The higher lipid productivity of *Rhodotorula glutinis* TISTR5159 was achieved by optimizing the pineapple pulp hydrolysis for releasing the high sugars content. The sequential simplex method operated by varied; solid-to-liquid ratio, sulfuric acid concentration, temperature, and hydrolysis time were successfully applied and the highest sugar content (83.2 g/L) evaluated at a solid-to-liquid ratio of 1:10.8, 3.2% sulfuric acid, 105 °C for 13.9 min. Moreover, the  $(NH_4)_2SO_4$  supplement enhanced the lipid productivity and gave the maximum yields of biomass and lipid of 15.2 g/L and 9.15 g/L (60.2%), respectively. The C16 and C18 fatty acids were found as main components included oleic acid (55.8%), palmitic acid (16.6%), linoleic acid (11.9%), and stearic acid (7.8%). These results present the possibility to convert the sugars in pineapple pulp hydrolysate to lipids. The fatty acid profile was also similar to vegetable oils. Thus, it could be used as potential feedstock for biodiesel production.

High lipid productivity of *Rhodotorula glutinis* from pineapple pulp residue has obtained via simplex optimization the acid hydrolysis and compositions of hydrolysate.

