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Original Research Article

Comparative analysis of collagen-1 amongst locally-farmed tilapia samples as potential xenograft for partial-thickness burns

Pinyapat Siriruck*, Woraphat Pongpoon

Nawamintharachinuthit Triamudomsuksanomklao School, Bangkok, Thailand

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***Correspondence:**
Pinyapat Siriruck,
E-mail: jasminesiriruck@gmail.com

ABSTRACT

Background: Tilapia farming is amongst the most lucrative in terms of yield and return of investments in the aquaculture industry in Thailand. The sustainable supply of tilapia (*Oreochromis spp.*) can be exploited to reconsider the fish species as a huge potential for Thailand's medical sector, particularly on xenograft as an innovative treatment for burns.
Methods: Collagen content quantification by high-performance liquid chromatography (HPLC) method is done on two samples: Chitralada I tilapia (Thai locally cultured strain of *Oreochromis niloticus*) and pink tilapia (commercially cultured strain by Charoen Pokphand Foods PCL) skin to compare the amount of type I collagen (COL-I) by measuring one of its major amino acids, hydroxyproline, with Nile tilapia skin which has been used as xenograft in Brazil.
Results: The results showed that there is no significant difference in the amount of hydroxyproline amongst the two strains. Chitralada I tilapia sample contains 9.01 g/100g (9.01%) and pink tilapia sample contains 18.55 g/100g (18.55%) of hydroxyproline respectively.
Conclusions: Since there are minor differences between the amount of hydroxyproline in Thai locally-farmed tilapia and Nile tilapia; therefore, Thai locally-farmed tilapia xenograft can be a possible choice for burn treatment.

Keywords: Collagen type-I, Xenograft, Burn treatment

INTRODUCTION

The Nile tilapia (*Oreochromis niloticus*) is a species of tilapia that is abundant in the northern half of Africa and can be found prominently in the Nile River.[1] In Thailand, various subspecies of tilapia are farmed including black tilapia, Mozambique tilapia, red tilapia, and pink tilapia.

Tilapia, among other fish species, is one of the most important fish farmed for economic purposes in Thailand. Since its introduction to the country in the 1960's, the demand for tilapia farming has remained robust and highly competitive in the supply chain, making Thailand amongst the world's top producers. According to Thailand Department of Fisheries, in 2017, the country produced 176,463 tons of tilapia from its various aquaculture sectors and 7,975.4 tons of tilapia and tilapia by-products ranging from frozen fillets to processed tilapia parts were exported worth more than 598.5 million baht. However, the mass exportation causes waste from fish skins, scales, fins, and fishbones.[2]

The sustainable supply of tilapia (*Oreochromis* spp.) can be exploited to reconsider the fish species as a huge potential as raw material for other useful by-products. In the recent years, tissue samples from tilapia have been used as innovative treatment for burns. The first medical use of Nile tilapia skin as a xenograft was first documented in Brazil. In 2017, scientists at the Federal University of Ceara have found that that tilapia skin possesses moisture, collagen, and antibacterial characteristics similar to those of human skin, and could be beneficial for healing purposes.[3]

The sustainable supply of tilapia stocks for supply chains in the country often overlook the huge amount of biowastes from tilapia processing companies. The researchers believed that this biowastes, though perceived as potential health and environment hazards can be processed and turned into lucrative products such as xenograft, which gains increasing acceptance to be one of the innovative treatments for burns. This prompted the researchers to explore this undertaking. The aims of this project are to characterize and quantify hydroxyproline (one of the major amino acids of COL-I) in Thai locally-farmed tilapia skin in order to make a comparison with afore researched Nile tilapia skin.

METHODS

This experimental study was performed at Food Quality Assurance Service Center, Institute of Food Research and Product Development, Kasetsart University, Bangkok, Thailand from 15th to 30th of August 2022. Pink Tilapia and Chitralada I tilapia samples were selected according to the inclusion and exclusion criteria. The inclusion criteria included the age of the fish as fully matured ones ranging from 5 to 10 months old were picked for the research. The exclusion criteria included other subspecies of tilapia other than pink tilapia and Chitralada I tilapia and were not purely bred from a creditable local farm.

Table 1: The properties of HPLC machine.

