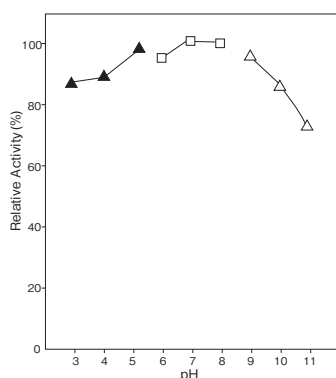


Table 1. Substrate specificity

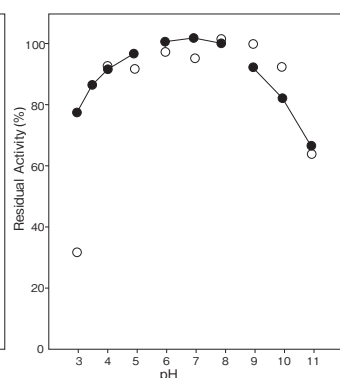
Substrate	Relative activity (%)
Triolein	100
Tripalmitin	22
Trimyristin	53
Trilaurin	103
Tricaprin	166
Tricaprylin	312
Tricaproin	156
Tributylin	94
Tripropionin	22
Triacetin	38

Fig.1 pH Optimum



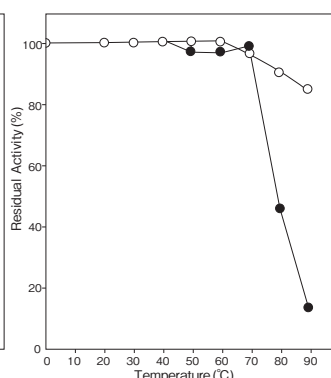
▲ : Mcllvaine buffer
□ : Phosphate buffer
△ : Borate buffer

Fig.2 pH Stability



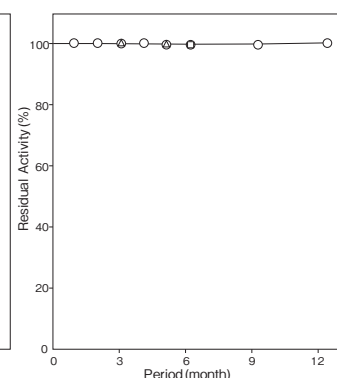
● : 37°C, 24 hr
○ : 50°C, 1 hr
pH 3, 4, 5 Mcllvaine buffer
pH 6, 7, 8 Phosphate buffer
pH 9, 10, 11 Borate buffer

Fig.3 Thermal Stability



pH 7.0, 10 hr
○ : Lyophilized powder
● : Aqueous solution

Fig.4 Storage (lyophilized powder)

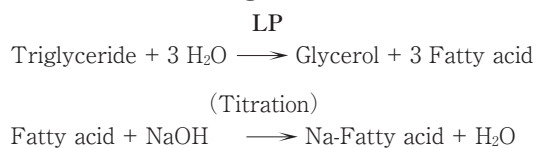


○ : -20°C
□ : 5°C
△ : 30°C

Assay

Principle

The assay is based on the titration of fatty acids liberated in the following reactions:



Unit definition

One unit is defined as the amount of enzyme which liberates 1 μ mole of fatty acid per minute at 37°C under the conditions specified in the assay procedure.

Reagents

- Substrate suspension (Olive oil and Adekatol SO-120)
50 g of Olive oil (Japanese Pharmacopoeia grade) and 50 g of Adekatol SO-120 are suspended with 150 ml of distilled water.
- Reaction stopper
Mixture of ethanol and acetone (1:1)
- Indicator
1% (W/V) Phenolphthalein-ethanol solution

4. Titration solution

50 mM NaOH solution

5. Enzyme dilution buffer

0.1 M KH_2PO_4 -NaOH buffer, pH 8.0 containing 0.1% (W/V) BSA and 0.1% (W/V) NaN_3

6. Reagents

Olive oil: (Japanese Pharmacopoeia grade)
Ethanol: (Japanese Pharmacopoeia grade)
Adekatol SO-120: ADEKA CORPORATION
BSA: Millipore Fraction V pH5.2 #81-053

Enzyme solution

Accurately weigh about 10 mg of the sample and add enzyme dilution buffer to make a total of 50 ml.

