Supporting Information

A highly efficient and enzyme-recoverable method for enzymatic concentrating omega-3 fatty acids generated by hydrolysis of fish oil in a substrate-constituted three-liquid phase system

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Table S1 The main fatty acid composition of tuna oil.

fatty acid	Content (w, %)
C14:0	4.26
C15:0	0.85
C16:0	18.54
C18:0	4.85
C20:0	0.52
C16:1	6.42
C18:1	11.00
C20:1	1.06
C22	0.34
C18:2	1.99
C20:2	1.56
C22:2	0.47
C18:3n3	0.81
C18:3n6	0.20
C20:4n6	1.72
EPA	8.25
DPA	1.30
DHA	23.17

Table S2 Number of phases in different systems

	PEG-400	PEG-600	$[BMIM]BF_4$	$[EMIM]EtSO_4$	methanol	ethanol	isopropanol	isoamyl alcohol
$(NH_4)_2SO_4$	III	III	III	III	II	III	III	II
Na_2SO_4	III	III	III	III	II	III	III	II

Table S3 Partition coefficients and recoveries of lipases as well as the yields of the FFAs in different SC-TLPSs contained ionic liquid.

	[DMIM]Cl/ Na_2SO_4[a]	[OMIM]Cl/ Na_2SO_4[a]	[EMIM]EtSO$_4$/ Na_2SO_4[a]	[OMIM]Cl/ Na_2SO_4[b]	[EMIM]EtSO$_4$ / $(NH_4)_2SO_4$[b]	[BMIM]Br / $(NH_4)_2SO_4$[c]	[EMIM]EtSO$_4$ / $(NH_4)_2SO_4$[c]
K_{lipase}	573.3	37.9	0	49.3	0	3.6	0
Y_{lipase} (%)	99.9	99.3	0	96.6	0	76.4	0
Y_{FFA} (%)	0	0	0.33	0	0.26	0.63	1.01

[a]: lipase AYS; [b]: lipase PL20000; [c]: lipase TL-100L. Y_{FFA}: the yields of the FFAs

Table S4 Changes in fatty acid content of the glycerides fractions and FFAs as well as the recoveries of n3 PUFA obtained by multi-repeated hydrolysis in the SC-TLPS.

		$W_{C14:0}$	$W_{C16:0}$	$W_{C18:1}$	$W_{C20:4n6}$	W_{EPA}	W_{DPA}	W_{DHA}	W_{PUFA}	R_{PUFA}
I	GB-FAs (%)	3.00	10.96	9.43	2.74	10.93	2.02	35.91	48.86	87.95
	FFA (%)	5.13	24.90	21.45	0.97	5.65	0.78	10.36	16.79	12.05
II	GB-FAs (%)	2.21	7.53	4.57	3.17	12.02	2.38	42.49	56.89	77.03
	FFA (%)	4.39	19.08	15.61	1.62	7.93	1.37	21.99	31.29	10.92
III	GB-FAs (%)	1.629	5.14	3.21	3.43	12.56	2.57	47.83	62.96	70.27
	FFA (%)	4.24	16.39	8.46	2.07	10.31	1.64	23.76	35.71	6.76
IV	GB-FAs (%)	1.17	3.51	3.05	3.66	12.74	2.705	52.52	67.97	62.58
	FFA (%)	3.72	14.41	6.94	2.37	12.22	1.84	25.48	39.54	7.68
V	GB-FAs (%)	1.06	3.28	2.64	3.68	12.24	2.72	54.59	69.55	58.54
	FFA (%)	3.46	12.26	6.10	2.70	14.26	2.04	26.92	43.23	4.04

GB-FAs: FAs on the glycerol backbone; W_{FA}: mass percent content of the FA; R_{PUFA}: recovery of FA.

Table S5 Diameters and specific surfaces of oil in water, middle phase and bottom phase of SC-TLPS.

	water	middle phase	bottom phase
D-specific surface (μm)	57.030	20.585	31.588
D-volume (μm)	65.991	28.236	49.531
specific surface (m^2 g^{-1})	0.105	0.291	0.19

Table S6 Comparison of achieved hydrolytic ratios of oils in different enzymatic reaction systems.

