Characterization of Collagen Hydrolysates Prepared Using Different Organic Acids and Dairy By-Product

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Abstract

In this study, collagen hydrolysates were prepared from limed bovine split wastes using organic acids, alkali product and by-product of dairy industry. They were lyophilized in a freeze dryer and their morphology was examined with TM1000 scanning electron microscopy (SEM). The structures of collagen hydrolysates were characterized by XRD and FTIR. Differences between alkali and acid-based hydrolysis have been found.

Keywords: collagen dissolution by products, XRD, FTIR, dairy by-products

Introduction

Leather processing has been considered as one of the highly polluting industries due to generation of significant quantities of tanned and untanned wastes. Untanned wastes are less contaminated with chemicals and a better source of high value products such as collagen than tanned or finished leather wastes.

In this study the collagen hydrolysates recovered from bovine limed splits as value added products will be characterized and compared in order to benefit from them as potential biomaterials or additives.

Methodology

In the present study, collagen dissolution products obtained by treating limed bovine splits with citric acid (Coll-C), formic acid (Coll-F), acetic acid (Coll-A), sodium hydroxide (Coll-S) and by-product of dairy industry (Coll-D) (Fig. 1) were lyophilized in a freeze dryer and their morphology was observed by TM1000 scanning microscopy. The structures of collagen hydrolysates were characterized by XRD and FTIR.

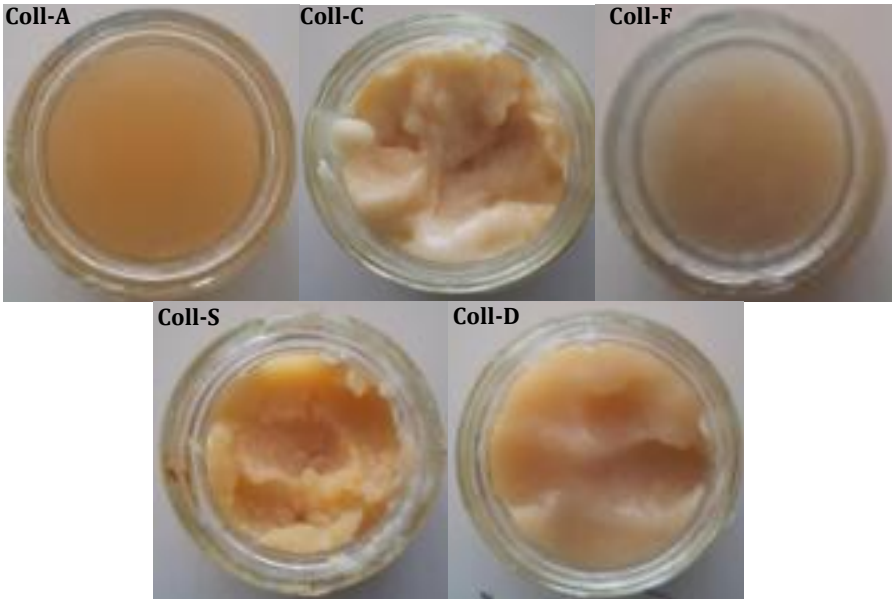

Figure 1. Collagen products obtained by treatment of citric acid (Coll-C), formic acid (Coll-F), acetic acid (Coll-A), sodium hydroxide (Coll-S) and by-product of dairy industry (Coll-D)

Coll-F showed a more transparent appearance and Coll-C has less homogenous texture than other collagen products. All hydrolyzates are yellow in colour, but Coll-S obtained by alkali treatment has the most intense yellow colour. The collagen hydrolysate derived from dairy by-product, Coll-D has a specific pungent smell.

Results and Discussion

Scanning electron microscopy (SEM)

The surface morphologies of the collagen hydrolysates obtained with various methods were greatly different from each other (Fig. 2). Collagen hydrolysates obtained with organic acids, Coll-A, Coll-C and Coll-F exhibit slightly less uniform porous structure than samples obtained with sodium hydroxide (Coll-S) and dairy by-product (Coll-D). Coll-S provided the minimum pore size.

