

# FACTORS AFFECTING LIPASE ACTIVITY

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# Introduction

- This presentation will discuss several factors that affect lipase activity determinations and must be controlled to ensure accurate and reliable results.

# What are lipases?

- A lipase is a water-soluble enzyme that acts at the oil-water interface to catalyze the hydrolysis of ester bonds in lipid substrates into free fatty acids and glycerol

# Industrial Uses of Lipases

- Pharmaceuticals
- Detergents
- Cosmetics
- Leather processing
- Production of aliphatic acids/other syntheses (enzyme is active in many organic solvents)
- Treatment of domestic and industrial wastes

# Sources of Lipases

## ⦿ Animal

- Porcine pancreas (pancrelipase, pancreatin)

## ⦿ Microbial

- *Achromobacter* sp., *Alcaligenes* sp., *Arthrobacter* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Chromobacterium* sp.

## ⦿ Fungi/Yeasts

- *Aspergillus niger*, *Candida antarctica*, *Candida rugosa*, *Candida lipolytica*, *Penicillium camembertii*, *Penicillium roqueforti*, *Rhizopus oryzae*, *Mucor javanicus*

# Lipase Mechanism of Action

- Hydrolysis mechanism involves a serine nucleophile, an acid residue (usually Asp) and a histidine [catalytic triad]
- Colipase – small (~10 kD) protein cofactor that prevents the inhibitory effect of bile salts on pancreatic lipase

