Preparation and Characterization of Modified Pectin: A New Insights Into Biodegradable Polymer for Collagen Stabilization

Gladstone Christopher Jayakumar, Jonnalagadda Raghava Rao*, Balachandran Unni Nair

Central Leather Research Institute, Council of Scientific and Industrial Research, Adyar, Chennai-600 020, India

Abstract

Pectin is a high value functional food ingredient widely used as a gelling agent and stabilizer. It is also an abundant, ubiquitous and multifunctional component of the cell walls of all land plants. The basic properties of pectin have been known for nearly 200 years, but recently there has been tremendous progress in understanding of the very complex fine structure of pectic polymers. The current study investigates the modification of pectin to (PD) Pectin Dialdehyde through selective oxidation method and used as a stabilizing agent for biomembrane preparation. PD is characterized using FTIR (Fourier Transform Infrared Spectroscopy), XRD (X-ray Diffraction) and DLS (Dynamic Light Scattering) to understand the critical possessions. Crosslinking of PD with protein involves in the formation of inter and intra crosslinking (Covalent and Hydrogen Bond Interactions) which endows higher stability against heat and enzyme. Conformational changes induced by PD in collagen have been evaluated through CD (Circular Dichroism). Influence of PD in nucleation centers in collagen has been determined through gelling time and turbidity assay. Water absorption of PD modified collagen membrane is estimated through swelling degree. The molecular toxicity of PD has been evaluated MTT assay. The modified pectin finds versatile application in leather industry as a new tanning system.

Keywords: Pectin; Collagen: Biopolymer; Stabilization: Biomembrane

---

*Author for correspondence Email: gc.jayakumar@gmail.com
Telephone+91 44 24411630, 2491386

Introduction

Type I collagen, the structural protein of skin, bone and tendon, is an important biomaterial finding widespread applications in various fields such as surgical sutures, cosmetics, wound healing and leather making [1]. This widespread use of collagen emphasizes the need of crosslinking of collagen to stabilize against biodegradation and heat. It is well known that collagen crosslinked with various crosslinking agents such as plant polyphenols, metal ions and aldehydes is made resistant against the degradation by collagenase and the thermal stability of collagen was increased owing to crosslinking. One of the alternative may be the modified biopolymers. Modified biopolymers were proved to be biodegradable and toxologically acceptable.

Pectin is all around us. It is a major component of the cell walls of all land plants and in a normal western diet around 4–5 g of pectin are consumed each day. Extracted pectin is widely used as functional food ingredient and it (or its EU code, E440) is listed among the ingredients of innumerable food products. Worldwide annual consumption is estimated at around 45 million kilograms, with a global market value of at least 400 million Euros. On all points he was quite correct and the study of this remarkable macromolecule has been pursued vigorously by both plant and food scientists ever since. In the food industry pectin is known primarily as a gelling agent and is widely used in the production of jams and jellies, fruit juice, confectionary products and bakery fillings. The other major use of pectin is for the stabilisation of acidified milk drinks and yogurts. In all application areas the fine structure of pectin profoundly affects its functionality. This is reflected in the fact that although most plant tissues contain pectin, commercial production is based almost entirely on just a few sources that have the required properties. Currently, citrus peel and apple pomace are the major sources of extracted pectin whilst other potentially valuable sources remain largely unused because of certain undesirable structural properties.

Even though, pectin and oxidized pectin were reported for several bioresource applications, attempts have not been made so far to use this oxidized pectin for collagen stabilization. Pectin dialdehyde, a modified biopolymer of pectin appeared to be an attractive option for stabilization of collagen as it is one of the eco-acceptable biopolymer.

In the present work, an investigation is made to study the effect of pectin dialdehyde on crosslinking efficiency and thermal stability of type I collagen matrix.

1. Material and Methods

Materials

The chemicals used for all the experiments were of analytical grade and purchased from SRL Chemicals, India.

Methods

The extracted collagen from rat tail tendon [3] was corsslinked with oxidized Pectin (Pectin dialdehyde) [4]. The crosslinking efficiency of the modified pectin was determined using TNBS assay and thermal stability studies using redissolution assay [5]. PD is

characterized using XRD (X-ray Diffraction) and DLS (Dynamic Light Scattering) to understand the critical possessions.

2. Results and Discussion

Crosslinking efficiency of pectin dialdehyde stabilized collagen membrane

The most probable reaction of aldehydes is the formation of schiff's base type compounds and the methylene linkages resulting from mannich reaction with the amino functional groups in the collagen. The extent of crosslinking brought about by pectin dialdehyde, crosslinking efficiency estimated by TNBS assay. The crosslinking efficiency of the pectin dialdehyde with collagen was studied at various concentrations. It was observed that increase in pectin dialdehyde concentration crosslinking increase [6]. This may be due to the formation of stable bond with amino groups of collagen [7].

