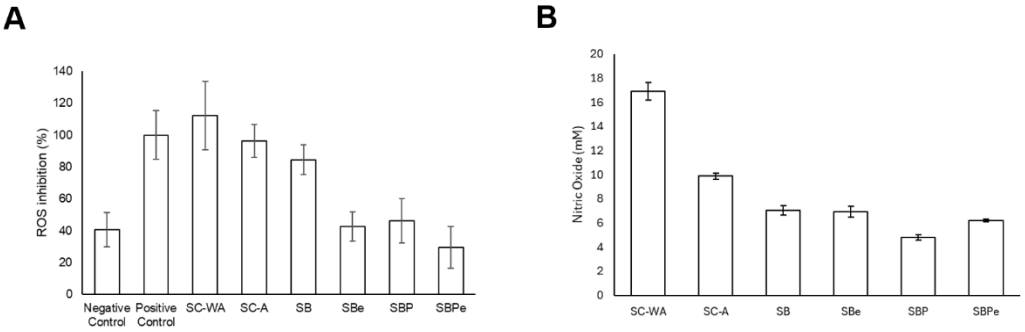


Figure 2. Effect of digested pork sausages on ROS **(A)** and Nitric Oxide **(B)** generation in Caco-2 cells co-cultured with RAW 264.7 stimulated with LPS.

IV. CONCLUSION

The incorporation of broccoli extracts, particularly when treated with PEF and encapsulated, enhanced the antioxidant and anti-inflammatory potential of pork sausages. These findings suggest that combining emerging technologies can improve the efficacy of natural additives in functional meat products, contributing to the development of clean-label formulations with health-promoting properties. Future research should explore *in vivo* validation and sensory impact of these novel formulations.

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