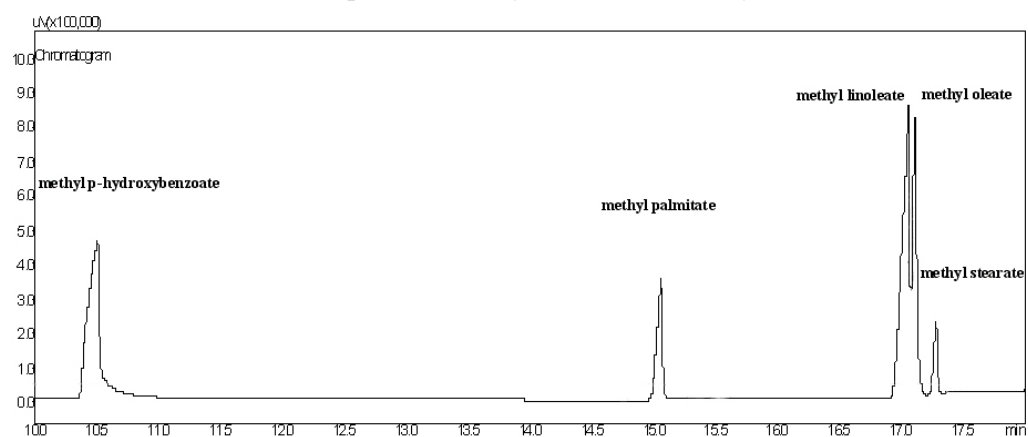


Separation of soybean oil FAMES by GC.



Scheme 1. Transesterification of soybean oil and methanol with Novozym 435 under microwave irradiation.

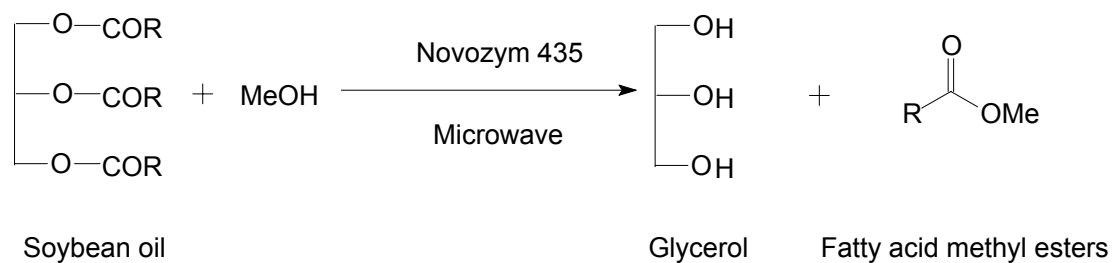


Fig. 1. Comparison of reaction process without enzyme under microwave irradiation (◆), with enzyme under microwave irradiation (■), and with enzyme under conventional heating (▲). Conditions: Reactions were carried out in *tert*-amyl alcohol (40 ml), soybean oil (40 mmol), methanol (240 mmol), Novozym 435 (3% based on the oil weight), water activity of 0.53 at 40°C and 200 rpm under microwave irradiation and conventional heating, respectively.

Reaction time (h)	FAME yield without enzyme under Microwave irradiation (%)	FAME yield with enzyme under Microwave irradiation (%)	FAME yield with enzyme under conventional heating (%)
0	0	0	0
2	0	28 ± 0.8	19 ± 0.6
4	0	51 ± 1.5	33 ± 1.0
6	0	65 ± 2.0	46 ± 1.4
8	0	79 ± 2.4	57 ± 1.7
10	0	88 ± 2.6	66 ± 2.0
12	0	95 ± 2.8	75 ± 2.3
16	0	95 ± 2.9	85 ± 2.6
20	0	96 ± 3.0	92 ± 2.7
24	0	96 ± 3.0	95 ± 2.9

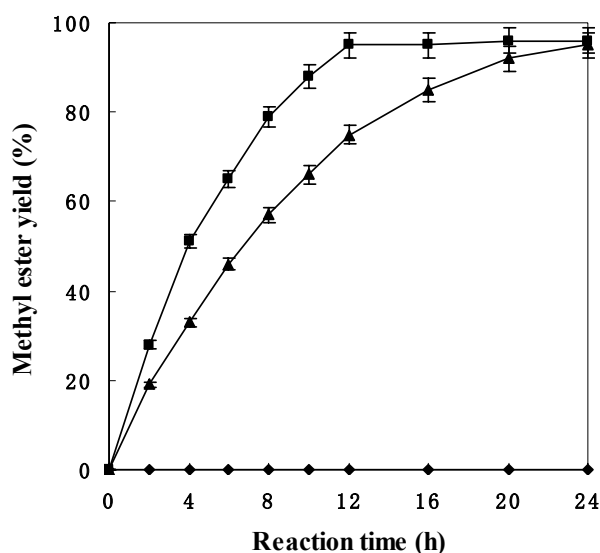


Fig. 2. Effect of water activity on FAME production. Conditions: Reactions were carried out in *tert*-amyl alcohol (40 ml), soybean oil (40 mmol), methanol (240 mmol), Novozym 435 (3% based on the oil weight) at 40°C and 200 rpm under microwave irradiation in 2 h. The water activity varied from 0.06 to 0.97.

