



OPTIMIZATION OF THE ENZYMATIC-AND ULTRASOUND-ASSISTED EXTRACTION OF HYDROLYZED COLLAGEN FROM BASA FISH (*P. bocourti*) BY RESPONSE SURFACE METHODOLOGY

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Abstract

This study aimed to optimize hydrolyzed-collagen extraction from basa skin fish (*Pangasius bocourti*) by Alcalase using Response Surface Methodology (RSM). First, fish skin is pretreated with NaOH, H₂O₂, and n-butanol to remove non-collagen protein, pigment and fat. The results showed that the hydrolyzed collagen with the Alcalase enzyme concentration of 0.15% stabilized in buffer pH 7 at 4 °C in 24 hours was 41.27 ± 1.71% in recovery yield. With the aid of ultrasound, using the optimized condition with 150 W in 60 minutes can recover 38.47 ± 1.31% of collagen. After that, the sample analysis experiments were conducted to test the specific properties of type-I collagen.

Introduction

Basa fish is a type of white fish, found in abundance in the waters of the Mekong River. This is a type of catfish, scientifically known as *Pangasius bocourti*.

Enzyme alcalase is a commercial protease preparation supplied by Novozyme - Denmark. Alcalase is an endopeptidase. Enzymatic parameters on product packaging:

