

A high-speed photograph of a water droplet hitting a surface, creating a series of concentric ripples. The droplet is captured mid-fall, just above the first ripple.

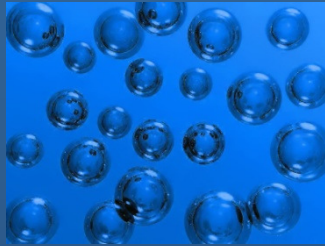
# **Development of Biotechnological Process for the Production *of* Novel Natural Antioxidants & Nutraceuticals**

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**McGill University**

**World Congress on Oils and Fats  
&  
28<sup>th</sup> ISF Congress**

***Sydney, Australia  
September 27-30<sup>th</sup>, 2009***

# *Outline*



## *Introduction*



## *Biosynthesis of Phenolic Lipids*

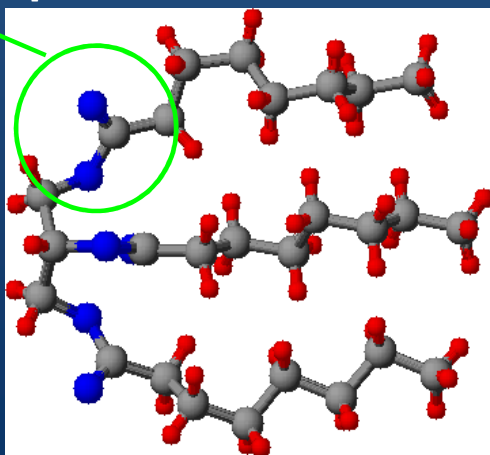


## *Conclusion*

# Phenolic Lipids

*Compounds that possess a hydrocarbon chain and an aromatic ring bearing one or more hydroxy substitutes attached via an ester bond*

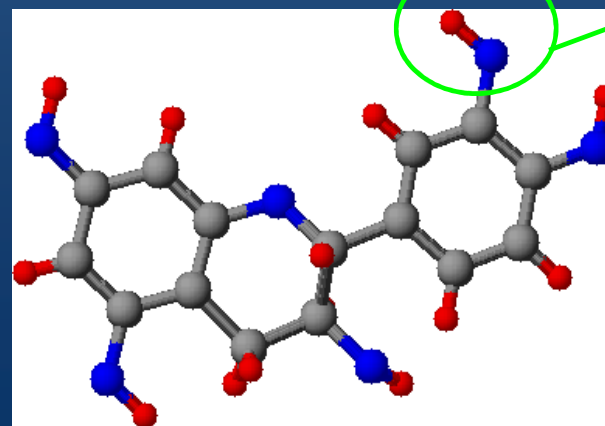
-COOH group



**Lipids**

+

-OH group



**Phenolic Compounds**

# Biosynthesis of Phenolic Lipids



*Selectivity*

*Milder  
Reaction Conditions*

*“Natural”*

# Sources of Polyunsaturated Fatty Acids (PUFAs)

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- Fish & Marine Oils

*(herring, anchovy, menhaden, seal & whale)*

- Edible Plant Oils

*(soybean, canola, safflower & corn)*

- Microorganisms

(algal: *Porphyridium cruentum*)

(fungal: *Mortierella* sp.)

# Fatty Acid Composition of Edible Oil

<b>Fatty acid</b>	<b>Canola Oil (%)</b>	<b>Flaxseed Oil (%)</b>	<b>Cod Liver Oil (%)</b>	<b>Soybean Oil (%)</b>
<b>C16:0</b>	5.0	5.7	27.6	10.0
<b>C18:0</b>	2.0	3.3	2.5	4.0
<b>C18:1n-9</b>	59.0	23.7	2.9	25.0
<b>C18:2n-6</b>	21.0	14.4	17.4	52.0
<b>C18:3 n-3</b>	18.0	52.6	3.4	7.0
<b>C20:5 (EPA)</b>	0	0	21.8	0.2
<b>C22:6 (DHA)</b>	0	0	18.2	0.3

# PUFAs Functionality

- **Essential Nutrients in Human Nutrition**
  - *Linoleic acid (C18: 2<sup>Δ9, 12</sup>) & ω-6 family*
  - *Linolenic acid (C18: 3<sup>Δ9, 12, 15</sup>) & ω-3 family*
- **Prevention & Modulation of Diseases**
  - *Cardiovascular disease*
  - *Immune function*
  - *Tumor growth*

# Sources of Plant Phenolics

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- Vegetables

*(red onions, radishes, green beans & potatoes)*

- Fruits & Berries

*(apples, pears, citrus fruit & blackberries)*

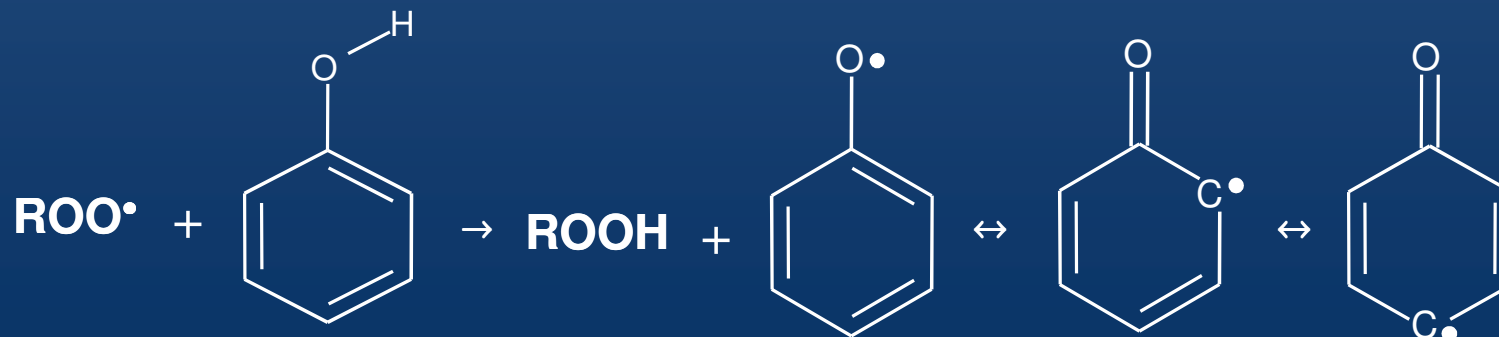
- Plant Seed Oils

*(safflower, canola, soybean, peanut & sesame)*



# Phenolic Compounds Functionality

- Possess Antioxidant Capacity
  - *Due to resonance delocalization of unpaired electron*
  - *Employed in prevention of off-flavors*
  - *Generally recognized as safe (GRAS)*



# *Properties of Phenolic Lipids*

## Functional & Physiological Properties of its Components

### Lipid Components

- PUFAs are linked to cardiovascular, cancer & inflammatory diseases
- Aid in memory and visual functions
- Physical properties: influence texture & mouthfeel

### Phenolic Compounds

- Antioxidant property, antitumor
- Generally recognized as safe (GRAS)