Water activity	Enzyme activity ($\mu\text{mol}/\text{min}\cdot\text{g}$)
0	0
0.06	124 ± 4
0.11	211 ± 6
0.24	256 ± 8
0.33	297 ± 9
0.53	335 ± 11
0.75	167 ± 5
0.97	75 ± 2

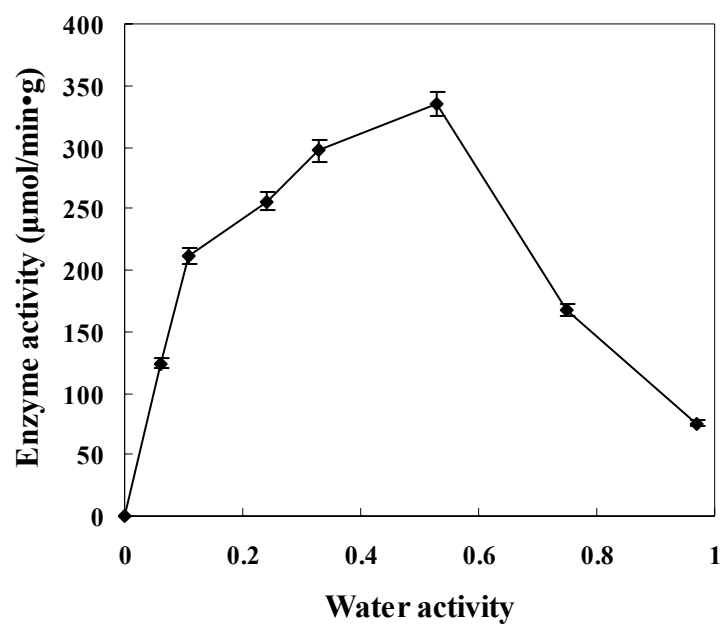


Figure. 3. Effect of *tert*-amyl alcohol concentration on FAME production. *Conditions:* Reactions were carried out in soybean oil (40 mmol), methanol (240 mmol), Novozym 435 (3% based on the oil weight) and water activity of 0.53 and 200 rpm at 40°C under microwave irradiation in 2 h. The *tert*-amyl alcohol/oil volume ratio varied from 0 to 4.

<i>Tert</i> -amyl alcohol/oil(v/v)	Enzyme activity ($\mu\text{mol}/\text{min}\cdot\text{g}$)
0	49 \pm 1
0.25	164 \pm 5
0.5	255 \pm 8
0.75	298 \pm 9
1	336 \pm 11
1.5	330 \pm 10
2	323 \pm 10
4	318 \pm 9

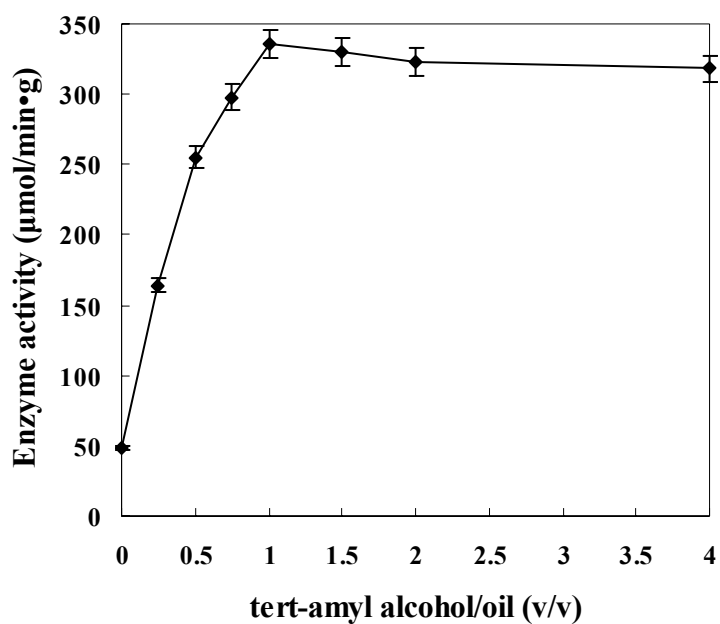


Fig. 4. Effect of substrate ratio on FAME production. *Conditions:* Reactions were carried out in *tert*-amyl alcohol (40 ml), soybean oil (40 mmol), Novozym 435 (3% based on the oil weight) and water activity of 0.53 at 40°C and 200 rpm under microwave irradiation in 2 h. The molar ratio of methanol/oil varied from 3 to 18.

