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Extraction of collagen from raw trimming wastes of tannery: a waste to wealth approach

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ABSTRACT

Discharge of huge quantities of raw skin/hide trimming wastes by leather industries has raised serious concerns on account of their environmental impacts. It is imperative to develop a simple method for the utilization of this waste to provide a practically feasible and economically viable solution. The trimmings contain high amount of fibrous protein, which are actually a valuable bioresource. The study presents the extraction of collagen, a product of high value, from raw trimmings. Experiments were carried out using propionic acid and acetic acid for the solubilisation of collagen from skin matrix. Of these acid extraction methods, propionic acid method showed higher yield of collagen (~93%) compared to the acetic acid extraction (~85%). The extracted collagen was further characterised by physicochemical techniques such as SDS-PAGE, circular dichroism and infrared spectroscopy. It was concluded that the collagen extracted from trimming waste using both acids were of type I and also found to have similar physicochemical properties. Therefore, the raw skin/hides trimming waste can be a cheap source of collagen, which has many high end applications like tissue engineering, biomaterials, cosmetics and pharmaceutical industry.

1. Introduction

Leather industry plays a notable role in today's global economy. Raw hides and skins are the by-products of the meat and meat products industry and serve as raw material to the leather industry (Langmaier et al., 1999). During the process of leather manufacturing, the hides and skins are trimmed to render them amenable for mechanical operations. This operation generates huge amounts of solid wastes (John Sundar et al., 2011). Raw hide trimmings account for a minimum 5% of the total quantity of raw material processed in leather industry. For every ton of raw material processed 50 kg of raw trimmings are generated. Globally on an average 6,500,000 tons of hides and skins are processed into leather (FAO, 2013). At 5%, about 325,000 tons of raw trimmings wastes are generated and considering minimum amount of 20% collagen content (% based on wet salted raw material), potentially 65,000 tons of collagen is available annually. One metric ton of wet salted hides/skins yield around 200 kg of end product, along with about 250 kg of chromium-containing solid waste, about 350 kg of chromium-free waste and about 100 kg of matter lost in waste water. These wastes bring about various problems for tanneries due to a lack of proper methods for complete utilization as well as treatment (Alexander et al., 1991). Globally, around 800,000 metric tons of chromium-free solid waste, a large proportion of which is raw trimming waste, are processed annually (Dong et al., 2008). Predominantly, dumping has been resorted to as the mode of disposal of this waste in developing and under developed countries. Skins or hide trimming wastes contain proteins and are also less contaminated by chemicals compared to the trimming of tanned and finished leathers. In some countries, raw trimming wastes are used as raw material for the production of various products such as glue, collagen peptides, industrial gelatin, feed and fertilizers (Dong et al., 2008; Olle et al., 2013). Ashokkumar et al. (2012) reported that pristine collagen bio-wastes raw material was introduced into high temperature to provide the synthesis of multifunctional nanocarbon structure for the sustainable approach for battery electrode development. These waste materials are a rich source of proteins and lipids. Collagen is the protein present in large level in raw hides and skin and it is tanned to

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produce leather (Kanagaraj et al., 2006). The raw trimming also contains chiefly collagen.

In animal hides and skins, Type I is the predominant of all types of collagen and it is also the major structural component of tendon, bone and connective tissue (Ricard et al., 2005). Till date, 29 types of collagens have been identified (named types I-XXIX) of which type I is the most abundant (Shoulders and Raines, 2009; Liu et al., 2010; Matmaroh et al., 2011; Chang et al., 2014). The common structural motif of all collagens is the triple helix structure in which each of the three parallel polypeptide strands with left-handed polyproline II (PPII)-type helical conformation coil around each other to form a right-handed triple helix. The tight packing of PPII helices within the triple helix mandates that every third residue be glycine (Gly), resulting in a repeating Xaa-Yaa-Gly sequence, where Xaa and Yaa can be any amino acid. This repetition pattern occurs in all types of collagen, although it is disrupted at certain locations within the triple-helical domain of non-fibrillar collagen (Brazel et al., 1987). The amino acids in the Xaa and Yaa positions of collagen are often occupied by Proline and hydroxyproline, respectively. Pro-Hyp-Gly is thus the most common triplet (10.5%) in collagen (Ramshaw et al., 1998).

Collagens apart from their utility in leather manufacture are associated with wide range of applications viz., cosmetics, biomedical materials, food and agriculture. Collagen has been widely accepted as a biomaterial with many unique characteristics such as high tensile strength, low antigenicity, bioresorbability, good biocompatibility, capable of inducing coagulation of blood platelets, effectiveness in cell differentiation, and effectiveness in wound healing (Stenzel et al., 1974; Lee et al., 2001). In pharmaceutical applications, collagen can be used for production of wound dressings, vitreous implants and as carriers for drug delivery. Murali et al. (2011) elucidated that the hybrid film obtained from skin waste collagen has a promising properties such as biocompatibility, mechanical and bio-stability with beneficial role in biomedical applications. In addition to that, collagen has been used for producing edible casings for meat processing industries (sausages/salami/snack sticks) (Kittiphattanabawon et al., 2005). However, in such cases it has not been attempted to extract or purify collagen in native form.