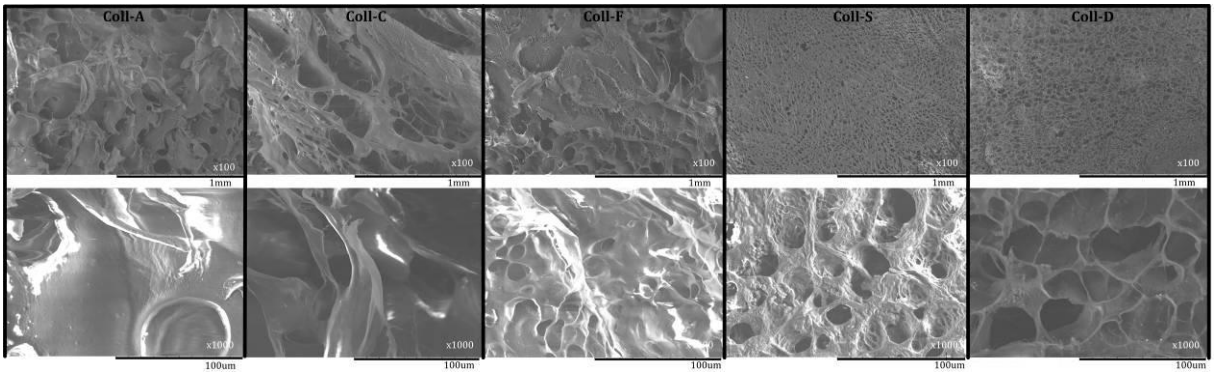

Figure 2. Surface morphologies of the collagen hydrolysates observed by SEM at 100x and 1000x magnifications.

Fourier Transform Infrared Spectra (FTIR)

The characteristics and changes in macromolecular composition of collagen products have been visualized with FTIR spectroscopy, and their spectrum was shown in Fig. 3.

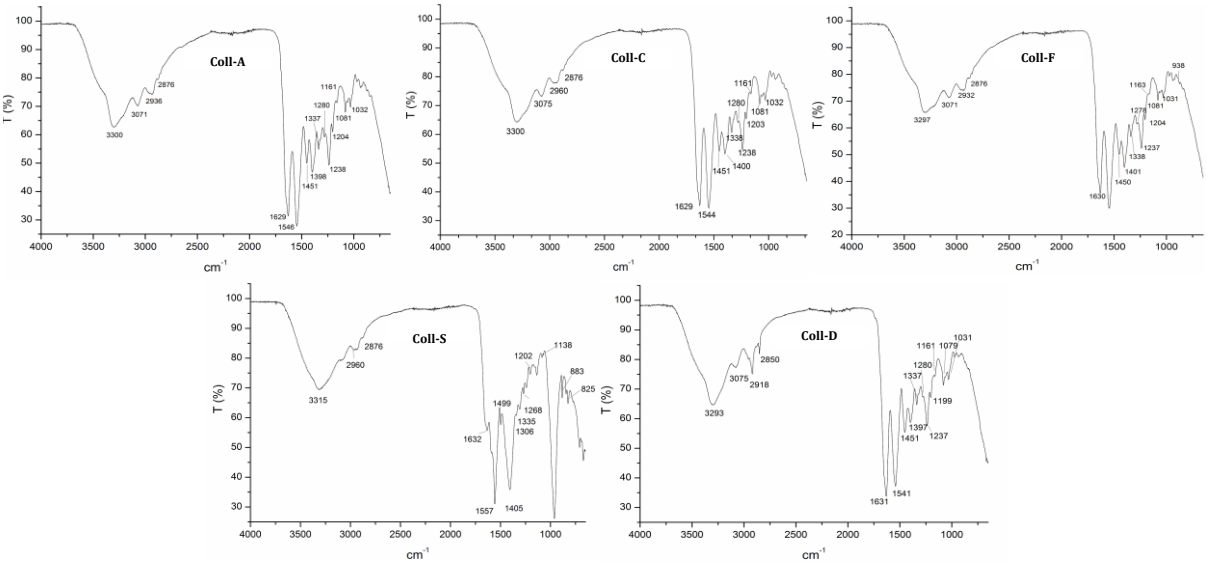

Figure 3. The FTIR spectrum of collagen hydrolyzates.