# Potential Applications of Phenolic Lipids



- **Nutraceutical Products**

- *Supplements imparting physiological benefits*

- **Bio-Ingredients**

- *Food ingredients providing biological benefits*

- **Enhancement of Physical Properties of Phenolics**

- *Increase solubility and miscibility in lipid systems*

# Overall Objective

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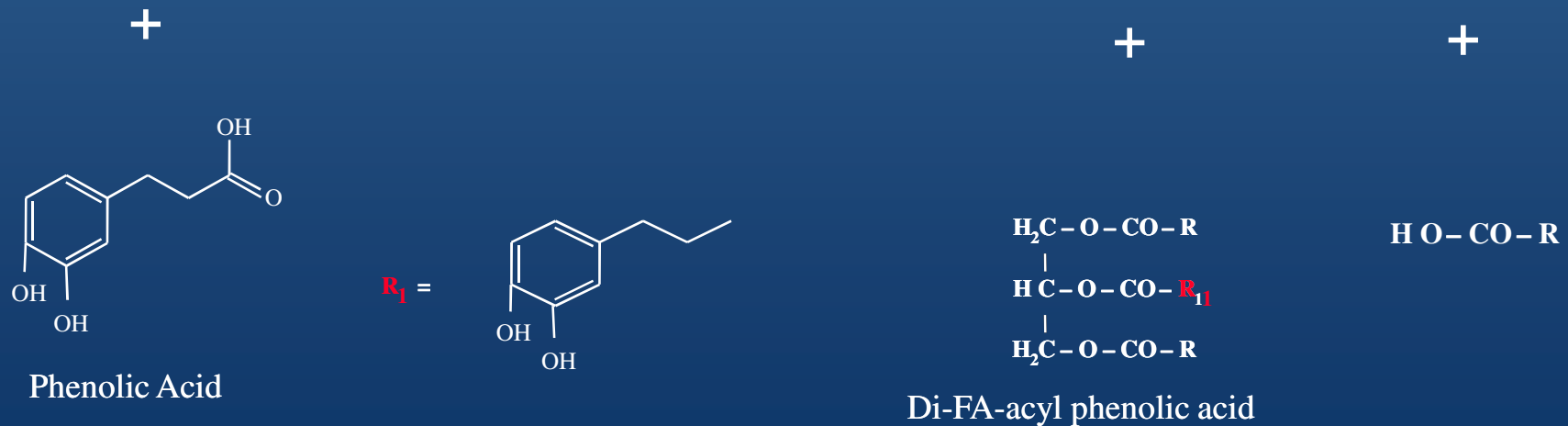
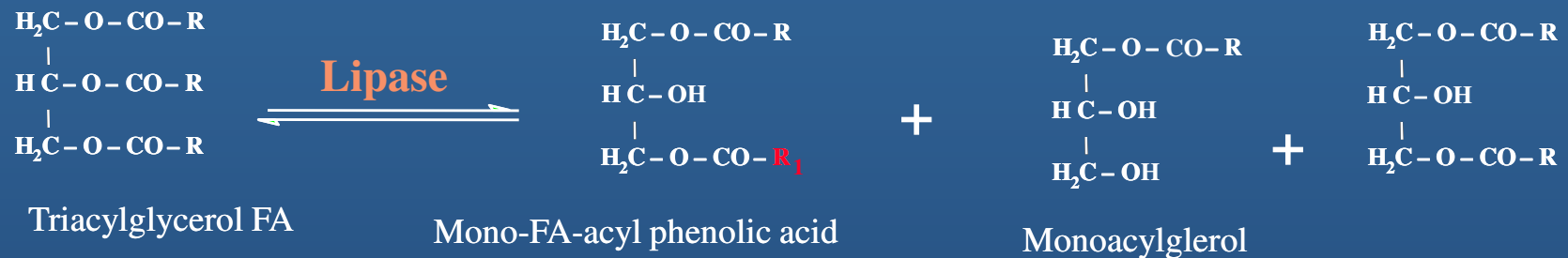
*Development of Biotechnological Process for the  
Production of Natural Nutraceutical and Bio-ingredients  
Using Edible Oils and Endogenous Phenolic Extracts*

# *Specific Objectives*

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- To optimize the process of production of *Phenolic Lipids* by determining the effects of selected parameters on the biosynthesis process
- To determine the structural and physico-chemical characteristics of synthesized *Phenolic lipids*
- To determine the anti-oxidant potential of *Phenolic Lipids*
- To develop a process for their encapsulation and their delivery

# Lipase-Catalyzed Transesterification Reaction



# Experimental Approach

- **Use of selected microbial lipases**
  - *Novozyme 435 from Candida antarctica*
  - *Lipozyme IM 20 from Mucor meihei*
  - *Lipase N from Rhizopus niveus*
- **Biocatalysis of lipase in non-conventional media**
  - *Organic solvent media (OSM)*
  - *Solvent-free medium*
- **Use of selected Substrates**
  - *Substrate models*
  - *Edible oils and endogenous phenolic extracts*

## ***Selected Parameters***

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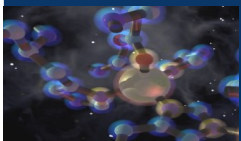
- Nature of Phenolic Acid***
- Organic Solvent Media***
- Temperature***
- Agitation***
- Surfactant***
- Water Activity***



