ENZYMATIC HYDROLYSIS OF RESIDUAL OIL FROM TILAPIA (*Oreochromis niloticus*) USING COMMERCIAL LIPASE *Thermomyces lanuginosus*

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ABSTRACT

In 2020, global aquatic animal production from fisheries and aquaculture reached 178 million tonnes, a marginal increase of 0.2% from the previous year but lower than the record 179 million tonnes in 2018. With the growth of fisheries, a lot of fish waste is generated, representing up to 15% of the total weight of the fish and up to 45% of the oil produced, a valuable resource for biodiesel. This study highlights these residues' potential for producing bioproducts, such as fatty acids for ester synthesis. The hydrolysis of waste oils, including fish oil, is a sustainable solution, using enzymes such as lipases, especially Thermomyces Lanuginosus lipase (TLL), for various biotechnological applications. Therefore, the present test carried out enzymatic hydrolysis using residual tilapia oil (*Oreochromis niloticus)* as a substrate and the commercial lipase TLL as a biocatalyst, increasing the acidity index equivalent to 127.72%, indicating the potential of residual oils in the synthesis of bioproducts. These promising results pave the way for future commercial applications, instilling a sense of hope and optimism in the scientific community.

Keywords: Waste fish oil. Residual oil. Hydrolysis. Biodiesel. Thermomyces Lanuginosus.

1 INTRODUCTION

In 2020, the global production of aquatic animals through fishing and aquaculture amounted to 178 million tons, indicating a marginal increase of 0.2% compared to the previous year. However, this figure was lower than the highest recorded amount of 179 million tons in 2018[1]. As fishing activity grows, much fish waste is generated, particularly offal. This waste, often overlooked, can account for 7.5% to 15% of the fish's total weight and up to 35% to 45% of the oil produced, which is a valuable resource for producing biodiesel[2].

The hydrolysis of waste oils, including fish oil, is emerging as a critical raw material for biodiesel production, offering a sustainable and efficient solution[3]. By leveraging this waste, we reduce wastage and contribute to producing renewable and sustainable energy, aligning with the objectives of reducing carbon emissions and promoting the circular economy. Hydrolysis is a chemical reaction in which a water molecule is used to break chemical bonds in another molecule (Figure 1)[4]. Chemical hydrolysis and biological hydrolysis differ mainly in their mechanisms and contexts of occurrence. Chemical hydrolysis involves breaking chemical bonds by adding water, generally occurring under controlled laboratory conditions or in industrial processes, where specific chemical reagents and temperature and pressure conditions are adjusted to facilitate the reaction[5].

The biological route appears as an alternative, characterized by the use of enzymes to obtain the bioproduct. Its use on an industrial scale presents advantages in terms of sustainability and efficiency[6,7]. Enzymes are biological catalysts responsible for promoting the transformation of chemical species in living systems. They can catalyze reactions under mild conditions with a very high degree of substrate specificity, thus reducing the formation of byproducts[8].

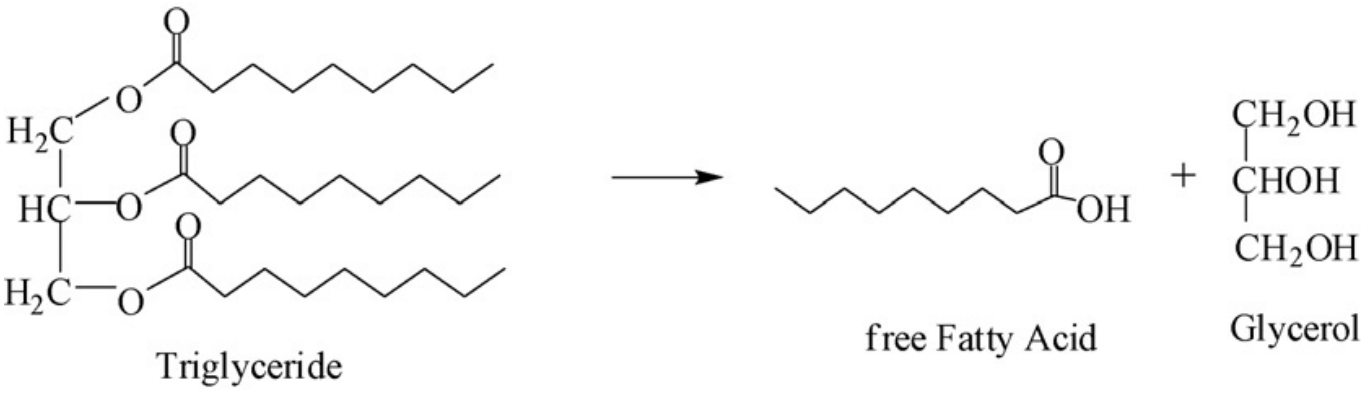

Figure 1 Hydrolysis reaction of a triglyceride (oil) obtaining fatty acids and glycerol as products.