# Activators and Inhibitors of Lipase Activity

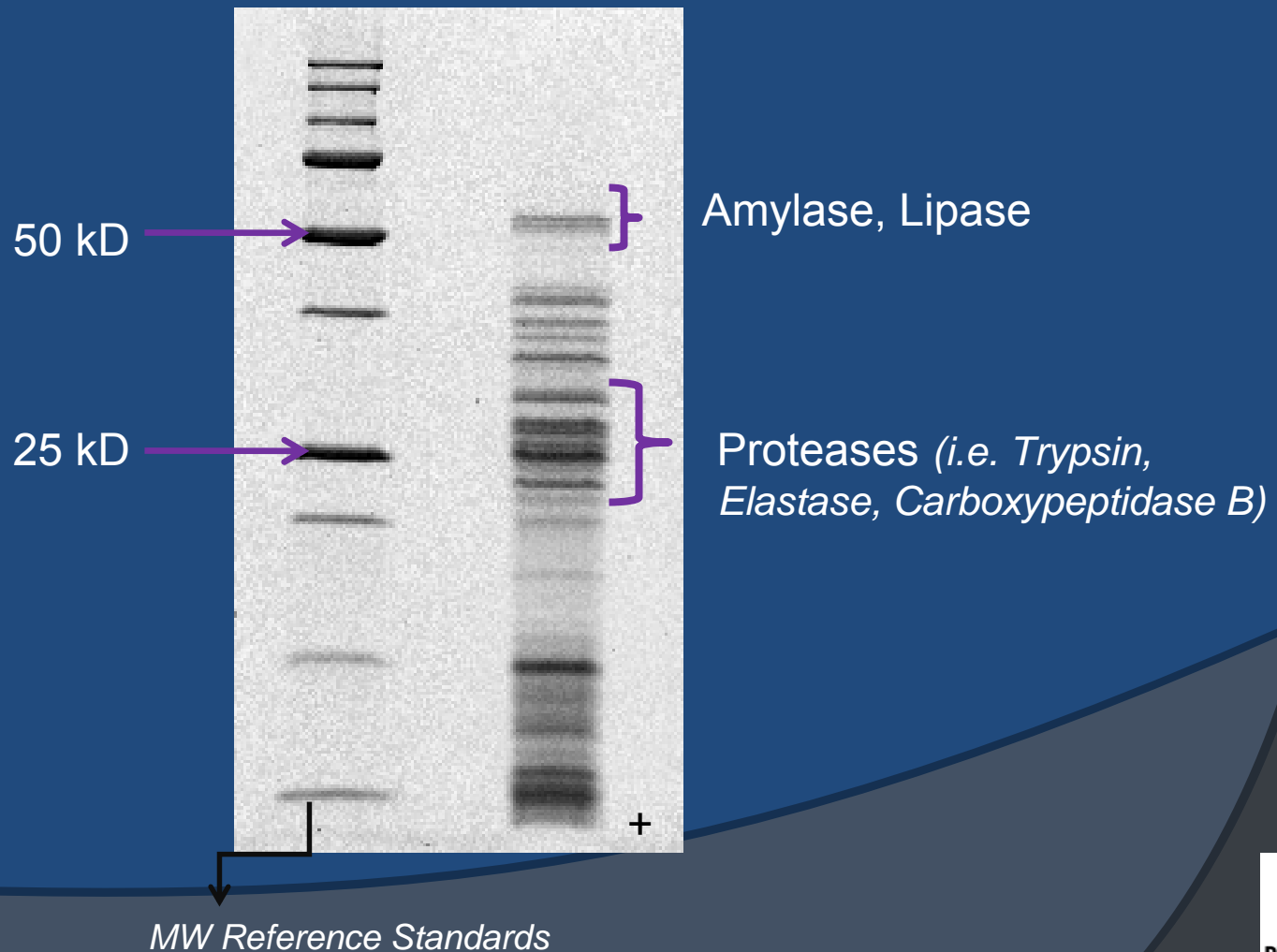
## ⦿ Activators

- $\text{Ca}^{2+}$  is required for activity;  $\text{Sr}^{2+}$ ,  $\text{Mg}^{2+}$  are less effective activators
- Colipase