# *Biosynthesis of Phenolic Lipids in OSM*

*Model Substrates*

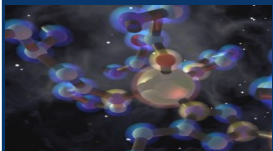
*Endogenous Edible Oils*



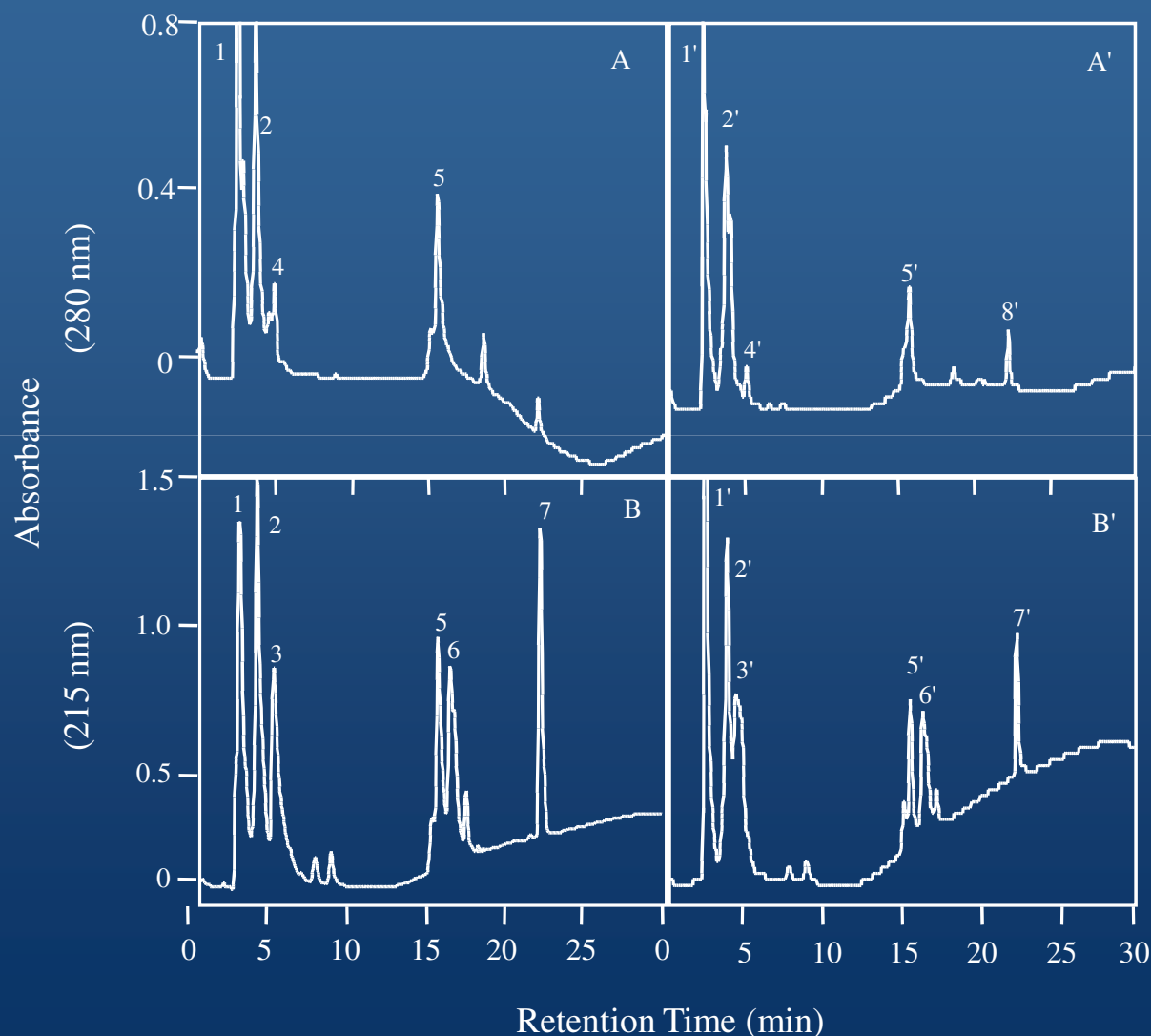
# *Biosynthesis of Phenolic Lipids*

## *Model Substrates*

*Selected Triacylglycerols & Dihydrocaffeic Acid*



# HPLC Analysis of Reaction Components



## Section

(A, B) Trilinolein

(A', B') Trilinolenin

## Peak

Dihydrocaffeic acid (1, 1')

Trilinolein (7)

Trilinolenin (7')

Phenolic monoacylglycerols (2, 2')

Phenolic diacylglycerols (5, 5')

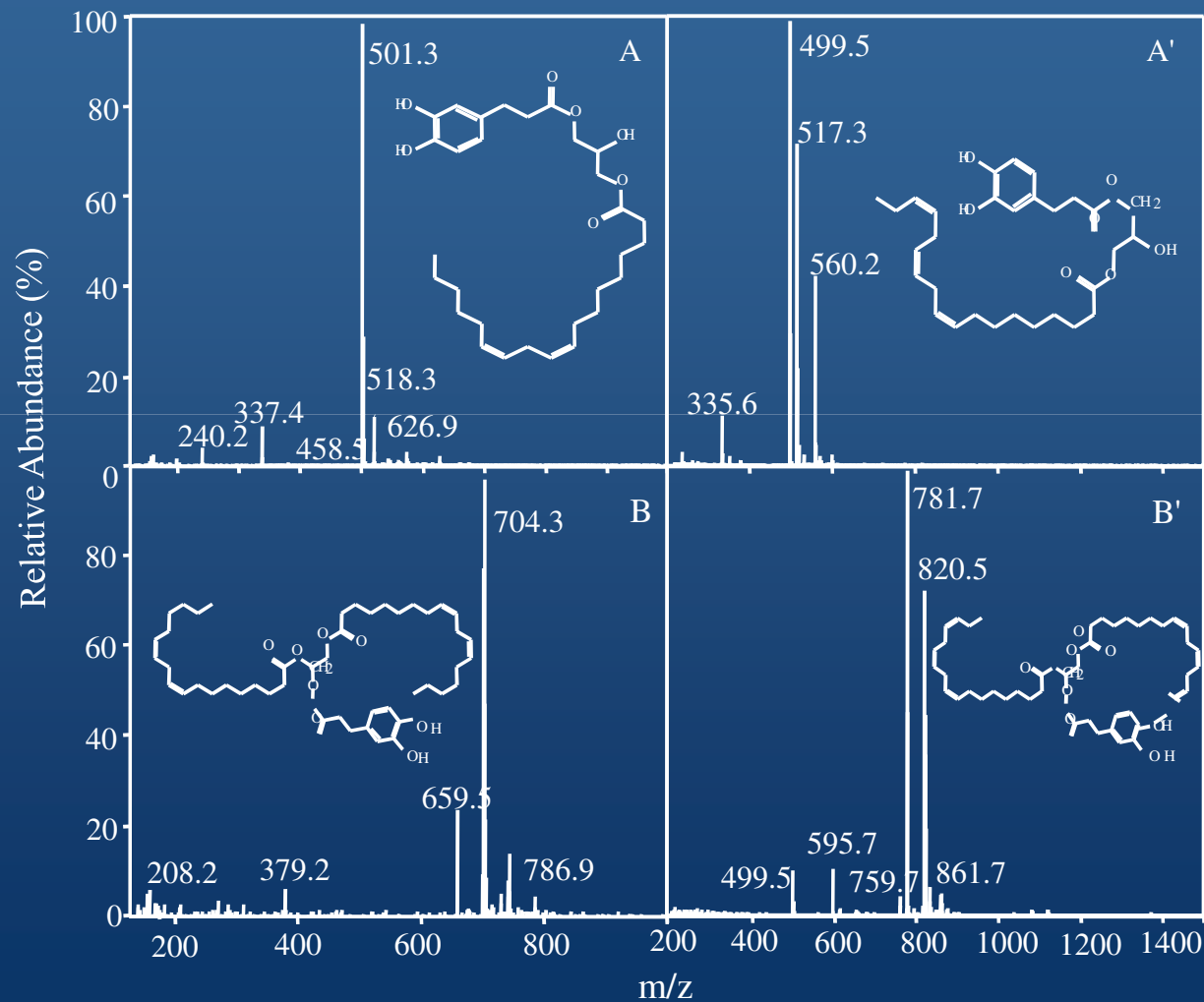
Monoacylglycerols (3, 3')

Diacylglycerols (6, 6')

Side reaction product (4)

Trilinolenin oxidation product (8)

