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(54) Title: ENGINEERING OF *RHIZOPUS ORYZAE* LIPASE TO INCREASE ITS THERMOSTABILITY FOR THE PRODUCTION OF STRUCTURED TRIACYLGLYCEROLS

First round of screening		Second round of screening	
A8E	Y187N	A7R	V175F
S31D	G193K	L113Y	G177C
S114N	Q197H	Q150F	T199V
N134Y	Q197Y	Q150H	F216Y
T136D	G228D	G155A	S223Y
P163K	G228S	S171F	F261Y
F173Y	S267L		

FIG. 2

(57) Abstract: Described herein are modified enzymes with increased thermostability. The modified enzymes of SEQ ID NO: 3 includes at least one amino acid substitution of SEQ ID NO: 3, the at least one amino acid substitution selected from the group consisting of A8E, S114N, N134Y, T136D, F173Y, Y187N, G193K, Q197H, Q197Y, G228D, S267L, A7R, L113Y, Q150F, Q150H, G155A, S171F, V175F, G177C, T199V, F216Y, S223Y, F261Y, and any combinations thereof. The modified enzymes may be used in methods of forming a first triglyceride by mixing a second triglyceride, a first fatty acid, and the modified enzyme to form the first triglyceride which is a mixture of fatty acids from the second triglyceride and the first fatty acid. Biodiesel may be produced by mixing a fatty acid or a triglyceride, a short-chained alcohol, and the modified enzyme to form a fatty acid ester of the short-chained alcohol.

[Continued on next page]

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