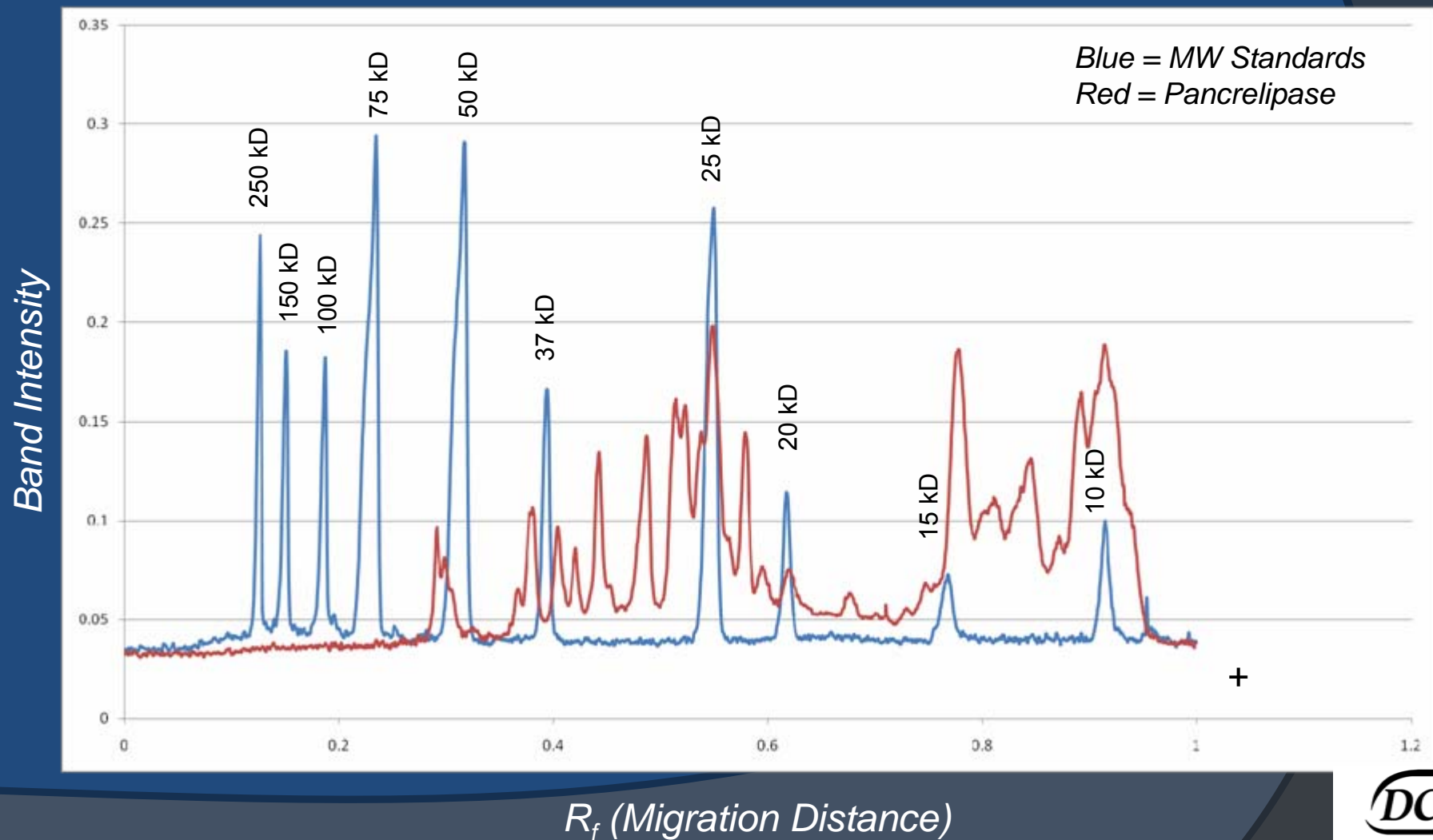
## ⦿ Inhibitors

- Orlistat – specific, irreversible inhibitor that binds at serine residue in active site
- EDTA,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Hg}^{2+}$ , iodine, p-chloromercuribenzoate (PCMB)
- Bile salts

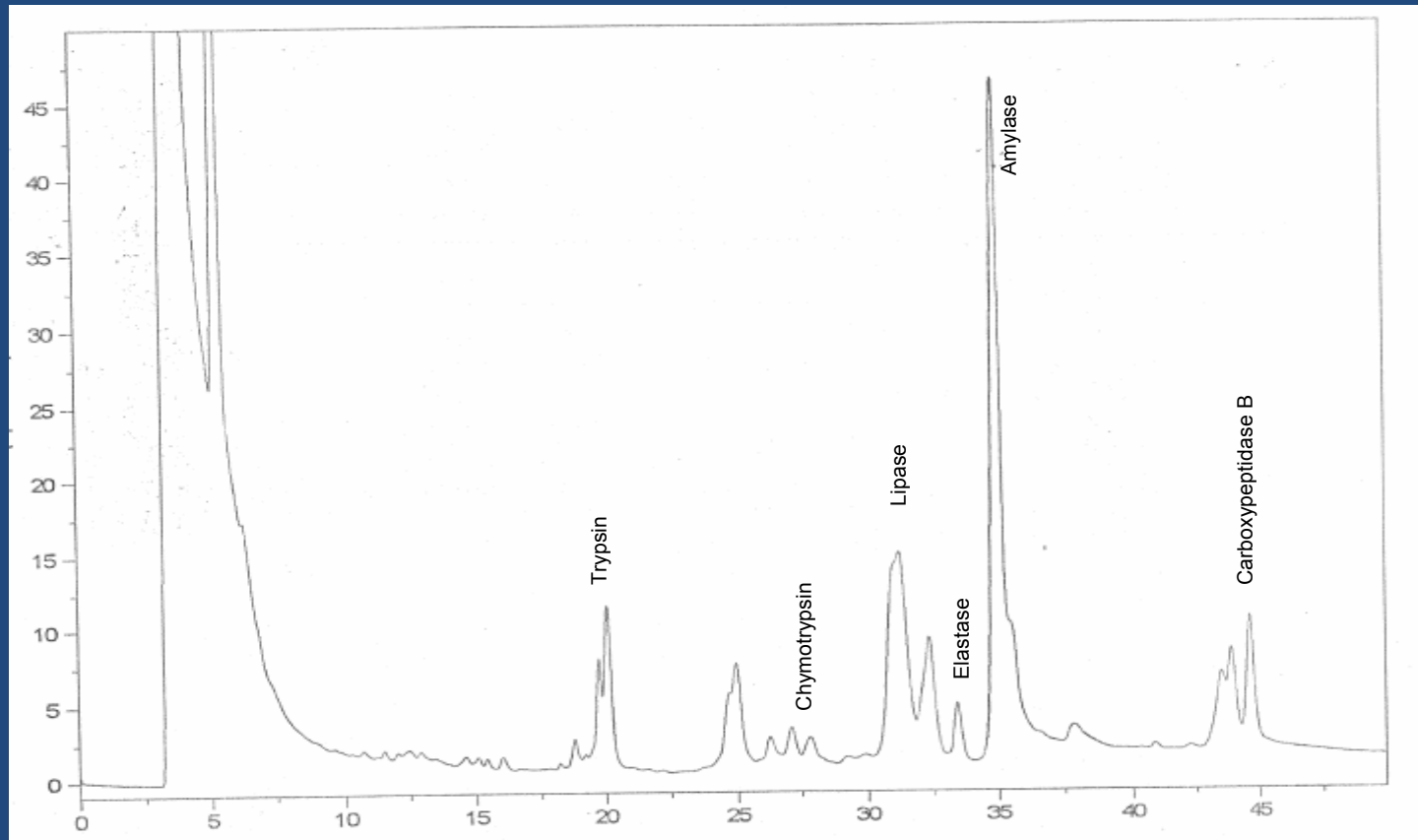
# Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis of USP Pancreatin Lipase RS, Lot # JOG363