# LC-MS Analysis of Phenolic Lipid Products



## Section

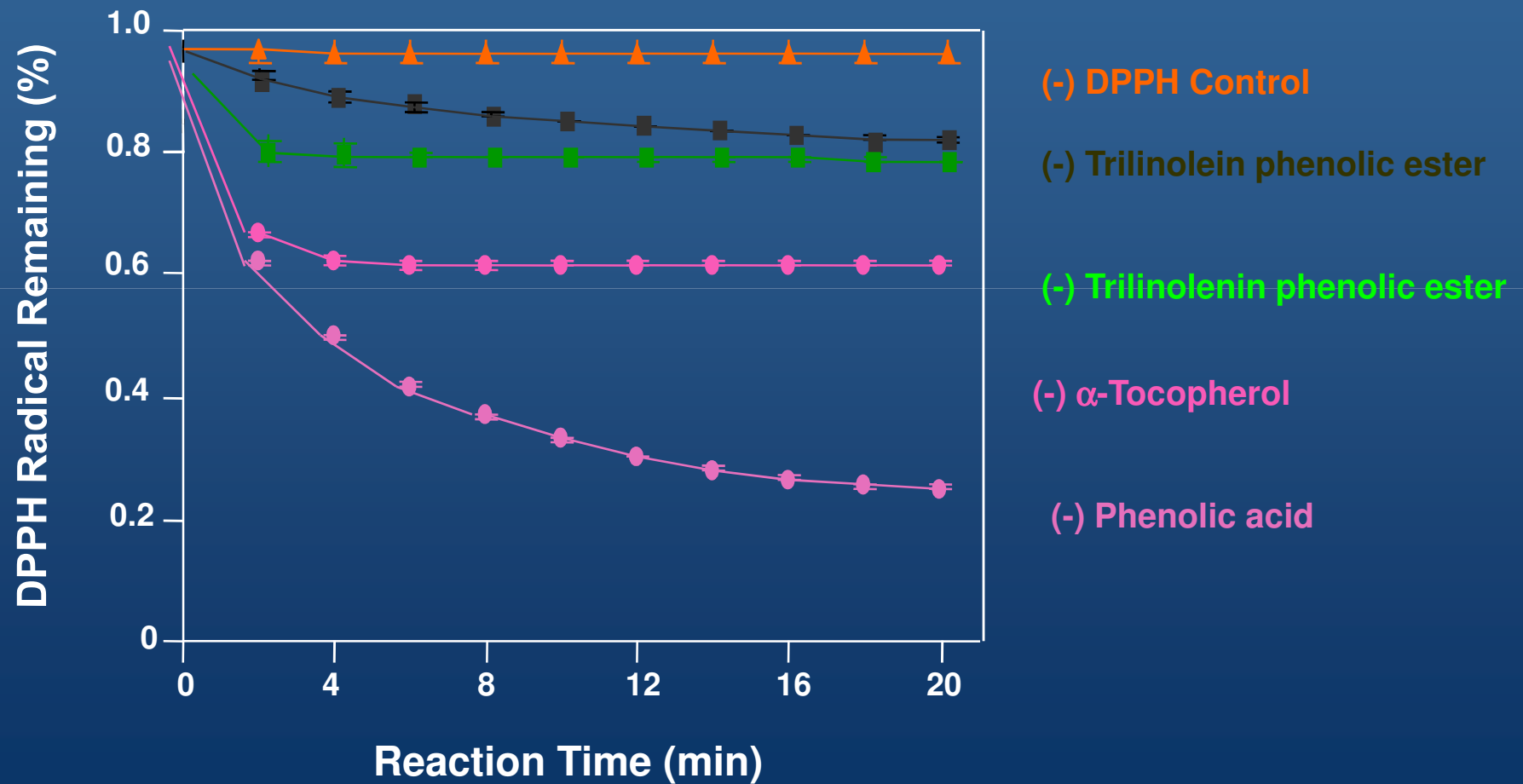
(A) Monolinoleyl dihydrocaffeate

(B) Dilinoleyl dihydrocaffeate

(A') Monolinolenyl dihydrocaffeate

(B') Dilinolenyl dihydrocaffeate

# Radical Scavenging Activity

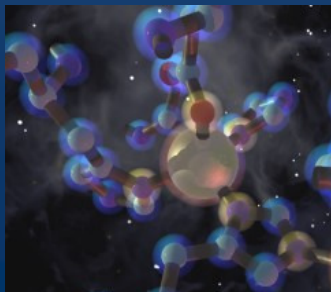


# *Biosynthesis of Phenolic Lipids*

*Transesterification*

*of*

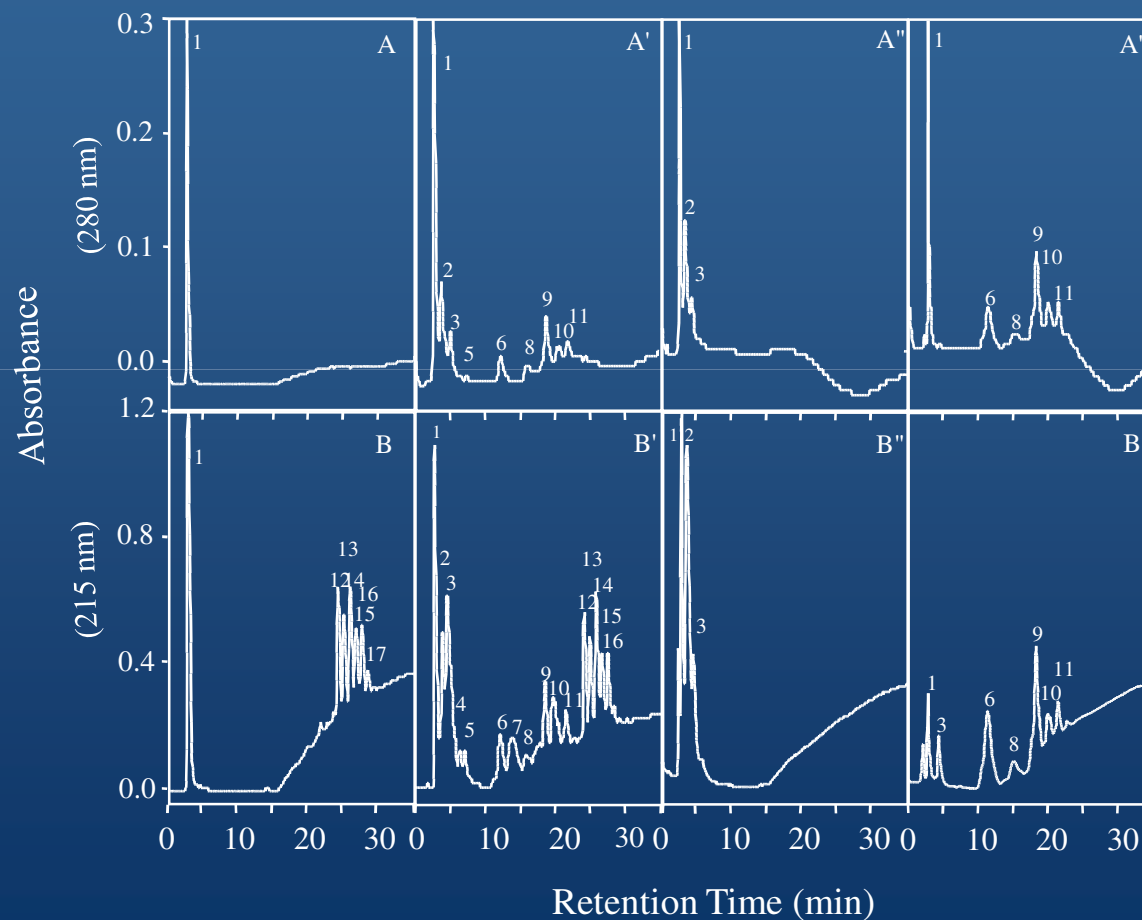
*Endogenous Edible Oils & Dihydrocaffeic Acid*



# *Biosynthesis of Phenolic Lipids*

## *Transesterification of Flaxseed Oil & Dihydrocaffeic Acid*

# HPLC Analysis of Reaction Components



## Section

(A and B) DHCA

(A', B') Flaxseed oil

(A'' and B'') Phenolic monoacylglycerols

(A''' and B''') Phenolic diacylglycerols

## Peak

Dihydrocaffeic acid (1)