Methanol/Oil molar ratio (mol/mol)	Enzyme activity ($\mu\text{mol}/\text{min}\cdot\text{g}$)
3	309 ± 10
6	325 ± 11
9	285 ± 9
12	173 ± 5
18	39 ± 1

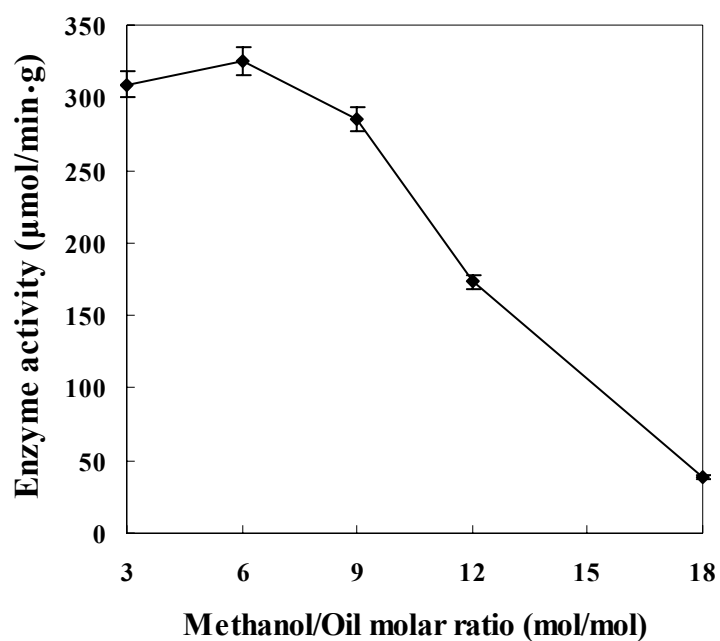


Fig. 5. Linear regression curves obtained from logarithmic representation of residual activity *versus* preincubation time.

Time	$\text{Log}(A/A^0 \times 100)$
0	2 ± 0.06
1	1.94 ± 0.05
5	1.89 ± 0.05
10	1.71 ± 0.04
30	1 ± 0.03

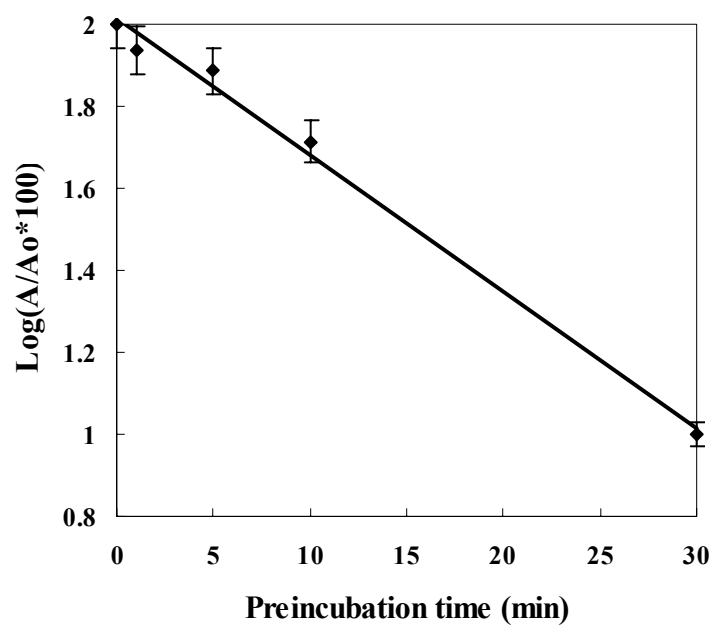


Fig. 6. Effect of enzyme dosage on FAME production. Conditions: Reactions were carried out in *tert*-amyl alcohol (40 ml), soybean oil (40 mmol), methanol (240 mmol), water activity of 0.53 at 40°C and 200 rpm under microwave irradiation in 2 h. The amount of enzyme varied from 0 to 4% based on the oil weight.

