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SHORT COMMUNICATION

# Technical note on the isolation and characterization of collagen from fish waste material

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**Abstract** The aim of this manuscript was to evaluate the major technical problems on the isolation and characterization of the collagen from fish waste materials that were usually faced by the growing researchers. Although the original research article published by authors contributed new information to the literature, some of them were failed to provide sufficient details in order to reproduce the study as well as could not adequately interpret/compared the results with other publications. Therefore, it is required to research the technical problems during the isolation and characterization of the collagen. This technical note provides the information which is crucial for the reader's and growing researchers for understanding as an essential part of the published research studies about the collagen extraction and characterization. Hence, this technical note may be helpful to those working on the collagen extraction and characterization from fish/marine waste materials.

**Keywords** Collagen · Extraction · Characterization · Fish waste

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## Introduction

Collagen is a major structural fibrous protein that contributes in the connective tissue and also has the various industrial applications (Pal et al. 2015). Due to the outbreak of the prions disease in the bovine and porcine collagen, most of the research focused on the extraction of the collagen from the alternative sources (fish, marine and seafood sources) (Pal and Suresh 2016a, b, 2017). There are several research groups which are working on the extraction of the collagen from alternative sources (Liu et al. 2012; Matmaroh et al. 2011; Pal et al. 2016). However, some researchers could not be able to extract the collagen systematically and also published the erroneous information in the reputed international journal. We published the technical comments on these articles to avoid the misunderstanding in the readers and researchers (Rani and Kumar 2016a, b; Rani 2016).

Here, we wish to elaborate the major technical problems in the recent articles published on the extraction and characterization of the collagen from fish and marine wastes. Therefore, the object of the present short communication is to remarks upon the various problematic inconsistencies in the recently published articles on collagen isolation and characterization.

## Technical comments on published articles on collagen extraction

Fish waste material-skin, scales, and fins from *Catla catla* and *Cirrhinus mrigala* were used for the proximate composition analysis and collagen extraction (Mahboob 2015), but the report did not adequately describe the fish waste material proximate compositions and characterization of

the extracted collagens. Some of the specific points are as following-

1. It was required to clarify that the proximate composition analysis of fish waste materials was done using lyophilized fish waste or fresh fish waste material.
2. The method used for acid soluble collagen extraction from fish fins was not able to follow the cited publications. As well as, the cited publications did not provide the information about the acid and pepsin soluble collagen extraction from the fish scales.
3. It was evident that removal of minerals (demineralization) is a critical step to extract the collagen from the fish scales (Liu et al. 2012; Matmaroh et al. 2011). However, the author did not remove the minerals from the fish scales and bones.
4. More information should be provided on the *Catla catla* and *Cirrhinus mrigala* waste material size (pieces), stirrer speed, and dialysis conditions (MW cutoff of dialysis tube/bag) was needed in order to reproduce the study.
5. Author did not compare the proximate analysis data with the relevant publications/references. For example, the fish scales proximate composition was not compared with carp/other fish scale proximate composition.
6. In this article, a low amount of ash content was reported in the *Catla catla* and *Cirrhinus mrigala* fish scales. Although, the fish scale contains a high amount of the ash content. It is evident that higher ash content was found in the scale due to the presence of calcium-deficient hydroxyapatite localized in two distinct regions; an upper osseous layer and a lower fibrillar plate of fish scale (Matmaroh et al. 2011).
7. The author stated that the acid and pepsin soluble collagen were characterized using the SDS-PAGE and amino acid composition. However, it was well known that collagen cannot be characterized on the basis of these two methods. In the gelatin extraction process may lead to the same results. Gelatin is a derived form of the collagen. Therefore, the secondary structure and fibril formation ability of collagen should be evaluated for the characterization of the collagen.
8. Additionally, the author has not reported the denaturation temperature (thermal stability) of extracted collagen. The author stated that protein bands below 70 kDa were observed. However, after extraction, the collagen was not purified. Therefore, the presence of small peptides may be due to the extraction of non-collagen proteins. In addition, the 70 kDa protein may be the degradation products of $\alpha$ chain.

A detailed overview of the self-assembly property of type I collagen prepared from tilapia (*Oreochromis niloticus*) skin by different extraction methods has been published (Yan et al. 2015). In this study, we were regretted to see that work presented by authors did not clearly described the published information and created a controversy in the procedure of extraction of collagen and gelatin. Some of the specific points are as following-

1. Type I collagen prepared by the hot-water method, authors stated that the extraction the collagen were performed as previously described by Yan et al. (2011) with slight modifications. However, Yan et al. 2011 did not describe any method for collagen extraction process. This reference was not cited correctly in the article.
2. Authors used the 0.05 M sulphuric acid for swelling of skin at room temperature. Here, we would like to point out that swollen process led to the removal of collagen.
3. Fish skin collagens are extracted at low temperature ($\sim$4 °C). The low-temperature extraction process of fish skin collagens were well reported by several researchers (Liu et al. 2012; Pal et al. 2015). However, authors reported the extraction of collagen at 25–45 °C. Some readers may get the mistaken impression that the research article describes the collagen extraction at a higher temperature. Authors used the hot water extraction method that was commonly used for the extraction of the gelatin (a hydrolyzed form of collagen) (Niu et al. 2013).
4. Authors described that minced skins were continuously stirred overnight in distilled water at high temperature. Gelatin was mainly extracted in the water due to its higher solubility (Niu et al. 2013). However, fish skin collagen was extracted in the acidic conditions.

## Conclusion

In this study, we have pointed out the major technical problems for the extraction and characterization of the collagen from fish and marine waste. We hope that these technical comments could be able to avoid the misunderstanding of the readers and researchers about the collagen extraction and characterization. In addition, we would like to suggest that growing researchers and authors should make the serious efforts to check the accuracy of the references cited in their research and review articles because it is essential for the scientific knowledge transmission.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that we have no conflict of interest.

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