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Topic of Research: **Characterization and applications of collagen from fish waste and effect of ionic liquids on its conformational stability**

Findings

My Ph.D. research starts with the extraction of collagen from the fisheries by-products. The extracted collagen was type-I and its structure were not destroyed during the extraction process, was confirmed by fourier transform infra-red spectroscopy (FTIR), circular dichroism (CD), and sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The collagen is made up of sheets with random coiled like structure as confirmed by scanning electron microscope (SEM). The extracted collagen was not contaminated with heavy metals was confirmed by energy dispersive X-ray (EDX). The effect of ionic strength and pH on collagen solubility was also investigated. The collagen showed high solubility at low ionic strength (up to 3% NaCl), and low pH (up to 3 pH). Thermal stability of collagen obtained from skin of freshwater fishes was higher as compared to marine water fishes and close to mammalian collagen. In addition, collagen shows efficient free radical scavenging activities.

Further, we utilized the extracted collagen in the development of biodegradable food packaging films by combining collagen obtained from *Sperata seenghala*, carboxymethyl cellulose (CMC), and *Berberis lyceum* root extract (BLRE). The addition of BLRE into CCMC film enhances the surface morphology, opacity, flexibility, UV light barrier, and antioxidant properties of the films, that are important characteristics of active food packaging materials. The films with BLRE showed good antioxidant properties and the film's exposure to various food simulants presented that the antioxidants are released from the film. In the acidic and alcoholic food simulants, films containing BLRE produced the highest release as compared to fatty food simulants. Moreover, mushrooms packed in BLRE loaded CCMC films were well-preserved longer than neat CCMC

films. Thus, the promising results in our present study open a new window for inspection of collagen-based films with bioactive agents for shelf-life extension of mushroom and other food products.

Moreover, we also investigated the use of collagen extracted from fisheries waste for the construction of films for drug release study. Poly (vinyl alcohol) was used as a crosslinker and [Cho]Ac as a plasticizer. Ciprofloxacin (CIP) was used as a model drug to study its release behavior. The [Cho]Ac increases the opacity, elongation at break, tensile strength and roughness of films and reduces their solubility and swelling as compared to glycerol-based films. The IL also induces antioxidative properties in the compositive films. Moreover, the antimicrobial studies against *S. aureus*, and *E. coli* showed that the CIP loaded films have outstanding antibacterial activity against both the gram-positive and gram-negative bacteria. According to the *in-vitro* release results, drug release can also be controlled for maximum time by using IL as a plasticizing agent.

Additionally, we also investigated the effect of imidazolium ILs on conformational stability of collagen. The effect of these ILs on collagen conformational stability was analyzed by using UV-vis, circular dichroism (CD), steady state fluorescence, time resolve fluorescence (TRF), synchronous fluorescence, dynamic light scattering (DLS), fourier transform infrared spectroscopy (FTIR) and molecular docking methods. The results obtained showed distortion and unfolding of collagen polypeptide structure at higher concentration, however, tenuous conformational changes were observed at lower concentration, resulting in less structural deformation in collagen. The destabilization propensity of ILs toward the collagen increases with increasing chain length in ILs. In addition, when organized in the order of providing destability to collagen triple helix structure, the cations of the ILs obeyed the Hofmeister series. Additionally, molecular docking study was also performed, and their results also support the experimental results, confirming the complex binding of long alkyl chain length of imidazolium cations, revealing disruption of collagen.