Although the amide group showed peaks in the bands between 3400-3440 cm^{-1} ranges (), due to binding to a peptide chain the vibrational mode of amide peaks switched to lower frequencies about 3300 cm^{-1}. This is verified with the spectrum obtained from the collagen products. The amide bands were observed at 3300 cm^{-1} for Coll-A and Coll-C, and at 3315 cm^{-1}, 3293 cm^{-1} and 3297 cm^{-1} for Coll-S, Coll-D and Coll-F respectively. The major bands between 2923-2926 cm^{-1} can be assigned to amid B band. Moreover sharp peaks around 1630 cm^{-1} observed at acid extracted collagen products represents amid bond due to C=O absorption. The observed bands confirmed the presence of α-helix. The reason for lower transmittance values of alkali extracted samples in the same peaks of acid extracted ones, and sharp peaks observed around 1000-1405 can be attributed to significant conformational change due to alkali effect (1, 2).

XRD Analysis

X-ray diffraction is known as a practical method for qualitative identification of crystal compounds. In the study the samples were exposed to the X-ray beam from X-ray generator running at 30 KV and 40 mA for 45 minutes. The scanning diffraction angles were 2-80 θ and step interval was 0.05°.

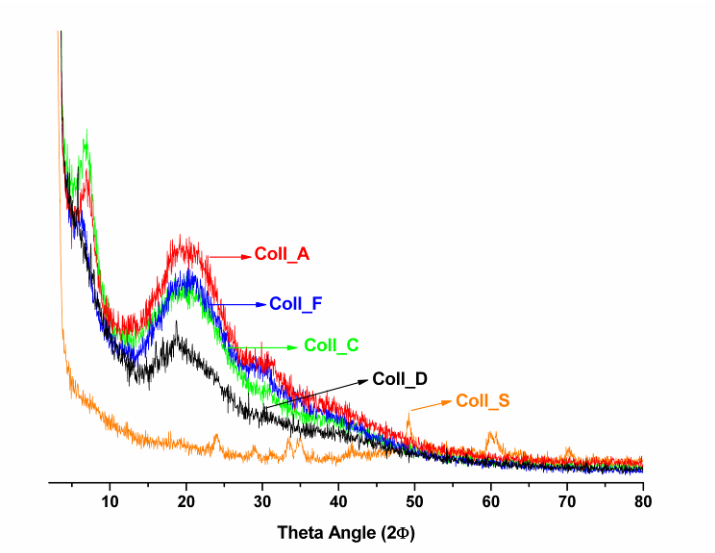

Figure 4. XRD graphs of collagen products

The X-ray diffractograms of the five collagen products are presented in Fig. 4. As can be seen from Fig. 4 XRD patterns of collagen products obtained from acidic treatments exhibited similarities, unlike alkali treated collagen products.

Conclusion

In the present study different types of collagen dissolution products were developed using various organic acids, sodium hydroxide and dairy by-product. The results suggest that although the products were all derived from bovine limed split wastes, they showed different characteristics in their morphologies and macromolecular compositions. The alkali derived collagen products exhibits only minor differences from acid extracted collagen, and that these differences can be attributed to a decrease in the inter- and intramolecular cross-linking.

Collagen derived from bovine limed split wastes has greater potential in biomaterial utilization than gelatin and collagen hydrolysate due to its special physicochemical properties, therefore characterization of collagen based products obtained with different chemicals is essential for investigating the applicability of these wastes in different industrial fields and economic benefits can be provided by conversion of an industrial solid waste into a valuable product.

Acknowledgements

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