Variables	
Column	C18, 250 mm, ID 4.6 mm
Detector	FLD at Ex: 270, Em: 316
Column temperature	30 °C
Flow rate	0.7 ml/min
Injection volume	20 µl
Mobile phase	Gradient monosodium phosphate: (methanol + acetonitrile + water)
Run time	35 minutes

The method of quantizing hydroxyproline was implemented from Hutson et al.[4] First, 100 g of sample were measured. Second, 2 ml of 6 M hydrochloric acid were added to the sample. Third, the samples were heated with block heater at 110 °C for 24 hours. Fourth, the water level was adjusted by adding deionized water then the sample was injected into HPLC machine. Fifth, make the derivative by pipetting 1 ml of borate buffer into test tube and add 0.25 ml of 50% fluorenylmethyloxy-carbonyl chloride then shake vigorously. Last, the sample was injected into the HPLC machine.

The specification of the HPLC machine was HP 1260, Detector: Fluorescence Detector (FLD) from Agilent Technologies, Germany. The chemicals used were hydroxy L-proline 99% (from Sigma-Aldrich), 0.5% fluorenylmethyloxycarbonyl chloride, monosodium phosphate, 6 M hydrochloric acid, borate buffer, acetonitrile, and methanol.

RESULTS

The amount of hydroxyproline from the selected subspecies of fish are as follows, pink tilapia contains 18.55 g/100g, Nile tilapia contains 10.28 g/100g, Chitralada I tilapia contains 9.01 g/100g respectively.

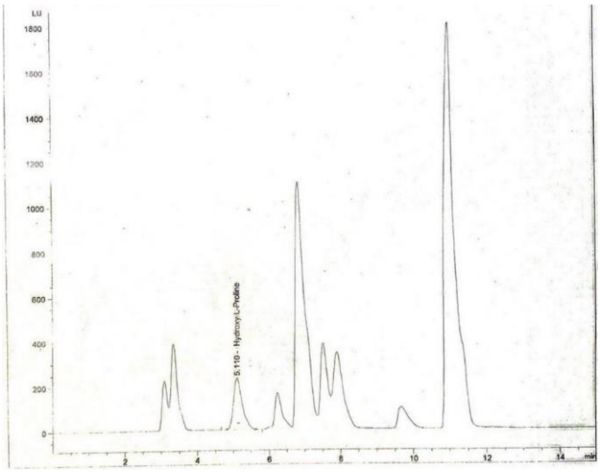

Figure 1: The chromatograms obtained from HPLC machine.

Table 2: The comparison of hydroxyproline amongst selected fish subspecies.

Subspecies	Hydroxyproline (g/100g)
Pink tilapia	18.55
Nile tilapia[5]*	10.28
Chitralada I tilapia	9.01

*Data obtained

DISCUSSION

Collagen is one of the major components that helps maintain the biological and structural integrity of the extracellular matrix.[6] Among more than 27 types of collagen, type I collagen (COL-I) is found to be most abundant in human bone, skin, teeth, fibrocartilage, and tendon and has a prominent ability to form highly-resistant fibrils.[7] In order to compare the amount of COL-I in the sample, hydroxyproline which is a major component of the protein collagen[8] and composing approximately 13 to 14 percent of mammalian collagen is quantified.[9] Xenografting has gained more popularity in the development of biomaterials which mostly contain collagen as their main constituent. Biomaterials have an important role in regenerative techniques like biological dressings used in burn therapy.[10] According to the research by Yamamoto et al, COL-I extracted from Nile tilapia skin is easier absorbed compared to bovine and porcine collagen, which is why Nile tilapia is often used in biomaterials and is a safe material with no following adverse skin reactions.[11] Moreover, COL-I from Nile tilapia dominates porcine collagen in inducing human mesenchymal stem cell, crucial in skin rejuvenating.[12] But ultimately, the reason behind the suggestion of the tilapia skin as a potential xenograft for the burn injuries is because the tilapia skin contain more properties such as large amount of COL-I, and alike morphological and histological structure to the human skin.[13] In addition, the tilapia skin promotes the healing of the patient's skin without hematological and biochemical changes.[14] Likewise, it can enhance the synthesis of epidermal and fibroblast which help reduce the pain from burn wounds, patient cost, and human resource since there is no need of dressing changes.[15] Hence, pink tilapia, Nile tilapia, and Chitralada I tilapia skin can be used as a potential xenograft substituting other costly and painful methods of treating burn wounds.

CONCLUSION

From the obtained Tilapia samples, the results showed that there is no significant difference in the amount of hydroxyproline amongst the three subspecies. Pink tilapia has the highest amount of hydroxyproline followed by Nile tilapia, and Chitralada I tilapia respectively. Pink tilapia has 8.27 g/100g higher yield of hydroxyproline compared to Nile tilapia. On the other hand, Nile tilapia has 1.27 g/100g higher yield of hydroxyproline compared to Chitralada I, whereas pink tilapia has 9.54 g/100g higher yield of hydroxyproline compared to Chitralada I tilapia.

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