Thermal stability of the crosslinked collagen for various concentrations of pectin dialdehyde is shown in the Figure 1. It was observed that increase in pectin dialdehyde concentration from 0.2 to 1.0 ratio, the thermal stability of collagen increases from 66 to 89ºC. From the above ratios it is evident that pectin dialdehyde as a crosslinking agent plays a crucial role in denaturation.

The collagen modified with pectin was measured by DLS, the increase in cross-linked points by pectin dialdehyde resulted in the limited movement of collagen molecules. Therefore, the increase in *D* with collagen concentration in the present study reflects the increase in the number of cluster points and hence the reduction in the mobility of clusters of collagen fibril network.

3. Conclusion

The present study concludes that modified pectin as a crosslinking agent plays a vital role in the stabilization of collagen. The study demonstrates that pectin dialdehyde brings about a significant crosslinking efficiency and increase in denaturation temperature.

5. References

[1] Nimni, M.E., Harkness, R.D., 1988, Molecular structures and functions of collagen. Vol. I. Florida: CRC Press, pp.128.

[2] Zhao, M.W., Yi, C.C., Po, H.L., Jian, Y.W., 2010, Ultrasonic treatment for improved solution properties of a high-molecular weight exopolysaccharide produced by a medicinal fungus, Bioresour. Technol, 101, 5517–5522p.

[3] Chandrakasan, G., Torchia, D.A., Piez, K.A., 1976, Preparation of intact monomeric collagen from rat tail tendon, J. Biol. Chem., 251, 6062–6067p.

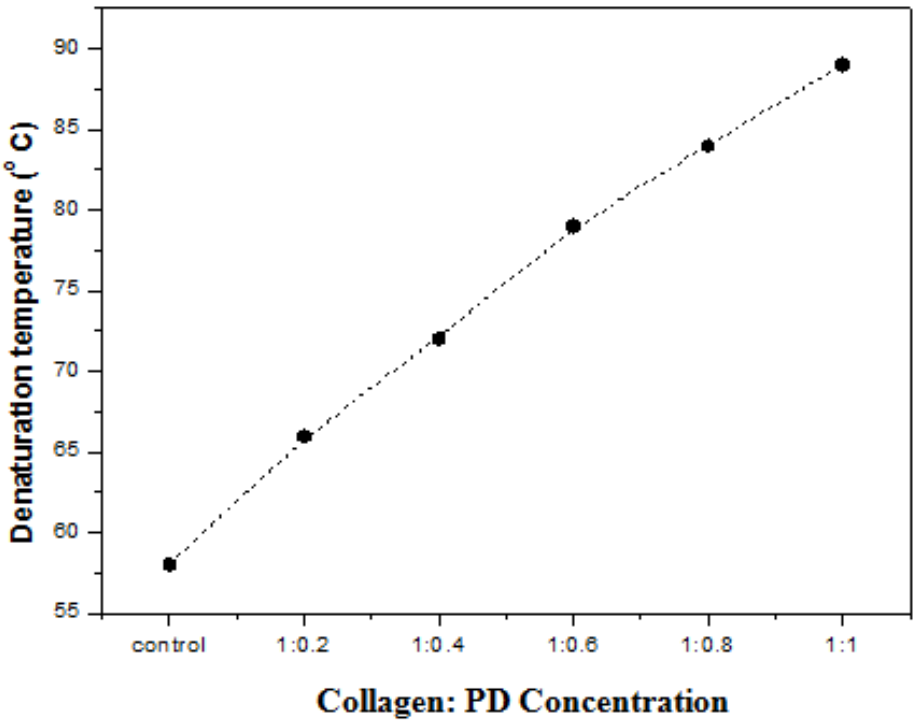
[4] Jayakumar, G.C., Kanth, S. V., Chandrasekaran, B., Raghava Rao, J., Nair, B.U., 2010, Preparation and antimicrobial activity of scleraldehyde from Schizophyllum commune, Carbohydr. Res., 345 (15), 2213-2219p.

[5] Bubnis, W. A., Ofner, C.M., 1992, The determination of ε-amino groups in soluble and poorly soluble proteinaceous materials by a spectrophotometric method using trinitrobenzenesulfonic acid, Anal. Biochem., 27, 129-33p.

[6] Calvini, P., Conio, G., 2006, Viscometric determination of dialdehyde content in periodates oxycellulose Part II. Topochemistry of oxidation, Cellulose 13, 571-579p.

[7] Jayakumar, G.C., Kanth, S.V., Purna Sai, K., Chandrasekaran, B., Raghava Rao, J., Unni Nair, B., 2012, Scleraldehyde as a stabilizing agent for collagen scaffold preparation, Carbohydrate Polymers 87,1482-1489p.

Figure 1


Denaturation temperature (°C)
90
85
80
75
70
65
60
55
control
1:0.2
1:0.4
1:0.6
1:0.8
1:1
Collagen: PD Concentration