Reaction system	**lipase origin**	**catalyst form**	**Substrate**	**T (°C)**	**Time (h)**	**$C_{n3\text{-}PUFA}$ (%)**	**catalyst reuse and stability**	**ref.**
SC-TLPS	*Candida rugosa*	free lipase (3.25%)	tuna oil	37	8	67.97	no significant losses of activity after 8 uses	this paper
reversed micelles	*Candida rugosa*	surfactant-coated lipase (3.3%)	tuna oil	40	24	49.8	-	1
O/W system	*Candida rugosa*	free lipase (6%)	salmon oil	45	8	50.58	-	2
O/W system	*Cryptococcus sp.*	free lipase (1000U/ml)	sardine oil	25	72	42	-	3
reversed micelles	*Candida rugosa*	immobilized lipase (30%)	tuna oil	40	10	16.5	80% activity after 5 uses (washed with tBA)	4
cyclohexane /water system	*Rhizomucor miehei*	immobilized lipases (25%)	fish oil	25	24	-	85% activity after 5 uses (washed with cyclohexane)	5
Reverse micelles	*Yarrowia lipolytica*	immobilized lipase (500U/g)	fish oil	25	24	36.8 (DHA)	80% activity after 20 uses (washed with PBS)	6
O/W system	*Yarrowia lipolytica*	immobilized lipase (64%)	*Chlorella protothecoides* oil	30	16	31.5 (DHA)	90% activity after 10uses (washed with PBS)	7

T: temperature; Y: the hydrolysis rate of oil; (DHA): C_{DHA}

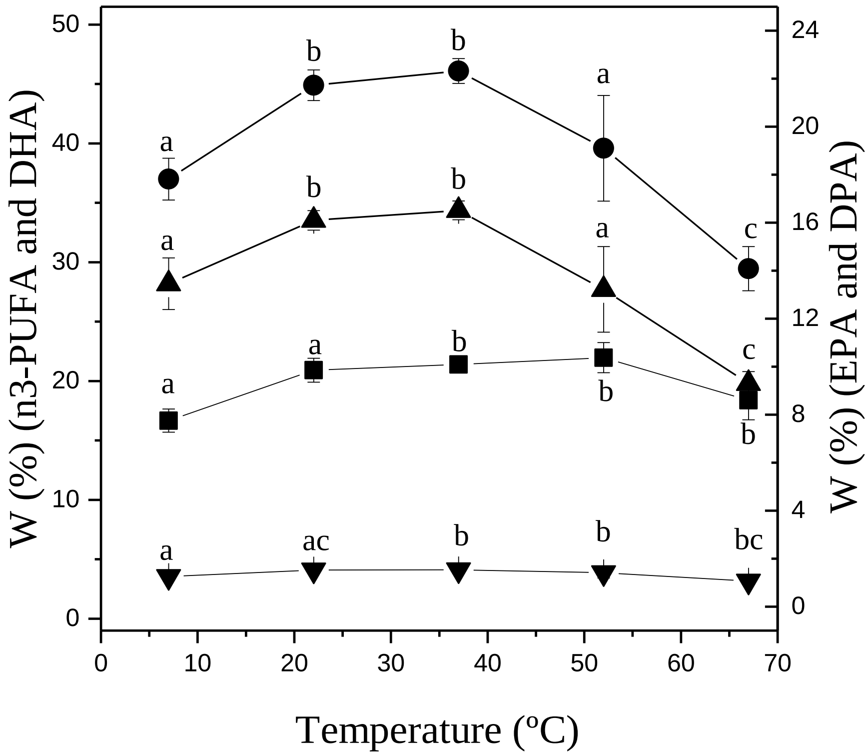

Figure S1. Effects of temperature of FFA in the PEG-400/Na_2SO_4 SC-TLPS contained lipase AYS. (●: n-3 PUFA; ▲: DHA; ■: EPA; ▼: DPA).

References

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