# Densitometric Scanning of SDS-PAGE Separation



# Reverse Phase-High Performance Liquid Chromatography of USP Pancreatin Lipase RS, Lot # JOG363



RP-HPLC Chromatogram of USP Pancreatin Lipase RS, Lot # JOG363

# Some Assays for Lipase Activity

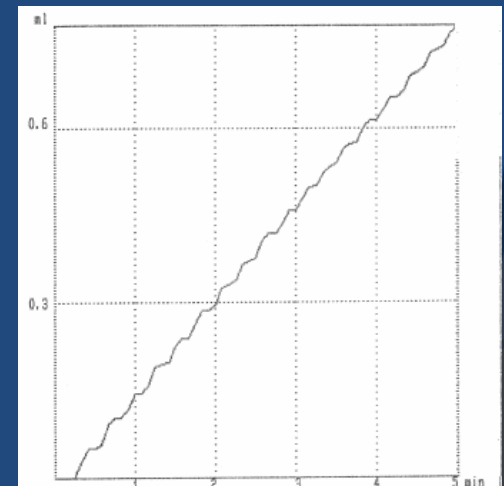
## ● Titrimetric

- pH-stat Method → Titrate free fatty acids released by lipase breakdown of triglycerides using sodium hydroxide solution
- Standard Assay Used