Many sources have been studied for the extraction of the collagen (Skierka and Sadowska, 2007), and the most popular method for extraction is solubilisation using acetic acid (Tanaka et al., 1988; Chandrakasan et al., 1976). But work on the extraction and utilization of collagen from raw hides and skin trimming wastes is limited. Therefore, the aim of this study is to develop an effective process for the extraction of collagen from the trimming waste of raw hides or skin, and also to explore a possible better alternative to acetic acid extraction. We chose to study propionic acid as an alternative because of its already known applications in the food industry. The efficacy of both propionic acid and acetic acid in extraction of collagen has also been studied. The process of extraction of collagen from raw hides/skin trimmings will not only provide scope for the secured disposal of the solid waste but also would be an option for producing a valuable product, which will bring about significant returns.

2. Materials and methods

2.1. Raw materials

Raw trimmings of goat skins were obtained from pilot tannery of CSIR-Central Leather Research Institute and the trimmings were washed with plain water to remove salt, dung and other impurities. The skin trimmings were soaked in water to increase the moisture content to 60–65%. Unhairing of rehydrated trimmings were carried out using 10% lime and 3% Na_2S and the percentage of chemicals was based on the weight of trimmings. After unhairing, the trimming matrix is opened up by treatment with 3% lime for 24 h. Then the trimmings pieces were subjected to deliming using 2% NH_4Cl for 120 min. The delimed pieces were washed thoroughly in water and were cut into small pieces and these were used as a starting material for collagen extraction.

2.2. Preparation of collagen from raw trimming waste

A set of known amount (~10 g) of delimed pieces were taken and collagen was extracted from these delimed pieces using two different organic acids viz., acetic and propionic acid, concurrently. The process sequence employed for the extraction of collagen from raw trimming wastes is presented in Fig. 1. Following is the procedure adopted for the extraction of collagen from delimed pieces. The delimed pieces were suspended in 0.5 M cold organic acid (acetic or propionic acid) at 1:250 w/v and kept overnight at 4 °C under mild stirring. This suspension was homogenised in cold condition at 15,000 rpm using homogeniser equipped with 100 g dispersing element (IKA T25, Germany) for 10 min and further kept under stirring for 12 h at 4 °C. The supernatant of the extracted solutions was collected by centrifugation at 12,000 g for 15 min at 4 °C, and then salted out by adding NaCl (5% w/v), incubated at 4 °C for 8 h followed by centrifugation under the same conditions. The pellet was re-dissolved in the corresponding organic acid used for extraction in cold condition at 0.5 M. For better solubilisation, the solution was kept under mild stirring for 12 h at 4 °C. The collagen solution was dialysed against 20 mM disodium hydrogen phosphate for 72 h at 4 °C. The dialysed sample was again centrifuged at 7000 rpm for 10 min. The collagen pellet was re-dissolved in 0.5 M organic acid under overnight stirring. The re-dissolved collagen solution was dialysed against its respective organic acid (0.1 M) for 72 h at 4 °C. The collagen solution extracted from propionic and acetic acid were adjusted to same volume at all stages of processing in order to highlight the differences, if any, in the amount collagen extracted by both acetic and propionic acid as the initial raw material taken for both remained constant.

2.3. Characterization of collagen extracted from the raw trimming waste

The collagen extracted from the trimming waste using both acetic and propionic acids were subjected to detailed characterization to determine the % yield and purity of collagen.

2.3.1. Quantitative estimation of acid extracted collagen

The amount of collagen extracted using acetic and propionic acids were determined based on the determination of hydroxyproline content. A known aliquot of acetic acid extracted collagen (AEC) and propionic acid extracted collagen (PEC) was hydrolysed using HCl (6 N) for 16 h at 105 °C. The hydrolysate was evaporated to dryness in a porcelain dish over water bath to remove excess acid. The acid free residue was made up to a known volume and the amount of hydroxyproline was determined by the method of Woessner (1961) using L-hydroxyproline (Sigma–Aldrich) as the standard. Hydroxyproline is a unique amino acid in collagen and it offers itself as a useful marker for estimating collagen in the presence of non-collagenous proteins. Woessner method of determining hydroxyproline involves the oxidation of hydroxyproline to pyrrole-2-carboxylic acid, which complexes with p-dimethylaminobenzaldehyde exhibiting maximum absorbance at 557 nm. The absorbance was read using a UV–Vis spectrophotometer (Carry win 100). Collagen content is 7.4 times of the amount of

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