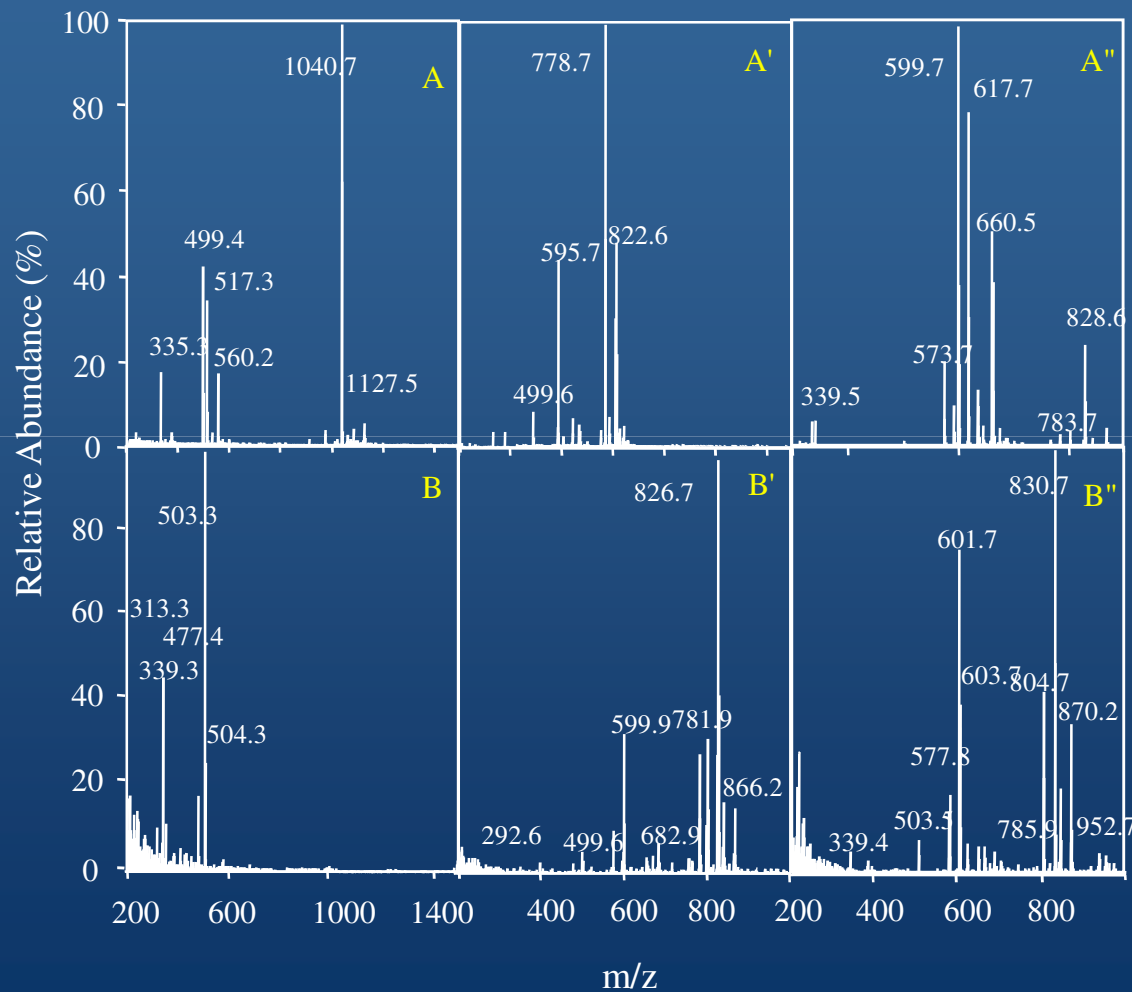
Flaxseed oil (11, 12, 13, 14, 15)

Phenolic monoacylglycerols (2, 3)

Phenolic diacylglycerols (6, 8, 9, 10)



