CHANGES IN COMPOSITION AND BIOLOGICAL ACTIVITY OF THE PHENOLIC COMPOUNDS FROM *Ribes magellanicum* AND *Ribes punctatum* AFTER *in vitro* GASTROINTESTINAL DIGESTION AND COLONIC FERMENTATION

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RESUMEN

The wild Chilean currants *Ribes magellanicum* and *Ribes punctatum* are a good source of polyphenolic compounds with interesting bioactivities in several *in vitro* models. The effect of simulated gastrointestinal digestion (GID) and *in vitro* colonic fermentation on phenolic content, composition and antioxidant capacity was determined. The inhibitory activity of the non-digested, digested, and fermented samples towards metabolic syndrome-associated enzymes (α-amylase, α-glucosidase, and lipase) was evaluated. The anti-inflammatory activities of the gastro-intestinal digested PEEs were assessed using differentiated human Caco-2 (clone C2BBe1) cells stimulated with interleukin 1β (IL-1β). The inhibitory effect of non-digested and digested PEEs towards human cyclooxygenase 1 (COX-1) and COX-2 and the gene expression of COX-2 and inducible nitric oxide synthase (iNOS) was determined. The potential prebiotic-like effect of the pre-digested PEEs was evaluated in a simulated colon model. Digested PEEs were submitted to a colonic fermentation with feces from healthy human donors. Samples were taken at 1, 4, 8 and 24 h of incubation, monitoring pH, ammonia, branched-chain fatty acids (BCFA), short-chain fatty acids (SCFA) and bacterial growth. FOS (fructooligosaccharides) and fecal slurry without treatments were positive and negative control, respectively. The total phenolic (TP) and flavonoid contents (TF) decreased by about 50 % at the end of the *in vitro* GID. Main anthocyanins and hydroxycinnamic acids were strongly affected by this process, with a loss of about 80 %. A decrease in the antioxidant activity was observed throughout the digestion steps, which was correlated with the reduction in the TP and TF content. After the *in vitro* GID of the samples, only the inhibition of α-glucosidase was preserved. The phenolic profiles of the fermented samples showed significant changes after 24 h incubation. Nine metabolites, derived from the microbial fermentation, were tentatively identified, including dihydrocaffeic acid, dihydrocaffeoyl-,

dihydroferuloylquinic acid, 1-(3,4-dihydroxyphenyl)-3-(2,4,6-trihydroxyphenyl)propan-2-ol (3,4-diHPP-2-ol), among others. The content of anthocyanins and hydroxycinnamic acids was most affected by simulated colonic conditions, with a loss of 71–92 % and 90–100 % after 24 h incubation, respectively. The highest antioxidant capacity values (ORAC) were reached after 8 h incubation. The inhibitory activity against the enzyme α-glucosidase was maintained after the fermentation process. The digested PEE from *R. punctatum* decreased the secretion of IL-8, IL-6, and TNF-α; whereas *R. magellanicum* reduced IL-6 and TNF-α in the Caco-2 cells ($p < 0.05$). Both digested extracts significantly down-regulated the mRNA expression of COX-2 and iNOS ($p < 0.05$). PEEs showed 60 % of inhibition towards COXs, with higher inhibition against COX-2. The PEEs from *R. punctatum* displayed better anti-inflammatory activity in all the experiments. Both *Ribes* species reduced ($p < 0.05$) both BCFA and SCFA at 24 h. *R. punctatum* promoted the growth ($p < 0.05$) of beneficial bacteria such as *Clostridium* cluster XIVa, and *Faecalibacterium prausnitzii*. A trend to increase *Akkermansia muciniphila* was observed. *R. magellanicum* increased ($p < 0.05$) *Clostridium* cluster XIVa population. Total bacteria, *Escherichia coli*, *Lactobacillus spp*. and *Bifidobacterium spp*. remained unaffected. Our results show that the simulated GID and colonic fermentation modified the polyphenolic composition, influencing their potential health-promoting properties of the studied currants. Polyphenols from *R. magellanicum* and *R. punctatum* might be useful against intestine inflammation and possibly modulate both, bacterial metabolism and selected gut beneficial bacteria under simulated conditions. Therefore, Chilean currants might be useful as supplements to maintain a healthy colon. However, further *in vivo* studies are needed to confirm their prebiotic-like and anti-inflammatory effects.