Thermomyces Lanuginosus lipase stands out for its remarkable performance in biotechnological applications[9]. This is due to its robustness and stability when used in extreme temperature and pH operating conditions[10]. Its ability to hydrolyze triacylglycerols

into fatty acids and glycerol and its versatility to catalyze transesterification, esterification, and interesterification reactions are worth highlighting[11]. Therefore, it is frequently used to produce biodiesel, food, cosmetics, and pharmaceutical products[12]. This work aims to explore the potential of residual tilapia oil as a raw material for the synthesis of high-value products such as free fatty acids using the commercial lipase *Thermomyces lanuginosus* as a catalyst. The evaluation will be carried out by comparing the initial acidity value of the oil and after enzymatic hydrolysis.

2 MATERIAL & METHODS

This study utilized the commercial lipase from *Thermomyces lanuginosus* obtained from Sigma-Aldrich Brasil Ltda in Cotia, São Paulo, Brazil. Additionally, all other chemical reagents used, which were of analytical grade, were provided by Synth and Vetec, both located in São Paulo, Brazil. Furthermore, the residual tilapia oil used in this research was obtained through collaborative efforts with fish farming companies in Ceará, Brazil.

The residual tilapia oil underwent enzymatic hydrolysis using *Thermomyces lanuginosus* (TLL) as the catalyst. The procedure followed the methodology proposed by Carvalho et al. (2021) with some adaptations[13]. A solution consisting of equal parts oil and water was prepared, and 0.4% of the biocatalyst was added to the oil. The mixture was continuously stirred for 4 hours at 40°C. Afterward, the solution was separated using a decantation funnel, and the free fatty acids were washed three times and heated at 80°C for 10 minutes. Anhydrous sodium sulfate was used to remove moisture, and the free fatty acids were stored in an amber glass container.

Equation 1 calculated the initial and final acidity index (AI) (after the hydrolysis step). Aliquots of 0.3 g were taken from the volume of the final mixture and diluted in 10 mL of ethyl alcohol. Three drops of phenolphthalein were added, and the mixture was then titrated with a 0.1 M sodium hydroxide solution.

$$IA\left(\frac{mgNaOH}{g}\right) = \frac{MM_{NaOh} \cdot M_{NaOH} \cdot f \cdot V_{NaOH}}{m} \quad (1)$$

$MMNaOH$ (g/mol) is the molar mass of NaOH; $MNaOH$ (mol/L) is the molarity of the NaOH solution; f is the correction factor determined through NaOH standardization; $VNaOH$ is the volume of NaOH used during the titration (L); and, m (g) is the mass of the sample to be studied. The conversion of free fatty acids (Equation 2) was determined taking into account the initial (IAi) and final acidity of the sample (IAf).

$$Conversion\ (\%) = \frac{I\,Ai - I\,Af}{I\,Ai} x100 \quad (2)$$

3 RESULTS & DISCUSSION

Obtaining fatty acids through the hydrolysis of oils is a way of economically taking advantage of these renewable substrates. Oils are part of the group of materials with fatty acid esters, and their hydrolysis by water generates free fatty acids[14]. The increase in the acid value of the residual tilapia oil was 41.56 mg NaOH/g to 94.64 mg NaOH/g, indicating that the objective of enzymatic hydrolysis was achieved. In other words, there was a release and increase in free fatty acids. Therefore, residual tilapia oil has the potential for biodiesel production in this study.

Table 1 Comparison between the acidity index before and after enzymatic hydrolysis**.**

	Acidity index before	Acidity index after
Tilapia waste oil	41.56 mg NaOH/g	94.64 mg NaOH/g

Santos et al. (2010) used tilapia oil to produce biodiesel and to obtain free fatty acids; they opted for chemical hydrolysis (alcoholic saponification with sodium hydroxide followed by hydrolysis with sulfuric acid) assisted by ultrasound[15]. Among the disadvantages of the chemical route for hydrolysis of oils or fats is the need to add separation and purification steps, which increases time and energy costs. In contrast, the enzymatic route offers advantages such as reducing the cogeneration of products without the need for adding subsequent steps.

4 CONCLUSION

The use of biocatalysts in the synthesis of high-value-added products has stood out due to their versatility, effectiveness, and yield. Therefore, tilapia waste oil presents a viable alternative for future commercial applications. Furthermore, the development of complementary methodologies for integration with the industrial sector will contribute to sustainable development.

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