Activity: 2.4 AU-A/g

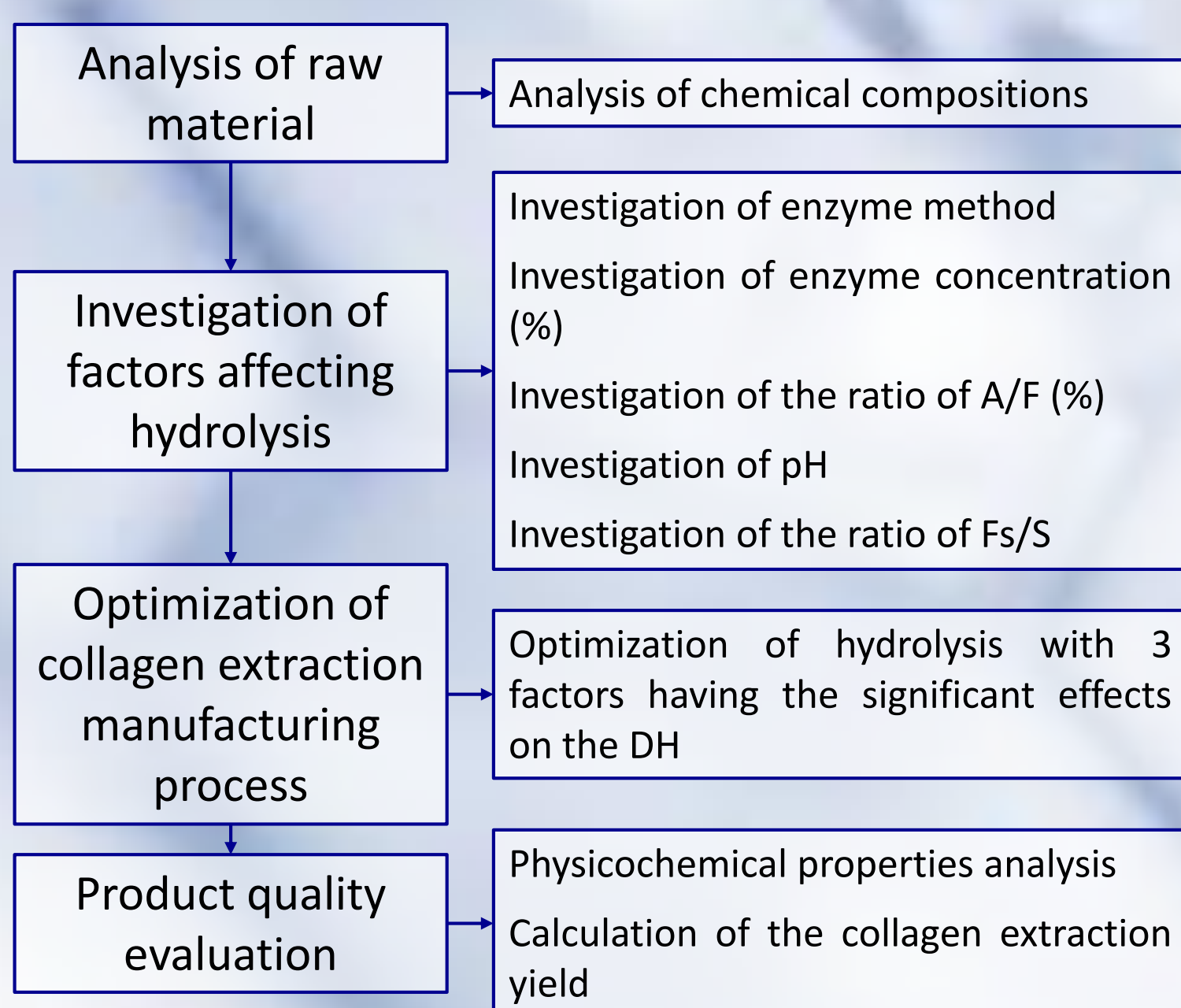
Optimum temperature: 30 oC – 65 oC

Active pH: 6 - 9



Basa fish (*Pangasius bocourti*)

