

Response surface methodology in the optimization of extraction conditions for *Gracilaria gracilis* extracts for use in thermoplastic food coatings

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Incorporation of antioxidant agents in edible films and packages often relies in the usage of essential oils and other concentrated hydrophobic liquids, with reliable increases in antimicrobial and antioxidant activities of the overall composite, and consequent improvements in product shelf life. These oils are often obtained from plant sources and the usage of organic solvents in their extraction has been criticized as to whether it constitutes a health hazard for consumers [1, 2, 3]. The use of water-soluble antioxidant agents is less common, due to lower activities and extraction yields. As such, using commonly available seaweeds as the source for both the main polymers and the supplementing antioxidant/antimicrobial agents in bioactive films can potentially reduce production costs and create a safer, more sustainable product [1].

Hydroethanolic extracts of commercially available red macroalgae *Gracilaria gracilis* were evaluated for their antioxidant potential and phenolic content, as part of the preliminary assays for the selection of algal biomass for the enrichment of thermoplastic films. The extracts were obtained through use of solid-liquid extractions, over which yield, DPPH radical reduction capacity, total phenolic content, and FRAP activity assays were measured [4, 5].

Solid to liquid ratio (SLR), extraction time, and ethanol to water ratio were selected as independent variables with experimental ranges and configurations obtained using a Box-Behnken design with three factors, resulting in 15 experimental conditions. Extraction duplicates were used [4]. Response surface methodology was then used to estimate the effect of each extraction condition on the tested bioactivities. Bioactivities were significantly ($p < 0.05$) and positively affected by the presence of ethanol in the extraction solvent, while extraction yield was reduced. Lower solid to liquid ratios (higher solvent proportions) resulted in higher yields, but had no significant ($p < 0.05$) impact on bioactivities. Time was not a relevant factor in any of the measured variables, and as such will be minimized in any further work. 100% ethanol with minimum extraction time (10 minutes) and 1g to 5mL SLR maximize antioxidant activities per gram of dry extract. Maximum ethanolic yields were obtained with a theoretical extraction of 100 minutes and a 1g to 25mL SLR. Aqueous extractions displayed similar results in terms of optimum conditions, but with overall lower bioactivities and higher yields, very likely due to high amounts of soluble polysaccharides.

While the high antioxidant activities from the ethanolic extracts encourage the selection of this solvent for extraction of bioactives, low yields may make them later unfeasible. Future studies over the compatibility of these extracts in the polymeric matrix of the films, as well as further optimization of the extraction process will be necessary before the definitive choice for bioactive origin and processing is made.

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