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Citrus pomace: source of bioactive compounds against *Salmonella*

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Salmonella is the major foodborne pathogen in Brazil, causing disease in humans by expressing virulence genes to invade the host epithelial cells. Thus, the search for bioactive compounds with anti-virulence and anti-adhesive effect could help to mitigate *Salmonella* infection. Agroindustrial residues are source of bioactive compounds, such as polyphenols, which display an array of mechanisms of action that interfere with microbial pathogenesis. In this study, polyphenolic extracts were obtained from citrus pomace (CP) by enzyme-assisted (CP-E) and conventional hydroalcoholic (CP-H) extraction methods. These extracts were evaluated on *Salmonella* Typhimurium virulence and adhesion to Caco-2 cells. The major components of CP-E and CP-H extracts were aglycone and glycosylated flavanones, respectively. The enzymes β-glucosidase and tannase led to deglycosylation of flavanones and improved extraction yield of polyphenols from the citrus matrix. CP-E and aglycone standards (naringenin and hesperetin) significantly reduced bacterial growth (up to 90%). CP-H and glycolsylated flavanones (naringin and hesperidin) showed no inhibitory effect. These results indicate that aglycones are responsible for the higher activity of CP-E over CP-H. At subinhibitory concentrations, CP extracts and pure flavanones were evaluated on *S.* Typhimurium adhesion to Caco-2 cells and mechanisms related to bacterial adhesion: adhesin structures (fimbriae and flagella), motility and virulence gene expression. Both CP extracts decreased *S.* Typhimurium adhesion to Caco-2 cells, and CP-H was the most effective, decreasing adhesion by 70%. Pure flavanones decreased bacterial adhesion by about 50%. Because of the high affinity between proteins and polyphenolic compounds, we evaluated if extracts and flavanones were able to bind to *Salmonella* protein adhesin structures (fimbriae and flagella). Agglutination assays confirmed the binding of extracts and pure flavanones to *S.* Typhimurium fimbriae and flagella. CP-E and aglycones also inhibited bacterial motility, suggesting that these compounds can act on flagellar structures preventing bacteria from reaching epithelial tissues and, consequently, adhering to host cells. The expression levels of *S.* Typhimurium fimbrial, flagellar, and virulence genes relative to housekeeping genes were determined by RT-qPCR. Both CP extracts and aglycones downregulated fimbrial (*fimA* and *fimZ*), virulence (*hilA* and *ssrB*), and effector protein (*sopD*) genes. These results suggest that CP extracts and flavanones inhibit *S.* Typhimurium adhesion by multiple mechanisms. Our outcomes advance the understanding of how aglycone and glycosylated flavanones impact *S.* Typhimurium virulence activity and adhesion, contributing to their application as functional ingredients against *Salmonella*.

Keywords: Bioactive compounds for health promotion; enzymatic extraction; flavanones

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