# LC-MS Analysis of Phenolic Lipid Products



## Section

(A) Monolinolenyl dihydrocaffeate

(B) Monooleyl dihydrocaffeate

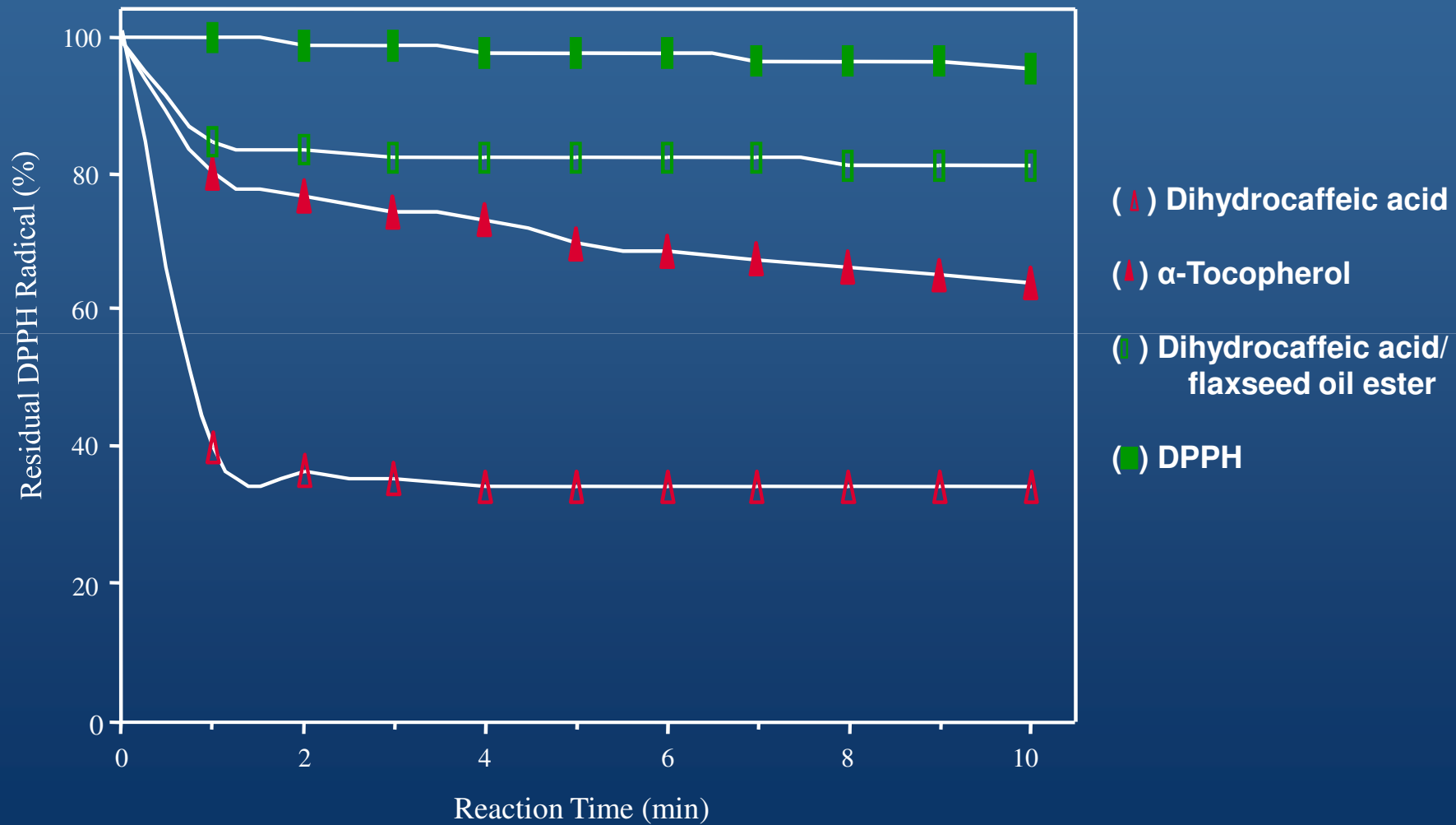
(A') Dilinolenyl dihydrocaffeate

(B') Dilinoleyl dihydrocaffeate

(A'') Oleyl linolenyl dihydrocaffeate

(B'') Dioleyl dihydrocaffeate

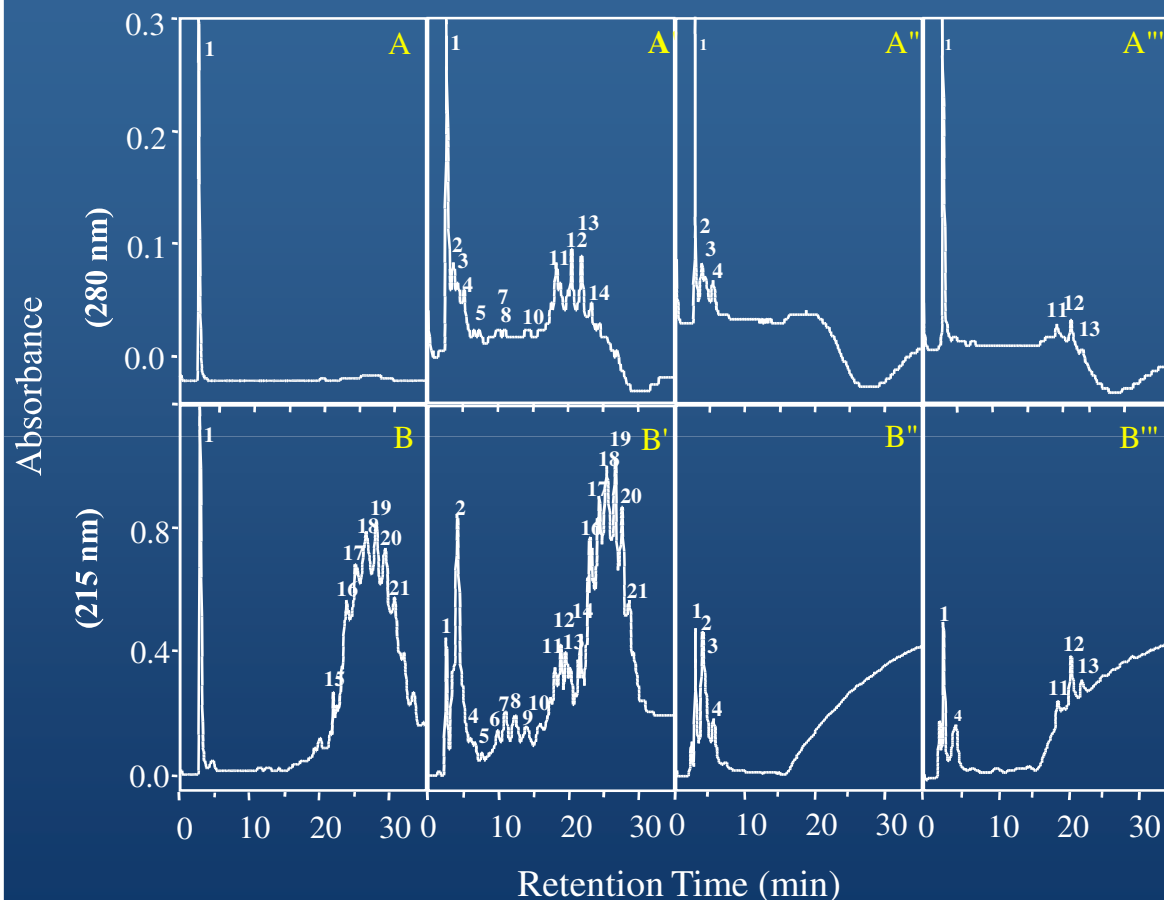
# Radical Scavenging Activity



# *Biosynthesis of Phenolic Lipids*

## *Transesterification of Fish Liver Oil & Dihydrocaffeic Acid*

# HPLC Analysis of Reaction Components



## Section

(A, B) Dihydrocaffeic acid

(A', B') Fish liver oil

(A'', B'') Phenolic monoacylglycerols

(A''', B''') Phenolic diacylglycerols

## Peak

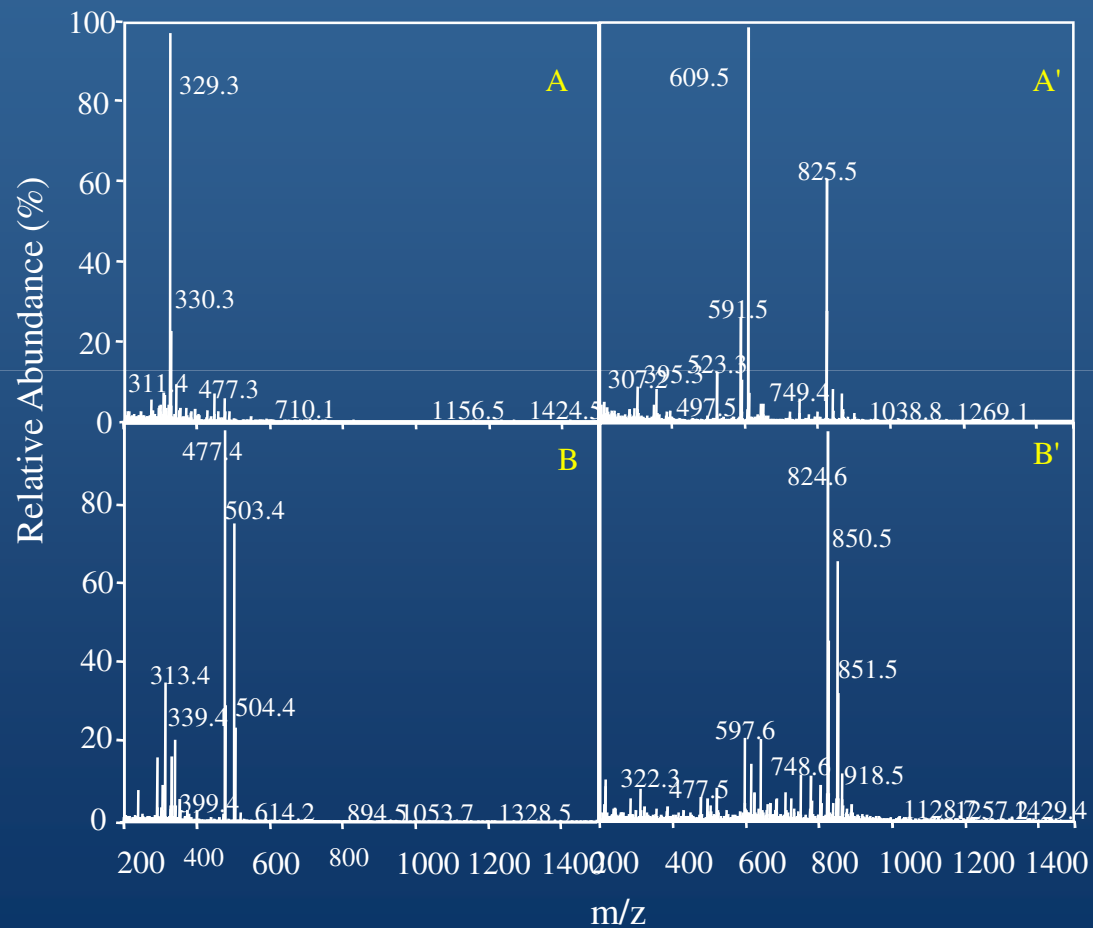
Dihydrocaffeic acid (1)