## ● Colorimetric

- 1,2-O-dilauryl-rac-glycero-3-glutaric acid-resorufin ester

## ● Immunological (ELISA)



# Preparation of Reagents for Titrimetric Lipase Assay

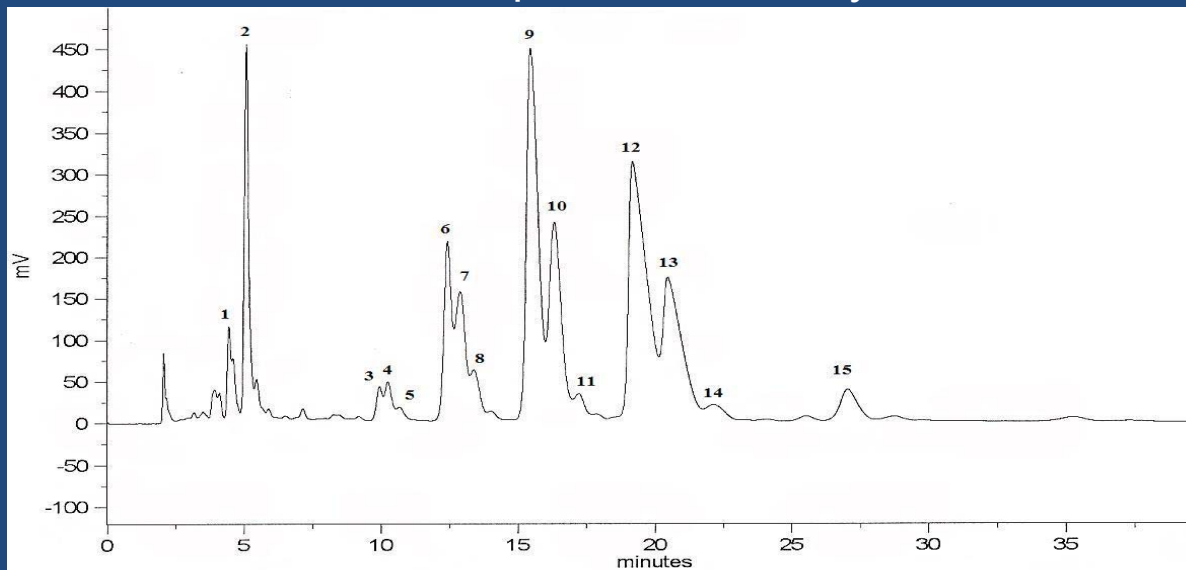
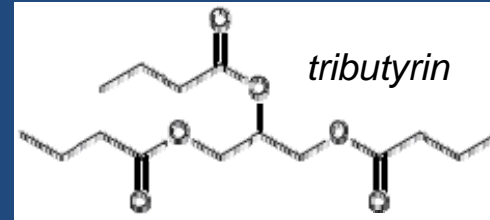
- Olive Oil Emulsion:
    - Olive oil (20 mL)
    - Acacia (165 mL of 10% Acacia solution)
    - Crushed Ice (15 g)
  - Bile Salt (Sodium Taurocholate): 80 mg/mL, 8% solution
  - Buffer: 5 mM Tris in 40 mM NaCl
  - Distilled Water
- \*\* Reaction: 10 mL Emulsion + 2.0 ml Bile Salt solution + 8.0 mL Buffer + 9.0 mL distilled water. Adjust pH to 9.2 and add 1.0 mL of Enzyme (Total Volume 30 mL) .

# Factors Affecting Lipase Activity

## Substrate Used

- Tributyrin vs. olive oil

- Lipases show higher specific activity with tributyrin substrate
- Olive oil composition can vary

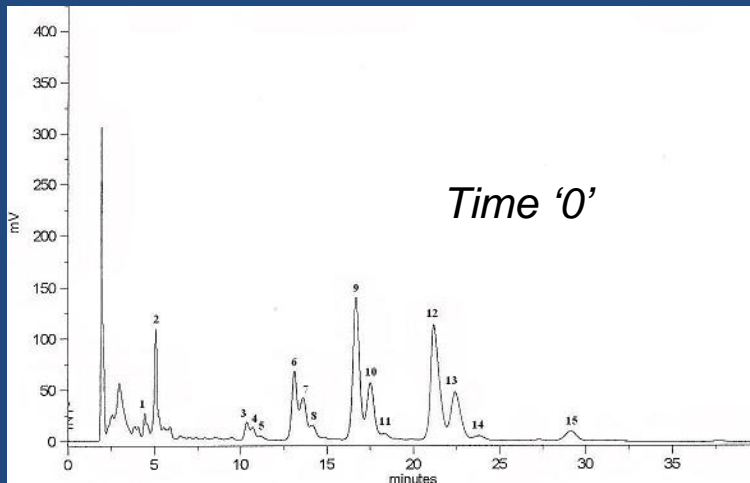


- #3 – LLL
- #4 – OLLn
- #5 – PLLn
- #6 – OLL
- #7 – OLLn + PoOL
- #9 – OOL
- #10 – POL
- #11 – PPL
- #12 – OOO
- #13 – POO + SOL
- #14 – PPO
- #15 – SOO

