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Bioaccessibility and Synthesis of Chronobiotics During In Vitro Gastrointestinal Digestion of Pistachio (Pistacia vera L.) to Mitigate Diseases Linked to Chronodisruption

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Objectives: This research aimed to evaluate the *in vitro* chronobiotic potential of Phyto-melatonin (PTM) during gastrointestinal digestion, its fermentative behavior (phenolic acids and other compounds), and potential production of chronobiotics (short-chain fatty acids or SCFAs production).

Methods: The chemical and nutraceutical composition of dry roasted and salted pistachios with seed coat (SC) (PN + SC) and without (PN) was evaluated. Both samples were digested under static *in vitro* simulated physiological conditions comprising oral, gastric, intestinal, and colonic stages. The PTM bioaccessibility during *in vitro* gastrointestinal digestion and colonic fermentation simulation was quantified. The identification and quantification of SCFAs and other colonic metabolites were conducted using SPME-GC-MS, followed by an untargeted metabolomic analysis.

Results: PN + SC had significantly ($p < 0.05$) lower lipids (−7.9) and protein (−1.1), but higher carbohydrate (+8.4) and total dietary fiber (+4.8) content (g/100g) than PN. PN + SC had highest content of total phenols (+42%), total flavonoids (+54%), and PMT (+21%) ($p < 0.05$) compared to PN. The bioaccessibility was low for both pistachio samples [Oral: 1.92 and 3.41%, PN + SC and PN; gastric: 0.83 and 1.63%; intestinal [60 min]: 1.79 and 2.55; colonic [6 h]: 0.32 and 0.36%). Chemo-informatics and an *in silico* analysis of PTM suggest that it was absorbed when chewed by the participants. The highest SCFAs were produced at 12 h during *in vitro* colonic fermentation for both pistachio samples, where PN + SC displayed the highest ($p < 0.05$) value (51 mmol/L), followed by PN (25.9 mmol/L). SCFAs, derived from bacterial fermentation of dietary fibers, can act as chronobiotics in peripheral clocks. The SCFAs molar ratio remained almost constant for both pistachio samples: butyric > propionic > acetic. Some metabolites with chronobiotic potential (e.g., indole, benzaldehyde, phenolic acids, and aliphatic/aromatic hydrocarbons) were detected, sample-dependent, through the untargeted metabolomics.

Conclusions: Pistachio's digestion increases the bioaccessibility of PTM and the biosynthesis of colonic metabolites (SCFAs, among others), all with chronobiotic potential to mitigate diseases linked to chronodisruption.

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