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The Effect of Fermentation on the Anti-inflammatory Activities Exerted by Olives in LPS-stimulated RAW264.7 Macrophages

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Abstract

Even if olive by-products have been considered a major environmental issue for many years, in the last decades numerous studies have deeply described their antioxidant, anti-inflammatory, immunomodulatory, analgesic, antimicrobial, antihypertensive, anticancer, antihyperglycemic activities (Albini et al., 2023). The fermentation of olives significantly affects their byproducts, especially wastewater and pomace, not only by changing their chemical and phytochemical composition, but also by reducing their environmental toxicity, thus offering opportunities for nutrient recovery (Sun et al., 2025). Therefore, it is essential to understand the impact that some processing methods, usually used during production of oil, such as fermentation, may exert on olives. This work aims to evaluate the effect of fresh and fermented olive (Carolea variety) on LPS-stimulated RAW264.7 macrophages. Total phenolic and flavonoids contents, as well as total antioxidant capacity and radical scavenging activity were determined. Viability test was assessed treating macrophages with a wide range (25 to 5000 µg/mL) of fresh or fermented extracts. Then, the effect of the extracts on extracellular NO production was evaluated, together with the protein and genes expressions of several inflammatory markers. In addition, eIF2α levels was measured as well. Finally, cellular metabolism was examined by analyzing phosphorylated AMPK (p-AMPK), PGC-1α expression and mitochondrial respiration. The results demonstrated that fermentation significantly reduced total polyphenols and flavonoids, while similar values were obtained for antioxidant and scavenging activities. On the other hand, cell viability was not affected after 24 h treatment with fresh and fermented extracts. In LPS-activated macrophages, both extracts at 250 and 500 µg/mL significantly attenuated pro-inflammatory mediators, such as extracellular NO production, iNOS, COX-2, TNF-α, and IL-6, while increased IL-10 production. Fresh and fermented extracts also improved cell metabolism, through the activation of AMPK-related pathways, and enhanced mitochondria functionality, through the increase of maximal oxygen consumption and spare respiratory capacity. The above findings indicate that even if fermentation affected the content of polyphenols and flavonoids, it displayed low cytotoxicity in macrophages, reduced the effect of LPS on NO generation, and modulated several keys signaling pathways, which are intricately involved in the inflammatory response. These results may suggest that fermented olives can be effectively targeted for preventive benefits.

Keywords: olive by-products, fermentation, re-use, health effects

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