HPLC Chromatogram of Olive Oil, NF

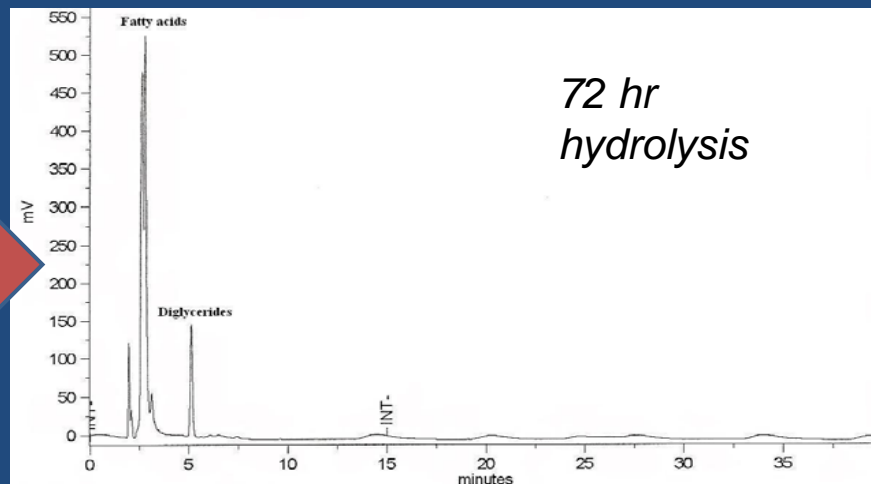
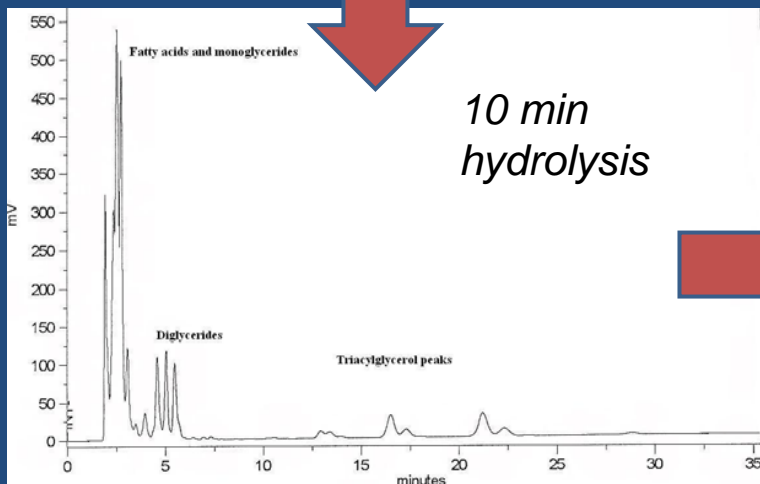
Note: P, Palmitic; O, Oleic; S, Stearic;  
L, linoleic; Ln, linolenic; Po, palmitoleic

# Enzymatic Hydrolysis of Olive Oil



RP-HPLC Chromatograms

Enzymatic Hydrolysis Conditions:  
37°C, pH 9.0



# Factors Affecting Lipase Activity (cont'd)

- ⦿ Substrate preparation
  - Concentration of substrate
  - Size of lipid droplets
  - Acacia (emulsifier) – natural product,  $\text{Ca}^{2+}$  composition may vary between lots
  - Stability of the emulsion
- ⦿ Assay Factors
  - Consistency of temperature, stirring rate, uniform enzyme preparation time