Methods

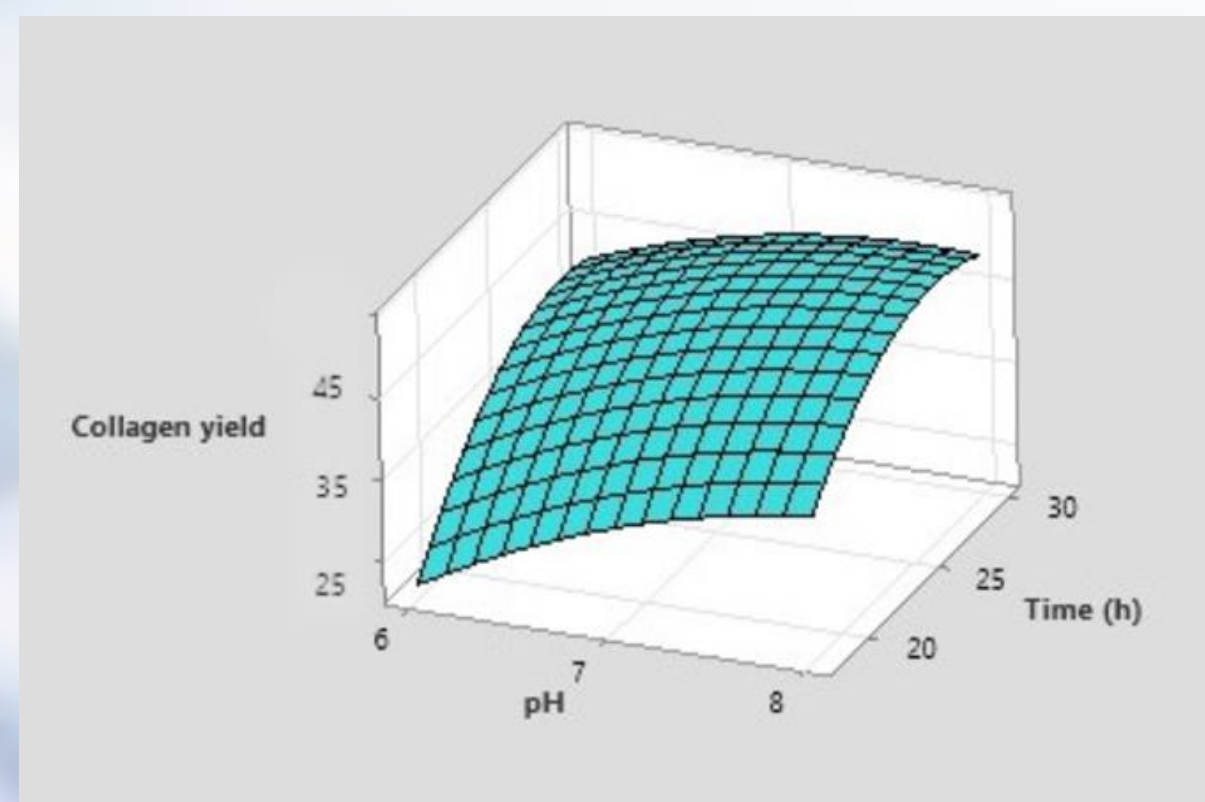


Results

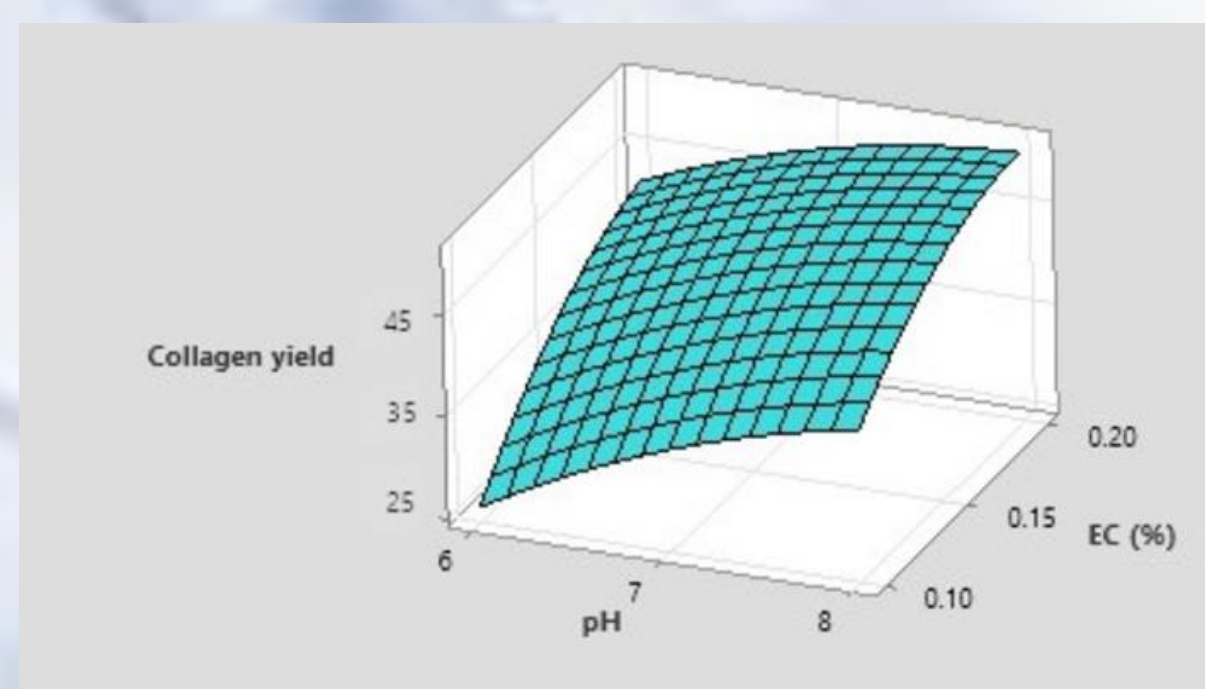
The hydrolyzed collagen



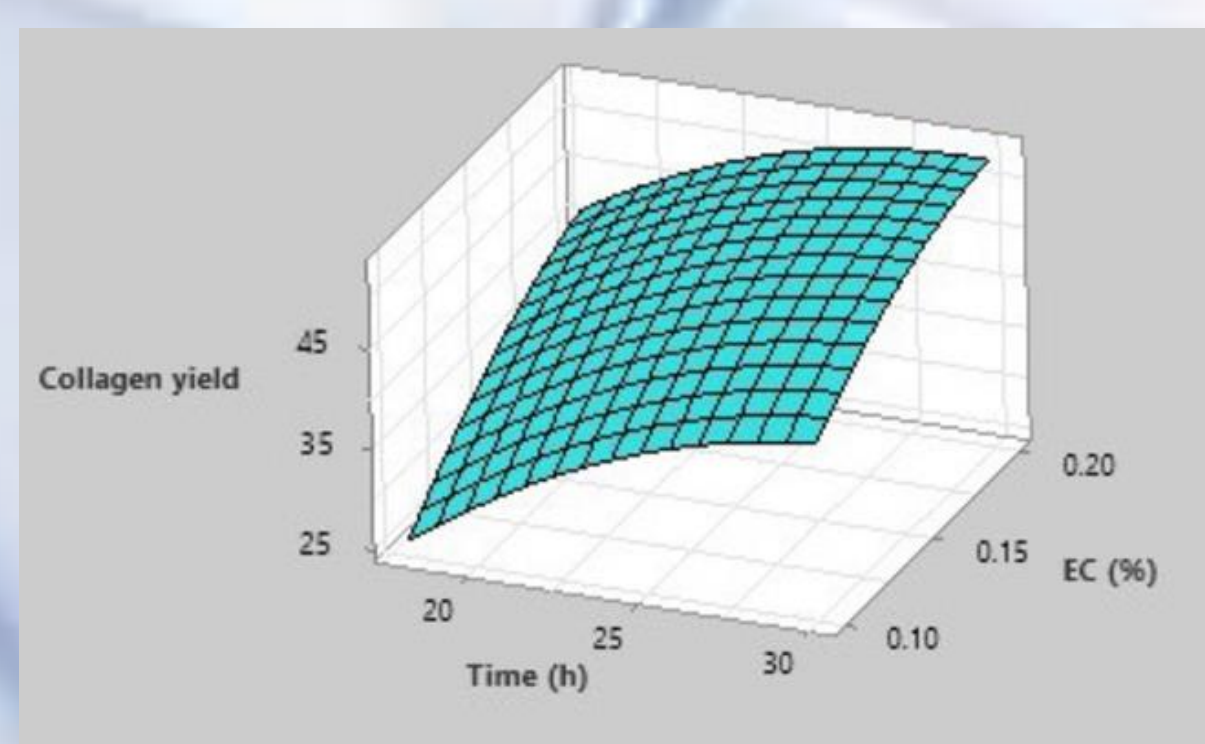
The surface response of collagen recovery is influenced by the combination of time hydrolysis and pH



