Development of collagen based biodegradable film from fish scale

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DEVELOPMENT OF COLLAGEN-BASED BIODEGRADABLE FILM FROM FISH SCALE

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Purpose

Plastic packaging is hazardous to the environment since it is non-degradable. Therefore, plastic pollution has become a burning question among the concerned people throughout the world. In contrast, starch- or protein-based film are degradable. As a result, scientists are now focusing on biodegradable film made from natural (plant and animal) sources for food coatings to prolong the shelf-life of food. The production of biodegradable film has many advantages in terms of environmental protection and effective utilization of wastes as well as value addition. For example, it can overcome the environmental pollution by replacing the plastic materials. In addition, biopolymers (collagen, gelatin etc.) can be obtained from by-products instead of discarding that may also result in environmental pollution. Moreover, biodegradable film made from natural polymer materials can provide nutritional value too. Fish processing companies produce a large amount (approximately 50-70%) of wastes such as skin, scale, bone, intestine, head, fins etc. annually and discard these. Researches exhibited that, commercially important collagen and gelatin can be obtained from these discarded materials especially from skin and scales. The extracted biomaterials from these by-products has numerous benefits compared to the conventional synthetic packaging. So, utilization of these vast resources is necessary. Therefore, the purpose of the study was to develop collagen-based biodegradable films with superior characteristics from fish scales.

Methodology

Fish scales were collected and cleaned with cold deionized water, placed in polyethylene bags and stored immediately at -30°C prior to analysis. Moisture, crude protein and ash content were determined following the standard protocol of AOAC (2000). Extraction of acid- and pepsin-soluble collagen (ASC and PSC, respectively) were carried out according to the method of Nagai and Suzuki (2000) with slight modifications. After pre-treatment by NaOH and Na_2EDTA, the scales were extracted with acetic acid. After extraction, the dialysate was lyophilized and stored.

Afterwards, amino acid composition (HPLC), SDS-PAGE, denaturation temperature (DSC) and effect of pH and NaCl were analysed. Then the film forming ability of both ASC and PSC were analysed following the method of Nunes et al. (2011). Collagen samples were dissolved in 0.5 M acetic acid to obtain film-forming solution and cast 4 g film. After drying, films were manually peeled off and conditioned. Then the thickness, mechanical properties such as tensile strength (TS) and elongation at break (EAB) by tensipresser, light barrier properties (spectrophotometer), water vapour permeability (WVP), moisture content and film water solubility, SDS-PAGE (Laemmli, 1970) and ATR-FTIR were analysed. Later, to improve the mechanical properties of the film phenolic compounds i.e., pyrogallol and curcumin were added at various concentrations and measured the film properties such as TS, EAB, WVP etc. Finally, to know the underlying mechanisms of improved film properties incorporated with phenolic compounds chemical analysis of the films were performed by SDS-PAGE and ATR-FTIR.

Experimental results

This study aimed to develop collagen-based biodegradable films, with high quality and functional properties. Based on the results of our study in Chapter 2, ASC and PSC were extracted from carp and lizardfish scales, which had necessary nutritional properties such that imino acids were found in adequate quantities and both extracted collagens were type-I collagen as revealed by SDS-PAGE. Pepsin treatment remarkably increased the recovery of collagen from the same fish scale after ASC extraction. Imino acid contents were set side by side with their living environment, and scales from the temperate-country fish had lower imino acid contents as well as *Td* and enthalpy change values than the sub-tropical country fish. The *Td* of PSCs were lower than their corresponding ASCs to some extent. Therefore, MW might be associated with the thermal stability of collagen. The collagen of carp scales contained superior characteristics i.e., imino acid content, MW, as well as Td than their counterpart lizardfish. ASC and PSC were soluble in acidic pH and the solubility decreased with increasing sodium chloride concentration.

Later in Chapter 3, lyophilized collagen was dissolved and formed film-forming solution. Both ASC and PSC films were able to form biodegradable films and were adequately transparent and exhibited good mechanical and physicochemical properties. Films had high vapor barrier properties and water solubilities. Besides, the characteristics of both ASC and PSC FFS were maintained in the corresponding films as evidenced by SDS-PAGE pattern. Moreover, the FTIR study confirmed that the nature of collagen was unimpaired during film formation. Therefore, collagen films prepared from scales of lizardfish showed comparable properties with LDPE films and could be applied in food and packaging industries.

Furthermore, in Chapter 4, phenolic compounds such as pyrogallol and curcumin were added to improve the mechanical, water vapor and light barrier properties of the prepared films. The addition of phenolic compounds significantly influenced the properties of ASC and PSC films. The pyrogallol and curcumin added films resulted in increased TS but decreasing trend was observed for EAB. Pyrogallol incorporated films were more transparent and exhibited higher mechanical properties than curcumin added films did. On the contrary, curcumin added films especially when added to ASC films showed substantial light barrier properties. Therefore, tensile performance and physical characteristics of both ASC and PSC films were improved by incorporating phenolic compounds in this study and the improvements exhibited potential for practical application.

Next, in Chapter 5, the films added with phenolic compounds were analyzed to see the molecular changes after addition of pyrogallol and curcumin. The SDS-PAGE and ATR-FTIR study revealed the relationship between collagen-phenolic compound film and improved properties (i.e., TS) of the treated films. The MW of pyrogallol and curcumin added films appeared to be higher than the control films regardless of the film types. Amide-I band detected the conformational changes of collagen films polypeptide chain. Hydrogen bonding occurred largely after adding the phenolic compounds.

Conclusion

The extraction yield of PSC was higher than ASC indicating that more collagen can be recovered from the same scale after the employment of pepsin. Glycine was the dominant amino acid found in all the collagen samples followed by alanine. The imino acid contents were different between temperate and sub-tropical country fishes resulting in the differences in denaturation temperature. The SDS-PAGE revealed the molecular mass differences among the samples and correlated with the corresponding denaturation temperature. Both ASC and PSC from lizardfish scales could form the films. The TS, total color difference of

ASC films was higher than the corresponding PSC films. This might be due to the differences between molecular weight of the ASC and PSC. After adding the pyrogallol and curcumin the TS, light barrier properties, color differences of the films showed increasing trend with the increasing phenolic compound concentration. This indicated that, hydroxyl group of phenolic compounds interacted with the collagen chain and changed the structure of the films. As a result, improved physical properties were observed. Afterwards, the SDS-PAGE and ATR-FTIR revealed that chemical structure was changed, and hydrogen bond was formed substantially and resulted in the differences of the properties. Finally, the results of our study suggest that ASC and PSC films prepared from lizardfish scales could be an alternative to mammalian derived biodegradable and low-density polyethylene films.

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