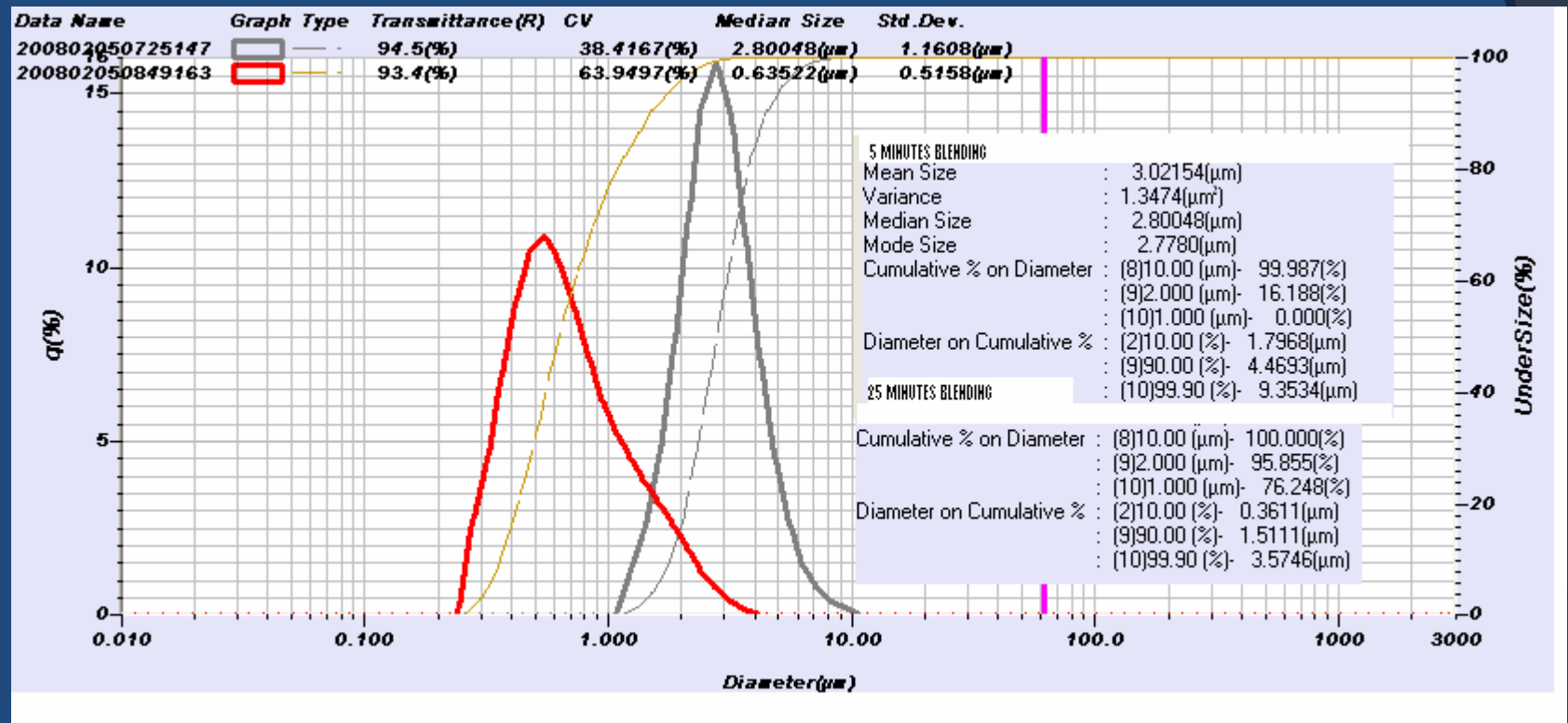
# Effect of Colipase on Lipase Activity of USP Lipase Reference Standard Candidate A, Lot# B060271