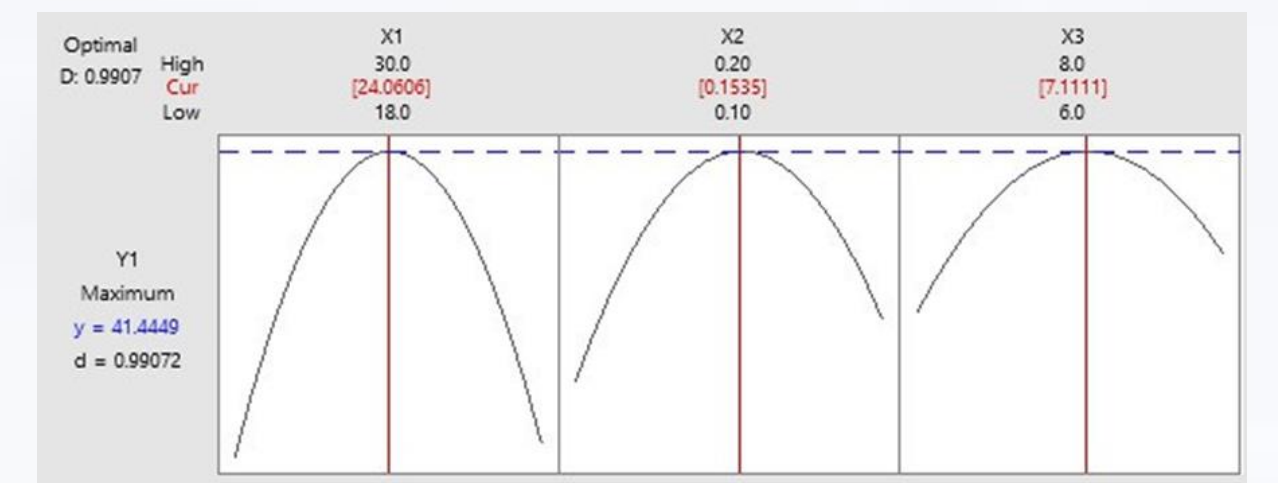
The surface response of collagen recovery is influenced by the combination of pH and enzyme concentration



The surface response of collagen recovery is influenced by the combination of time hydrolysis and enzyme concentration



The optimal values of the three extracted factors are predicted from the model



Conclusion

Under the same optimal conditions for hydrolysis, alcalase yields a collagen recovery efficiency that is more than two times higher than that of pepsin (41.27 ± 1.71% compared to 14.08 ± 1.71%). The three-factor BBD designed experiment predicted that with an enzyme concentration of 0.15%, in pH 7 and a duration of nearly 24 hours, the collagen recovery efficiency will be approximately 41.44%. By adhering to the optimal conditions set by the model, the actual collagen recovery reached an impressive 41.27 ± 1.71%. In particular, the correlation between the experiment and predicted model is very high (99.59%). Specifically, 60 minutes with a power ultrasound of 150 W, the recovery efficiency is 38.47 ± 1.31%, which is nearly equal to 24 hours of enzymatic hydrolysis.

Glycine is the most abundant amino acid found in collagen from basa fish skin, with a content of 25.2g (out of a total of 83.52g of amino acids), equivalent to 30.17%. The collagen extracted from basa fish skin has been analyzed to contain 11.95% of imino acid content. When the imino acid content is higher, the supercoiled structure of collagen becomes more stable and the melting temperature of collagen increases.

References

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