## Title:

Bioaccessibility and prebiotic potential of apple pomace and its hydroethanolic extract after simulated gastrointestinal digestion

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Apple pomace (AP), a bio-residue from cider production, is rich in fibers and phenolic compounds with potential health-promoting properties. This study aimed to evaluate the bioaccessibility of phenolic compounds and the prebiotic potential of lyophilized AP and its hydroethanolic extract (HE AP) before and after simulated gastrointestinal digestion to support its valorization as a sustainable functional ingredient. In vitro digestion was performed according to the Infogest 2.0 protocol, simulating oral, gastric and intestinal phases followed by dialysis to separate the bioaccessible fraction from the colon-available residue. Phenolic profiles were analyzed using HPLC-DAD-MS/MS to quantify individual compounds before and after digestion. The prebiotic potential was evaluated in vitro by monitoring the growth of Lactobacillus casei, L. plantarum, L. acidophilus LA-5 and Bifidobacterium animalis spp. lactis Bb12 in media supplemented with AP or HE AP, compared to inulin and fructooligosaccharides as positive controls. HE AP showed significantly higher total phenolic content after digestion (364  $\pm$  10 mg/100 g dw) compared with AP (64  $\pm$  1 mg/100 g dw), with marked enrichment of phloridzin and quercetin derivatives. However, AP retained matrix-bound phenolics likely released in the colon and exhibited approximately 79% bioaccessibility of total phenolics. AP supported greater bacterial growth than HE AP across all strains both before and after digestion, achieving profiles comparable to established prebiotics. Dialyzed AP retained notable prebiotic activity post-digestion, indicating persistence of fiber-phenolic interactions. These findings show that while HE AP provides a concentrated source of rapidly bioaccessible phenolics, AP offers a complex matrix promoting sustained phenolic release and superior prebiotic effects, revealing how different processing strategies modulate bioactive release and gut microbiota support. These findings advance the concept of transforming apple pomace from an underutilized bio-residue into a scientifically validated, multifunctional ingredient, underscoring its innovative potential for next-generation functional foods and nutraceuticals targeting gut health.