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Influence of the maturation stage on the chemical composition and bioactive properties of *Cynara cardunculus* L. var. *altilis* seeds

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A good understanding of dynamics involved in food production is of great importance for improving food quality and security. *Cynara cardunculus* L. is commonly called cardoon and comprises three varieties, the wild cardoon or variety *sylvestris*, the globe artichoke or variety *scolymus*, and the domesticated cardoon or variety *altilis*. This perennial plant is well adapted to the Mediterranean environment [1]. It is used in the Mediterranean cuisine and in several different sectors, including the production of cheese, paper pulp, biodiesel, biomass, and bioenergy [2,3]. The wide industrial applications and the consequent associated commercial interest have been the main contributions to the increase in the economic value and cultivated area of this multi-purpose crop [3]. Among its edible parts, the seeds are particularly rich in high quality edible oil and health-promoting antioxidants [4], which have encouraged the nutraceutical exploitation of this species. Therefore, it is important to explore the potential of cardoon seeds at different maturity stages to better direct them to the most suitable sector and also to reduce the waste of this natural resource and thus contribute to crop valorisation.
For this study, *Cynara cardunculus* L. seeds were collected in Greece at four principal growth stages (PGS), ranging from PGS 6/7 (immature seed sample S1) to PGS 8 (mature seed sample S4). After seed grinding and extraction, their chemical constituents were analysed by different chromatographic techniques. The individual profiles of fatty acids, tocopherols, organic acids, and free sugars were characterized. The phenolic composition was assessed by HPLC-DAD-ESI/MS in hydroethanolic seed extracts. Regarding *in vitro* bioactivities, the antioxidant activity was measured through the cell-based TBARS and OxHLIA assays, which evaluate the ability to inhibit lipid peroxidation and oxidative haemolysis, respectively; the cytotoxic potential was tested by the sulforhodamine B assay against four tumour cell lines (HeLa, MCF-7, NCI-H460, and HepG2) and a primary cell culture (PLP2); the anti-inflammatory activity was determined through the extracts' capacity to inhibit the production of the pro-inflammatory mediator nitric oxide by a lipopolysaccharide-stimulated murine macrophage cell line; and the antimicrobial activity was evaluated by the microdilution method against several foodborne bacterial and fungal strains.
Six phenolic compounds were tentatively identified in the cardoon extracts and the content increased with increasing seed maturity (from 23.2 to 53 mg/g extract). Caffeoylquinic and dicaffeoylquinic acids were the major polyphenols. Our results revealed that mature seeds (sample S4) presented the highest content in lipids (23 g/100 g dw) and tocopherols (29.62 mg/100 g dw). This mature sample also showed the greatest capacity to inhibit lipid peroxidation, nitric oxide formation, and tumour cell proliferation. On the other hand, sample S3 was the one that best inhibited oxidative haemolysis and the growth of the tested bacteria. Regarding antifungal potential, sample S1 which corresponds to a less advanced state of maturation showed the best results. Overall, this work allowed to characterize the chemical composition and bioactive properties of cardoon seeds harvested at different maturity stages. According to the obtained results, the analysed quality attributes of cardoon seeds may differ depending on their maturation. However, additional studied are necessary to correlate the specific detected compounds responsible for the observed biological potential.

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