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Abstract

In Vitro Antioxidant Activity Determination of a Microencapsulated Synergic Polyphenols—Polysaccharide Mixture †

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The aim of this work was to obtain a mixture of polyphenols and polysaccharides, extracted from forest fruit pomace (a side stream of forest fruit juice production) and chia seeds with a synergic antioxidant activity that will be further encapsulated and used in a fortified protein bar. We also intended to demonstrate the in vitro antioxidant effect, including in cell cultures, of the synergistic mixture encapsulated and subjected to a simulated digestion process. Forest fruit pomace is the main source for polyphenols (PPH) and chia seeds for polysaccharides (PSH). PPH extraction was performed in 70% ethanol, at room temperature, 48 h with agitation and PSH extraction was achieved by the Soxhlet method. Antioxidant activity of PSH and PPH combinations with synergic effects was determined using 3 different methods ABTS, DPPH and CUPRAC. The PPH and PSH combinations with the higher synergic antioxidant activity were encapsulated using whey protein and inulin as a matrix. Microencapsulated synergic polyphenols –polysaccharide mixture was analyzed by a HITACHi SU 1500 scanning electron microscope (SEM) operated at 15 kV (samples were fixed with glutharaldehyde, dehydrated in succesive ethanol concentrations and contrasted with osmium tetraoxide). The microencapsulated mixtures were also subjected to a simulation of gastrointestinal digestion in vitro. After digestion, the cells (primary human colonic tumor cells—Caco-2) viability, respectively, the estimation of the cellular antioxidant capacity of the samples, was measured by Neutral Red assay, with or without induced oxidative stress [1–3]. In the forest fruit pomace the polyphenol content (PPH) measured 304 mg gallic acid/g dw., and the polysaccharide content in chia seeds (PSH) measured 135 mg glucose/g dw. The antioxidant activity of forest fruit pomace was determined by 3 methods (ABTS—1.32 mM TE/mg dw.; DPPH—IC50 456.17 µg/mL; CUPRAC—4.96 mM TE/mg dw.) Regarding chia seed polysaccharides, the results were: ABTS—0.038 mM TE/mg dw., DPPH—IC50 5000.6 µg/mL, CUPRAC—1.96 mM TE/mg dw. The PPH and PSH combinations exhibited a higher synergic antioxidant activity especially in the ratio of 2:1 and 5:1. Through scanning electron microscopy analysis, we observed the presence of microcapsules on the outer layer of the matrices obtained by freeze drying. The average size of the microcapsules ranged from 5–10 microns. In vitro, we observed that Caco-2 cells, pre-treated with an encapsulated synergic mixture of PPH and PSH and subjected to simulated digestion, had a viability up to 20% higher compared

to the control treated only with H_2O_2, thus the studied mixture exhibited antioxidant protective effects on cells against the oxidative agent. We observed that the synergic mixture of the PPH and PSH, with proved antioxidant activity, maintained this activity after encapsulation. The antioxidant capacity of the microencapsulated mixture renders it suitable for introduction as a new formula in functional food.

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