Enzyme dosage (%)	FAME yield (%)
0	0
0.5	3 ± 0.1
1	11 ± 0.3
1.5	18 ± 0.5
2	24 ± 0.7
2.5	29 ± 0.8
3	33 ± 0.9
3.5	34 ± 1.0
4	35 ± 1.0

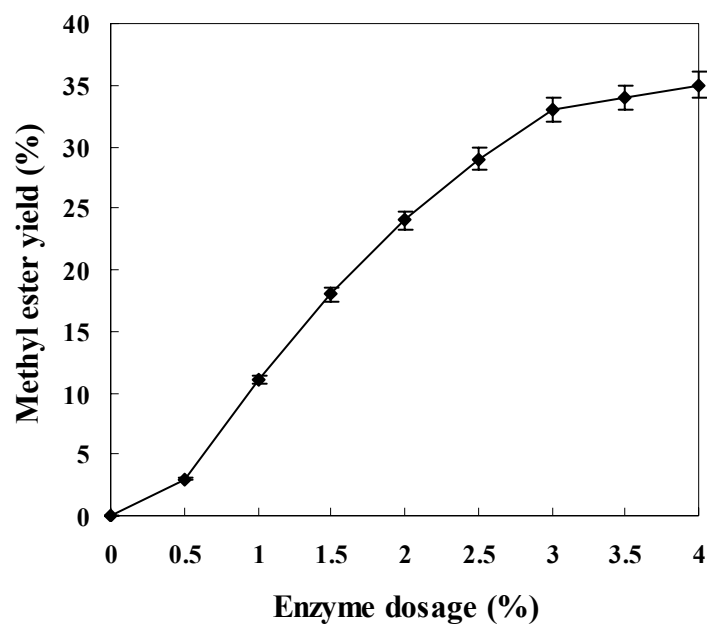


Fig. 7. Effect of temperature on FAME production. Conditions: Reactions were carried out in *tert*-amyl alcohol (40 ml), soybean oil (40 mmol), methanol (240 mmol), Novozym 435 (3% based on the oil weight) and water activity of 0.53 and 200 rpm under microwave irradiation in 2 h. Temperature varied from 30 to 70°C.

Temperature (°C)	Enzyme activity ($\mu\text{mol}/\text{min}\cdot\text{g}$)
30	97 ± 3
40	322 ± 10
50	267 ± 8
60	256 ± 7
70	86 ± 3

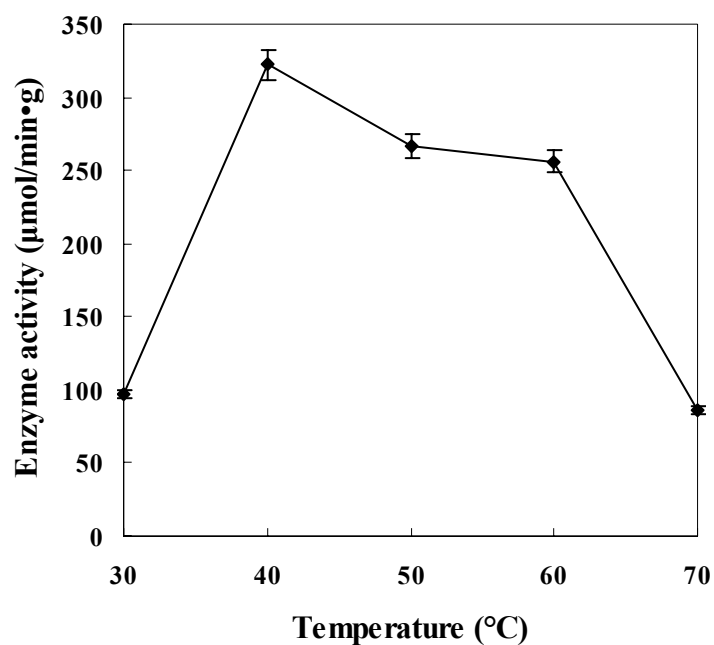


Fig. 8. Reusability of enzyme on FAME production. Conditions: Each cycle was carried out in *tert*-amyl alcohol (40 ml), soybean oil (40 mmol), methanol (240 mmol), Novozym 435 (3% based on the oil weight) and water activity of 0.53 at 40°C and 200 rpm for 2 h under microwave irradiation (◆) and under conventional heating (■).

Cycle number	Enzyme activity ($\mu\text{mol}/\text{min}\cdot\text{g}$) under microwave irradiation	Enzyme activity ($\mu\text{mol}/\text{min}\cdot\text{g}$) under conventional heating
1	338 ± 10	229 ± 7
2	335 ± 10	225 ± 6
3	333 ± 10	219 ± 6
4	330 ± 10	213 ± 5
5	325 ± 9	206 ± 5

