Supercritical Fluid Extraction of Polyphenols from Almond Oil Industry By-Products: Evaluating Bioaccessibility and Bioavailability Through *In Vitro* Digestion

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Supercritical fluid extraction (SFE) has emerged as an advanced green technology for the recovery of bioactive compounds, offering enhanced extraction efficiency while eliminating the need for toxic organic solvents. In this context, industrial residues from almond oil production were subjected to CO_2 SFE by ISANATUR (Navarra, Spain), a company specializing in by-product valorization. Specifically, almond press cake ($APBF_+$) from cold press extraction was submitted to SFE using 20 MPa and 24 MPa for further oil extraction, yielding two additional by-products ($APBF_{20}$ and $APBF_{24}$, respectively).

Given the potential application of these by-products (APBF+, $APBF_{20}$ and $APBF_{24}$) in functional food formulations and nutraceuticals, this study aimed to assess and compare the bioaccessibility of polyphenols following *in vitro* gastrointestinal digestion. The samples were digested using the standardized INFOGEST 2.0 static protocol. Polyphenolic compounds and their bioaccessible fraction (post-dialysis) were characterized by HPLC-DAD-ESI-MS. Additionally, both non-digested and digested samples were evaluated for their antioxidant capacity using DPPH and TBARS assays. The prebiotic potential of the non-bioaccessible fraction was assessed *in vitro* using three *Lactobacillus* and one *Bifidobacterium* strains. Finally, the bioaccessible fraction was subjected to Caco-2 cell transport assays to evaluate intestinal permeability.

A total of 22 polyphenols were identified in all almond by-products with the SFE-treated samples exhibiting only around 10% loss of polyphenols. After digestion, an average of 64% of total polyphenols in $APBF_+$ remained bioaccessible. Notably, SFE-treated samples exhibited enhanced polyphenol bioaccessibility, reaching 73% and 72% for $APBF_{20}$ and $APBF_{24}$, respectively. Interestingly, the digested extracts exhibited a reduction in EC_{50} values in both antioxidant assays, indicating enhanced antioxidant capacity after *in vitro* digestion. Both non-digested and digested samples showed prebiotic activity, yielding higher optical density values compared to the positive control (fructooligosaccharides), which suggests that the digested almond by-products can promote gut health.

This work shows that SFE by-products retain a high content of polyphenols, with high bioaccessibility and antioxidant activity after *in vitro* digestion, thus reinforcing the potential of almond by-products as sustainable sources of functional ingredients for the food and nutraceutical industries.

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