Fish liver oil (15, 16, 17, 18, 19, 20, 21)

Phenolic monoacylglycerols (2, 3, 4)

Phenolic diacylglycerols (11, 12, 13, 14)

# LC-MS Analysis of Phenolic Lipid Products



## Section

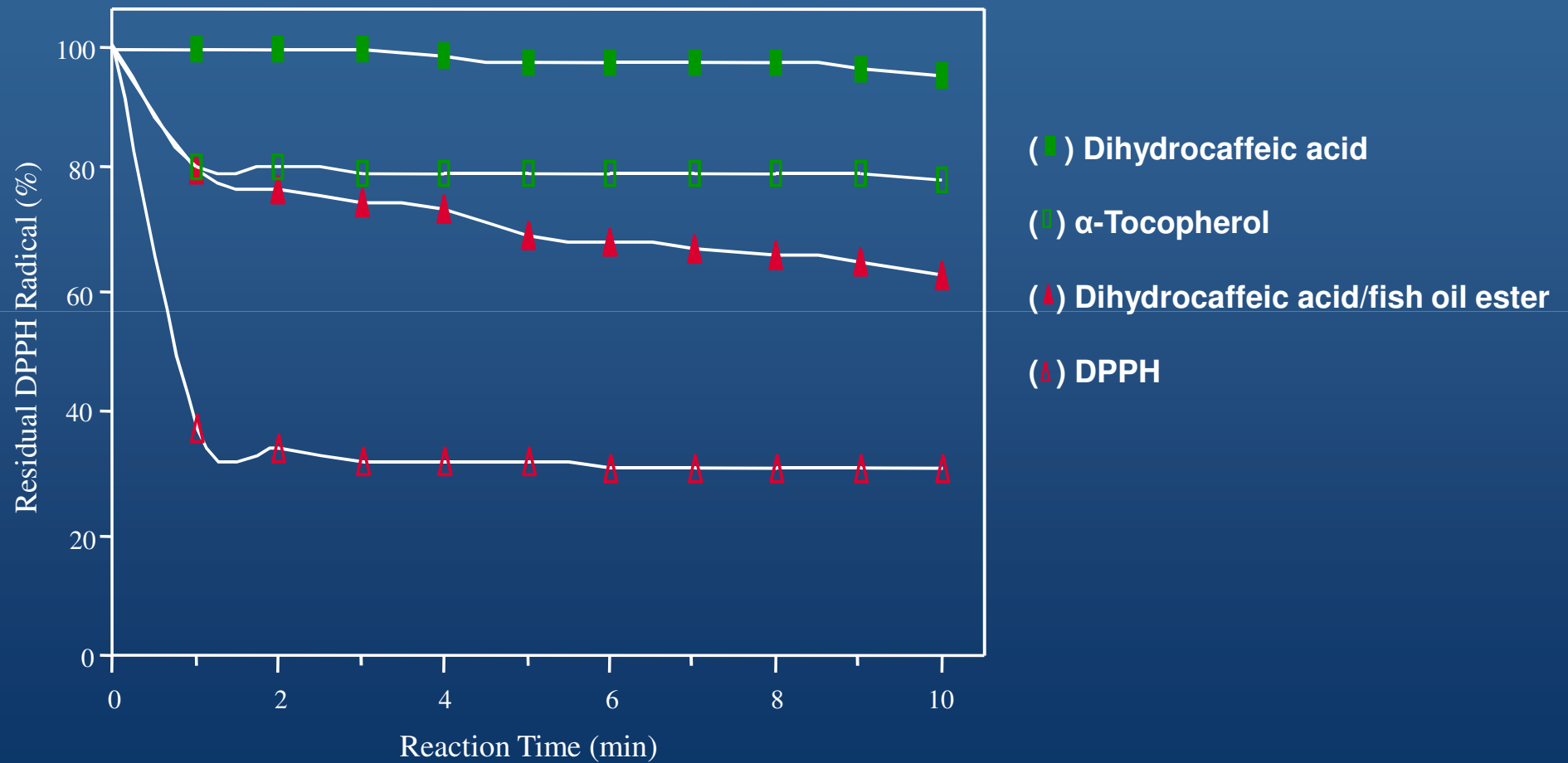
**(A)** Monopalmitoyl dihydrocaffeate

**(B)** Monooleyl dihydrocaffeate

**(A')** Dieicosapentanoate dihydrocaffeate

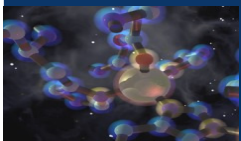
**(B')** Eicosapentanoate docosahexanoate dihydrocaffeate

