

Abstract

Chitin is a biopolymer commonly found in the environment, synthesized by insects, fungi, molluscs, sponges, and crustaceans. It can be modified in the process of deacetylation into chitosan. Chitosan, thanks to its free amide groups, can bind to various molecules and surfaces and exhibit various biological activities. It is considered a biocompatible compound characterized by low toxicity. Its biological activity is determined by parameters such as molecular weight and degree of deacetylation, which vary depending on the polymer source. Furthermore, the chitin extraction and deacetylation method can impact chitosan properties. The chitin extraction method used on industrial scale is considered environmentally harmful due to energy consumption, and production of large amounts of wastewater. Furthermore, strong reagents, used in the process can cause degradation of the isolated polymer. One of the alternative methods for chitin extraction is based on mixtures made with natural deep eutectic solvents (NADES).

The aim of this study was to obtain and analyse the physicochemical properties of chitin and chitosan polymers from four crustacean species found in Polish waters: the narrow-clawed crayfish *Pontastacus leptodactylus* (Eschscholtz, 1828), the spinycheek crayfish *Faxonius limosus* (Rafinesque, 1817), gammarid *Dikerogammarus villosus* (Sowinsky, 1894), and mysid *Neomysis integer* (Leach, 1814), and to assess their potential as alternative sources of these polymers. Additionally, chitin was extracted from these same species using natural deep eutectic solvents (NADES). The antibacterial and cytotoxic activity of the obtained chitosan was assessed.

This study demonstrated that *P. leptodactylus*, *F. limosus*, and *D. villosus* constitute attractive sources of chitin and chitosan. All tested chitosan samples demonstrated antibacterial activity higher than the chitosan used as a reference. The microstructure of chitosan samples obtained from *P. leptodactylus* and *F. limosus* was characterized by desirable characteristics in medicine in terms of tissue engineering, wound dressings, and drug applications, such as porosity and the presence of microfibers. All tested chitosan samples demonstrated cytotoxic activity at concentrations above 250 µl/mL and an exposure time of 24 hours. Based on the molecular dispersity index, *N. integer* was rejected as an attractive source of the tested polymers. The NADES mixture used in this study proved effective only for the extraction of chitin from *P. leptodactylus* shells. Chitin from *F. limosus*, *D. villosus* and *N. integer* wasn't fully separated from proteins.