

Transesterification and Quantification by ¹H NMR of Triester Oleate by *Candida antarctica* Lipase

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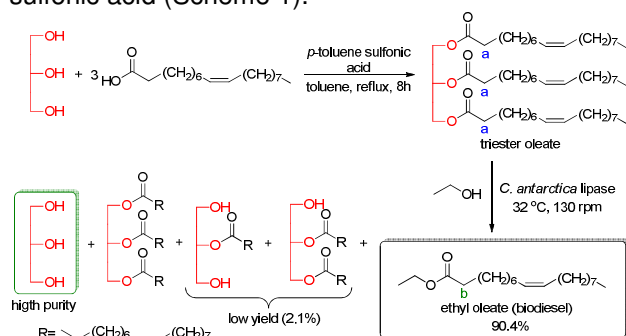
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INTRODUCTION

The Biodiesel is defined as a fatty acid alkyl ester obtained by a transesterification process using homogeneous or heterogeneous catalysis¹. Recent studies show that lipases can be used as catalysts for biodiesel production, thus, the production is "clean" and need less purification steps compared to the homogeneous chemical methods². In this work, we studied the enzymatic transesterification reaction of the oleate triester with ethanol and the products were quantified by ¹H NMR.

RESULTS AND DISCUSSION

First the glycerol triester oleate was prepared from glycerol and oleic acid catalyzed by p-toluene sulfonic acid (Scheme 1).



Scheme 1. Enzymatic synthesis of ethyl oleate from oleic acid and glycerol.

The triester oleate was then subjected to the enzymatic transesterification using different reaction times (1, 4, 8, 15 and 24h). The reactions were carried out in Eppendorf tubes where was added the oleate triester (250 mg, 0.282 mmol), ethanol (750 μ L, 12.85 mmol) and lipase (5.0% m/m in relation to triester oleate). The reaction remained in an orbital shaker at 32°C and 130 rpm. The products were washed with distillate water (3 x 1 mL), dried with Na₂SO₄ and the excess of the solvent was removed by evaporation at low pressure. The conversion to the products was determined by ¹H NMR based on signals the α -CH₂ protons of the carbonyl groups (a to triester oleate at 2.36 ppm and b to ethyl oleate at

2.31 ppm). The results obtained were compared with the obtained by GC-FID quantification (Figure 1).

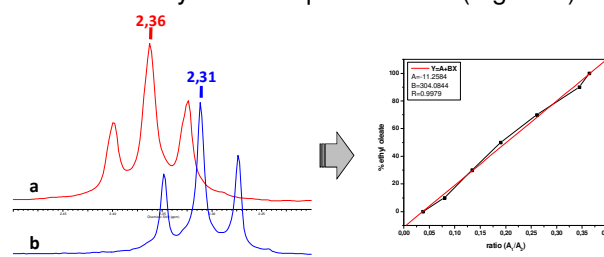


Figure 1. Analytical curve obtained by ¹H NMR and employed on quantification of transesterification of the triester oleate.

The conversions are show in a table 1.

Table 1. Comparison between GC-FID and ¹H NMR quantification.

Entry	Time (h)	GC-FID (%)	¹ H NMR (%)
1	1	39.1	41
2	4	56.8	59
3	8	65.4	66
4	15	80.3	81
5	24	90.4	93

It is possible to observe that good conversions were obtained with 5.0% of lipase at 24 h of reaction and the two techniques of quantification (GC-FID and ¹H NMR) showed similar results.

CONCLUSION

The biodiesel was produced by transesterification using lipase from *C. antarctica*. The quantification by ¹H NMR showed to be more efficient when compared with the GC-FID analysis.

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