Dilute it with enzyme dilution buffer to adjust the concentration to within 2-4 U/ml.

Procedure

- Pipette accurately 5 ml of substrate suspension and 2 ml of distilled water into a test tube (24 mm i.d. \times 200 mmH) and mix to start the preincubation at 37°C.
- After 10 min, add 0.5 ml of enzyme solution and mix to start the reaction.
* In the case of a test blank, add 0.5 ml of enzyme dilution buffer in place of enzyme solution.

- After 20 min, stop the reaction with 16 ml of reaction stopper.
- Add 3 drops of indicator and titrate the whole mixture with under nitrogen gas bubbling.

※ End point of titration: Appearance of the same color as that of the blank

Titration volume sample : V_s
 blank : V_c

$$\Delta V = (V_s - V_c) \leq 2.5 \text{ ml}$$

$$V_c \leq 0.6 \text{ ml}$$

■ Calculation

$$\text{Activity (U/mg of powder)} = \frac{\Delta V \times F}{20} \times 50 \times \frac{1}{0.5} \times \frac{1}{X}$$

20 : reaction time (min)

F : factor of titration solution (50 mM NaOH)

50 : concentration (mM) of titration solution (50 mM NaOH)

0.5 : the volume of enzyme solution (ml)

X : concentration of the sample in enzyme solution (mg/ml)

Storage

Storage at -20°C in the presence of a desiccant is recommended. Enzyme activity will be retained for at least one year under this condition (Figure 4).

References

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LP 活性測定法 (Japanese)

I. 試薬液

- 基質懸濁液 (オリーブ油とアデカトール SO-120 の懸濁液)

「局方」オリーブ油 50.0g とアデカトール SO-120 50.0g を精製水 150ml に懸濁する。

- 反応停止液

エタノール-アセトン (1:1) 混液

- 指示液

1% (W/V) フェノールフタレン-エタノール溶液

- 滴定液

50mM NaOH 液

- 酵素溶解希釈用液

0.1% (W/V) BSA と 0.1% (W/V) NaN_3 を含む 0.1M KH_2PO_4 -NaOH 緩衝液 pH8.0

- 試薬

オリーブ油:「局方」

エタノール:「局方」

アデカトール SO-120: ADEKA 製

BSA: Millipore 製 Fraction V pH5.2 #81-053

II. 酵素試料液

検品約 10mg を精密に量り、酵素溶解希釈用液に溶解して全容 50ml とする。

その液を酵素溶解希釈用液で 2~4U/ml 濃度となるように適宜希釈する。

III. 測定操作法

- 試験管 (24mm i.d. × 200mm H) に基質懸濁液 5ml と精製水 2ml を正確に分注して攪拌混和後、 37°C で予備加温する。

- 10 分経過後、酵素試料液 0.5ml を加えて混和し、 37°C で反応を開始する。

※ 盲検は酵素試料液の代わりに酵素溶解希釈用液 0.5ml を加える。

- 20 分経過後、反応停止液 16ml を加えて反応を停止する。

- 指示液 3 滴を加えて N_2 ガスで攪拌しながら滴定液で滴定する。

※ 滴定の終点は盲検時と同色を呈した時点とする。

求められた滴定量を試料液は V_s 、盲検液は V_c とする。

$$\Delta V = (V_s - V_c) \leq 2.5 \text{ ml}$$

$$V_c \leq 0.6 \text{ ml}$$

IV. 計算

$$\text{活性 (U/mg)} = \frac{\Delta V \times F}{20} \times 50 \times \frac{1}{0.5} \times \frac{1}{X}$$

20 : 反応時間 (min)

F : 滴定液 (50mM NaOH) の Factor

50 : 滴定液 (50mM NaOH) の濃度 (mM)

0.5 : 反応に供した酵素試料液量 (ml)

X : 酵素試料液中の検品濃度 (mg/ml)