<b>Enzyme concentration: 8.0 USP units/ml</b>				
<b>Run #</b>	<b>Colipase Added (µl)</b>	<b>Slope (ml/min)</b>	<b>Activity USP u/mg</b>	<b>Average USP u/mg</b>
<b>1</b>	<b>0</b>	<b>0.088</b>	<b>102.35</b>	<b>103.17</b>
<b>2</b>	<b>0</b>	<b>0.089</b>	<b>104.00</b>	
<b>3</b>	<b>5</b>	<b>0.091</b>	<b>106.32</b>	<b>105.38</b>
<b>4</b>	<b>5</b>	<b>0.089</b>	<b>104.45</b>	
<b>5</b>	<b>10</b>	<b>0.089</b>	<b>104.16</b>	<b>103.44</b>
<b>6</b>	<b>10</b>	<b>0.088</b>	<b>102.72</b>	
<b>7</b>	<b>20</b>	<b>0.087</b>	<b>101.56</b>	<b>101.59</b>
<b>8</b>	<b>20</b>	<b>0.087</b>	<b>101.61</b>	
<b>9</b>	<b>40</b>	<b>0.088</b>	<b>103.22</b>	<b>103.44</b>
<b>10</b>	<b>40</b>	<b>0.089</b>	<b>103.66</b>	

**Colipase was added to the reaction vial 1 minute before the initiation of reaction.**

\* Concentration of colipase solution was 0.04 mg/mL

# Effect of Blending Time on Lipid Droplet Size (5 minutes and 25 minutes blending)

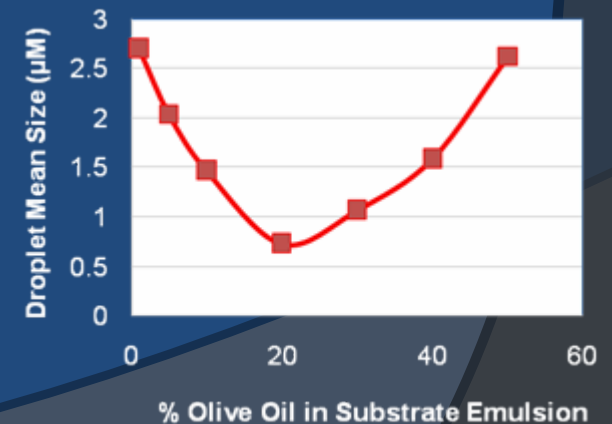
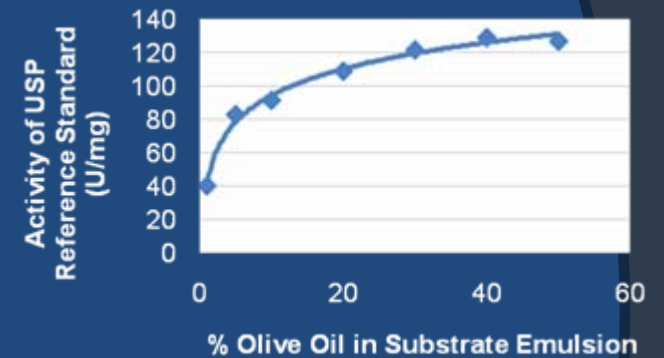


Emulsion size by HORIBA, LA 950 V2 Laser Scattering Particle Size Distribution Analyzer

# Effect of Olive Oil Concentration on Lipase Activity & Lipid Droplet Size

- Concentration of olive oil in substrate affects lipase activity and lipid droplet size

% Olive Oil	Activity (U/mg)	% Difference *	Droplet Mean Size ( $\mu\text{M}$ )
1	40.265	-56.8	2.71
5	83.15	-10.9	2.04
10	91.66	-1.8	1.47
20	109.2	17.0	0.73
30	122	30.8	1.07
40	129.4	38.7	1.59
50	127.16	36.3	2.62



\* Claimed Activity of USP Pancreatin Lipase Reference Standard, Lot # JOG363 = 93.3 U/mg

# Conclusion

- Important lipase assay factors include consistency of temperature, stirring rate, uniform enzyme preparation time
- Substrate preparation must be controlled to ensure a consistent and accurate assay for lipase activity
- Key parameters include lipid droplet size and concentration of olive oil
- Precision of tightly controlled lipase assay is ~ 5%