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Enzymatic Hydrolysis of Bovine Leather Collagen for Use as Protein Supplement.

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Introduction

Increased concern with aesthetics and prevention of early degenerative diseases has led consumers to seek improvement in eating habits. Hydrolyzed collagen has been used as a dietary supplement in order to suppress collagen loss that occurs with age and has been used for therapeutic purposes, improving blood circulation, reducing joint problems and reducing gastrointestinal ulcers (Kim et al. 2005). Collagen in its native form has low absorption by the human organism, being a polypeptide chain protein long and high molecular weight. To obtain an easily absorbed product it is necessary to hydrolyse the collagen using different enzymes and physical treatments (Zhang et al., 2014). Collagen is obtained from a variety of sources, such as fish hide, chicken skin, cowhide among others (SHIGEMURA et al., 2014). Brazil is the 4th largest producer of bovine leather in the world (FAO, 2008). The bovine leather scrap presents an easily obtainable raw material, since it is a co-product of the tanning industry. In this context, the present work aimed to hydrolyze enzymatically the collagen obtained from bovine leather scrap in small peptide fractions of easy absorption and to evaluate among the different enzymes used, which is the most efficient for this hydrolysis.

Methods

For the hydrolysis of the collagen, 6 different enzymes: alcalase, neutrase, papain, trypsin, collagenase and pepsin were used. They were added at concentrations of 0.5 and 1% on the dry weight of the substrate (type I collagen) and incubated under agitation for three different times, (1, 2 and 4h) followed by lyophilization. To determine the molecular masses, proteins electrophoresis in gel of polyacrylamide and sodium duodenum sulfate (SDS-PAGE) was performed as described by Laemmli (1970).

Results

The treatments with the 6 different enzymes were divided into three groups: high, medium and low molecular weight. The enzymes collagenase and pepsin produced high molecular weight peptide fractions, regardless of the enzyme concentration and the time of treatment, these enzymes produced fractions ranging from 235 to 5.6 kDa for collagenase and between 235 to 40kDa for pepsin. Neutrase and trypsin enzymes were classified as medium molecular weight, where the best treatments were the most drastic with 1% E / S for 4 hours incubation, where strong and visible bands between 14 and 50 kDa were produced with neutrase, and bands between 5.6 and 70 kDa with trypsin. In the treatments with neutrase it was possible to observe that with an increase of the treatment time and enzyme concentration with a better visualization of the bands and less drag in the gel was observed. The trypsin enzyme produced a large fractionation range, but at lower concentrations did not hydrolyze the collagen completely, and for all treatments, gel was formed after cooling, as also observed by Zhang et al. (2014).The enzymes papain and alcalase has produced peptide fractions with low molecular weight when compared to the previous enzymes, none of the treatments formed a band above 50 kDa but both had different characteristics. In the treatments with papain it was possible to observe a large drag on the gel bands. The fractionation intervals decreased with increasing concentration and treatment time. The alcalase enzyme proved to be very efficient from the moment of incubation, in which in only 10 minutes, it was no longer possible to observe the presence of solid particles and the solution after cooling did not form gel. Zhang et al. (2014) hydrolyzed collagen using several enzymes, and in hydrolysis with alcalase, thery observed a phenomenon similar to this work. Alcalase treatment produced the best fractionation between 5,6 and 15 kDa, and it was more stable producing similar fractions in all treatments. Alcalase treatments formed clearer and more visible bands, Figure 1(A) shows that in 1% E/S (Enzyme/Substrate) concentration there was no visible difference, and that 0.5% E/S for 4 hours it was equal to the treatments with 1% E/S. The comparison gel in Figure 1 (B) was produced with the treatments of each enzyme with 1% E/S for 4 hours. The treatment with pepsin was not included due to its high molecular weight. In the comparing gel, the treatments were applied in decreasing order of size of the fragments formed, in this gel it is possible to observe the large drag formed by the papain without a well defined band, and all the bands formed by the other treatments.

Conclusion

It was concluded that the alcalase enzyme was the most efficient and stable in the collagen hydrolysis of bovine leather, producing peptide fractions of less than 25 kDa. Therefore this enzyme shows promise for the production of hydrolyzed collagen and for application in the food and cosmetic industries.

References

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Notes

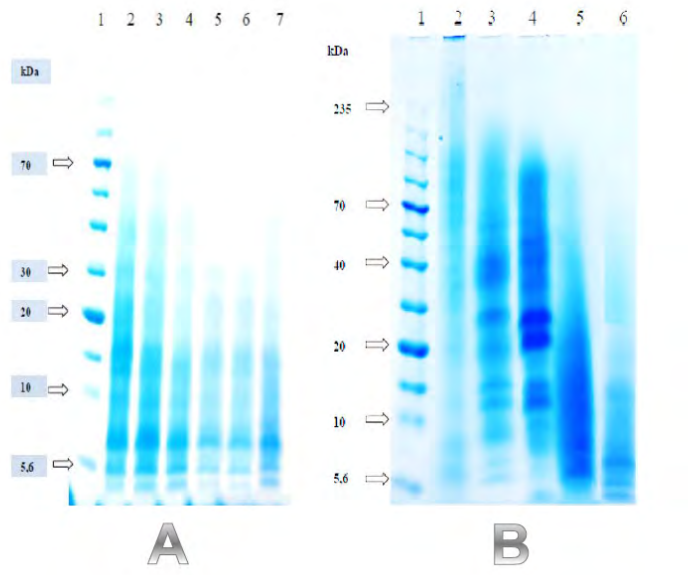

Figure 1
A). Gel electrophoresis, alcalase treatment. (1) molecular weight marker, (2, 3 and 4) 0.5% E/S for 1, 2 and 4h respectively, (5, 6 and 7) 1% E/S for 1, 2 and 4h respectively. (B) Comparison gel between the treatments of collagen hydrolysis of bovine scrap with different enzymes. (1) molecular weight marker, (2) collagenase, (3) trypsin, (4) neutrase, (5) papain and (6) alcalase.

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