# Radical Scavenging Activity

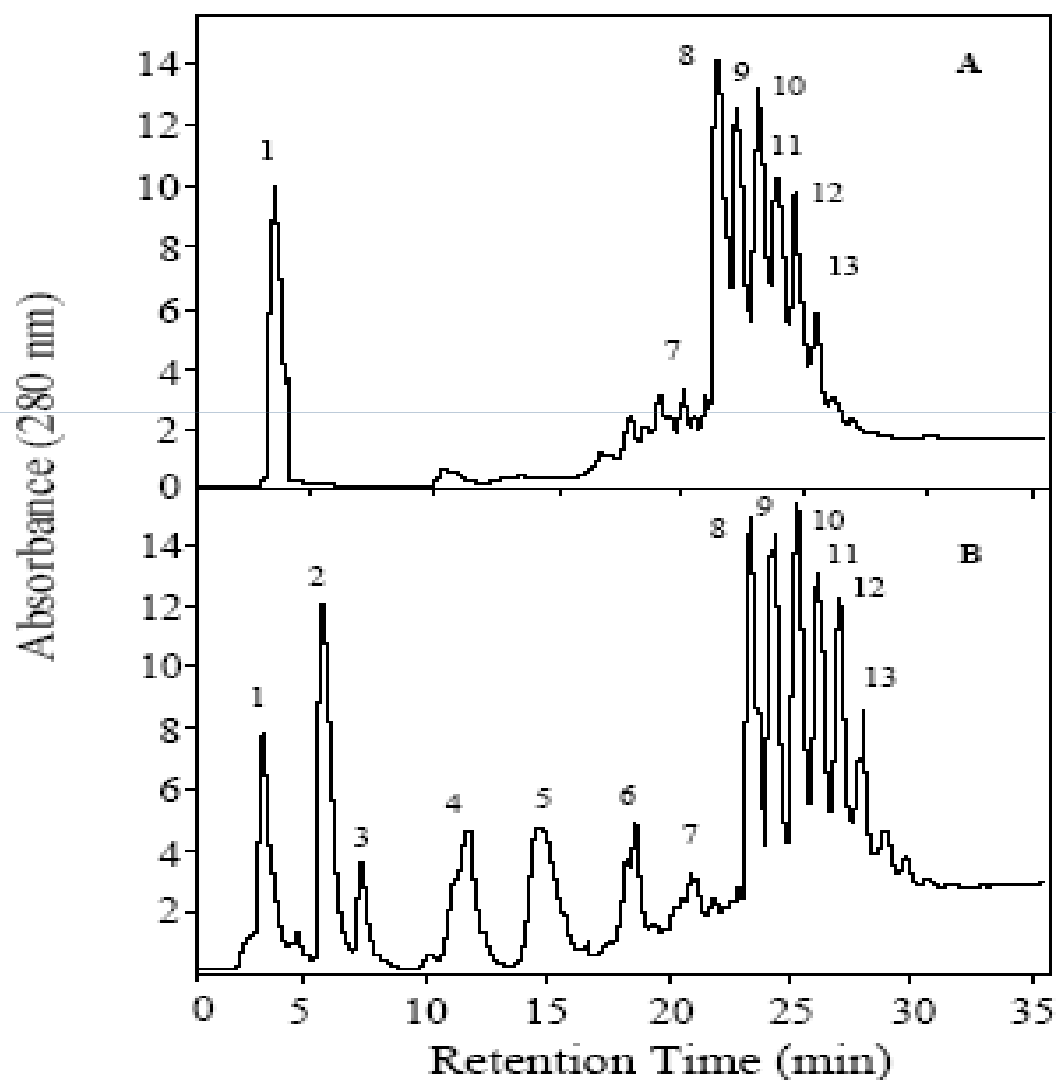


# *Biosynthesis of Phenolic Lipids in Solvent-Free Medium*

## Incorporation of 3,4-Dihydroxyphenyl Acetic Acid *into* Flaxseed Oil



# HPLC Analysis of Flaxseed Oil

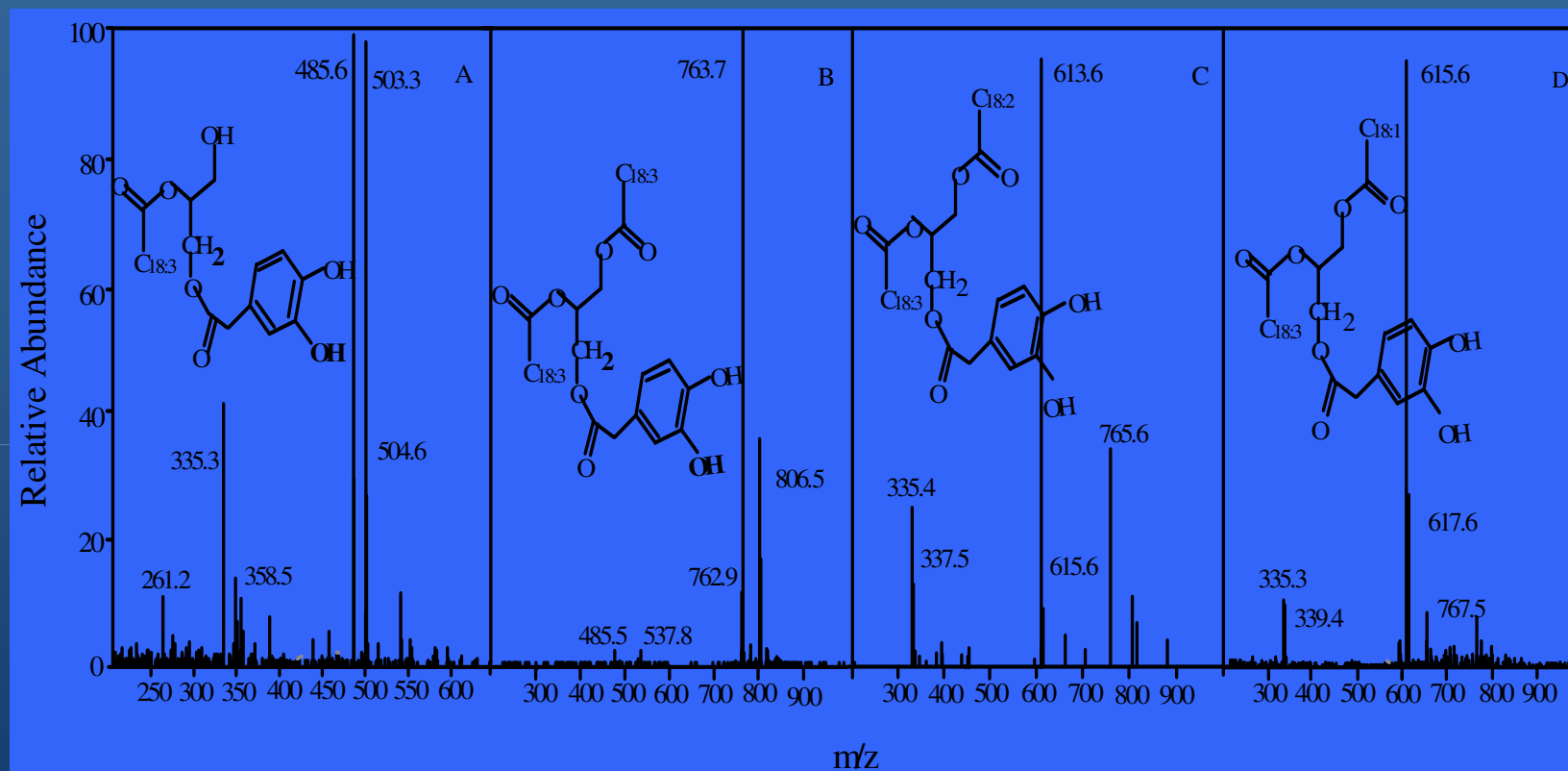


(A) Blank

(B) Reaction Mixture



# LC/MS Characterization of Phenolic Lipids



**A = Monolinolenyl dihydroxyphenyl acetate**

**C = Linoleyl linolenyl dihydroxyphenyl acetate**

**B = Dilinolenyl dihydroxyphenyl acetate**

**D = Oleoyl linolenyl dihydroxyphenyl acetate**

# Relative Fatty Acid Composition

## Relative Fatty Acid (%)<sup>a</sup>

<i>Fatty Acid</i>	<i>Flaxseed Oil</i>	<i>Phenolic MAGs</i>	<i>Phenolic DAGs</i>
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C16:0	4.7	0.1	0.7
C18:0	3.5	0.4	2.7
C18:1	18.0	7.9	14.6
C18:2	16.5	16.2	17.4
C18:3	57.7	75.2	64.4

<sup>a</sup>Relative % was determined as the peak area of selected FA over that of total FAs.

# Radical Scavenging Activity of Phenolic Lipids

Phenolic Compounds	Steady State (min) <sup>a</sup>	IC <sub>50</sub> <sup>b</sup>
<i>3,4-Dihydroxyphenyl acetic acid</i>	3.5 – 11.0	33
<i>3,4-Dihydroxyphenyl acetoxyated</i>	11.0 – 13.0	184
<i>Referenec (<math>\alpha</math>-tocopherol)</i>	6.0 – 12.0	123

<sup>a</sup>Range of concentrations is 30 to 250  $\mu$ M.

<sup>b</sup>IC<sub>50</sub> represents the concentration needed to reduce 50% of the initial amount of DPPH<sup>•</sup>; it was expressed in  $\mu$ M.

# Conclusion

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The overall results, obtained to date, could lay the groundwork for the development of biotechnological process for the production of “*Phenolic Lipids*” that can be used as natural nutraceutical compounds and bio-ingredients, with *scavenging activity compared to that of phenolic compounds, but very close to that of  $\alpha$ -tocopherol.*

# Acknowledgements

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Rosalie Karam (M.Sc.)

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