

# Recovery of Health-Promoting Proanthocyanidins from Berry Co-Products by Alkalization

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Berry Health Benefits Symposium



# Berry pomace

- Millions of pounds of pomace produced each year
- Most disposed of in landfills or used in animal feed
  - Low pH
  - Low protein



# Berry Pomace Polyphenolics

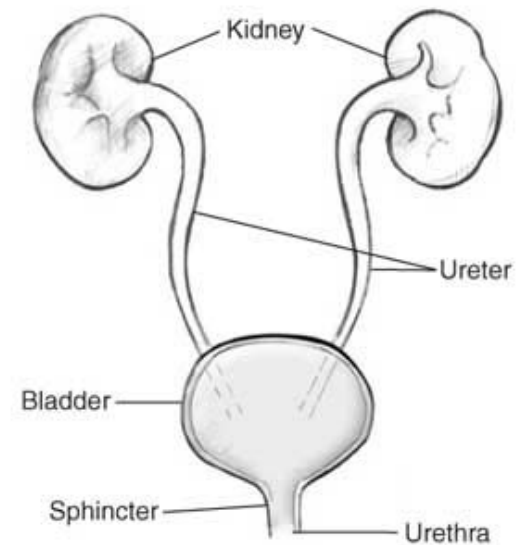
- Rich source of anthocyanins, flavonols, procyanidins
- Procyanidins may contain A or B-type linkages

B-type dimer

A-type dimer

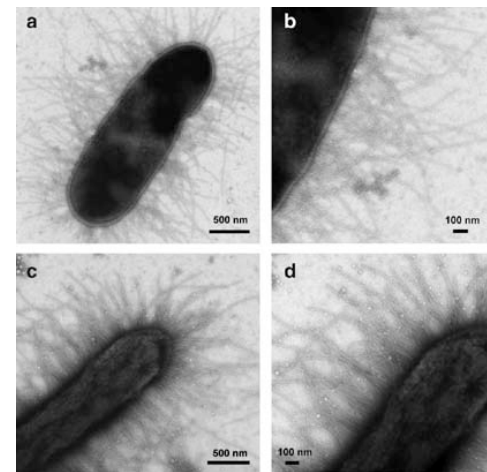
# Urinary Tract Infections (UTI)

- For centuries, cranberry juice has been consumed to prevent recurrent infections
- Bacteria adhere to the urinary tract including the bladder and kidney
- Affects millions of people each year including men and women.



# UTI Prevention Mechanism

- Acidification of urine by benzoic acid
- Procyanidins containing A-type linkages
- Prevents P-fimbriae bacterial adherence to epithelial cells in the urinary tract
- Preventative rather than curative



# Bound Procyanidins

- Procyanidin levels decrease drastically during fruit ripening
  - Metabolized
  - Bound to other cell components
- Bind to cell wall material
  - Hydrophobic interactions causing phenols to reside in pockets
  - Hydrogen bonding between hydroxyl groups of phenols and oxygen present in polysaccharides
  - Covalently bound to polysaccharides

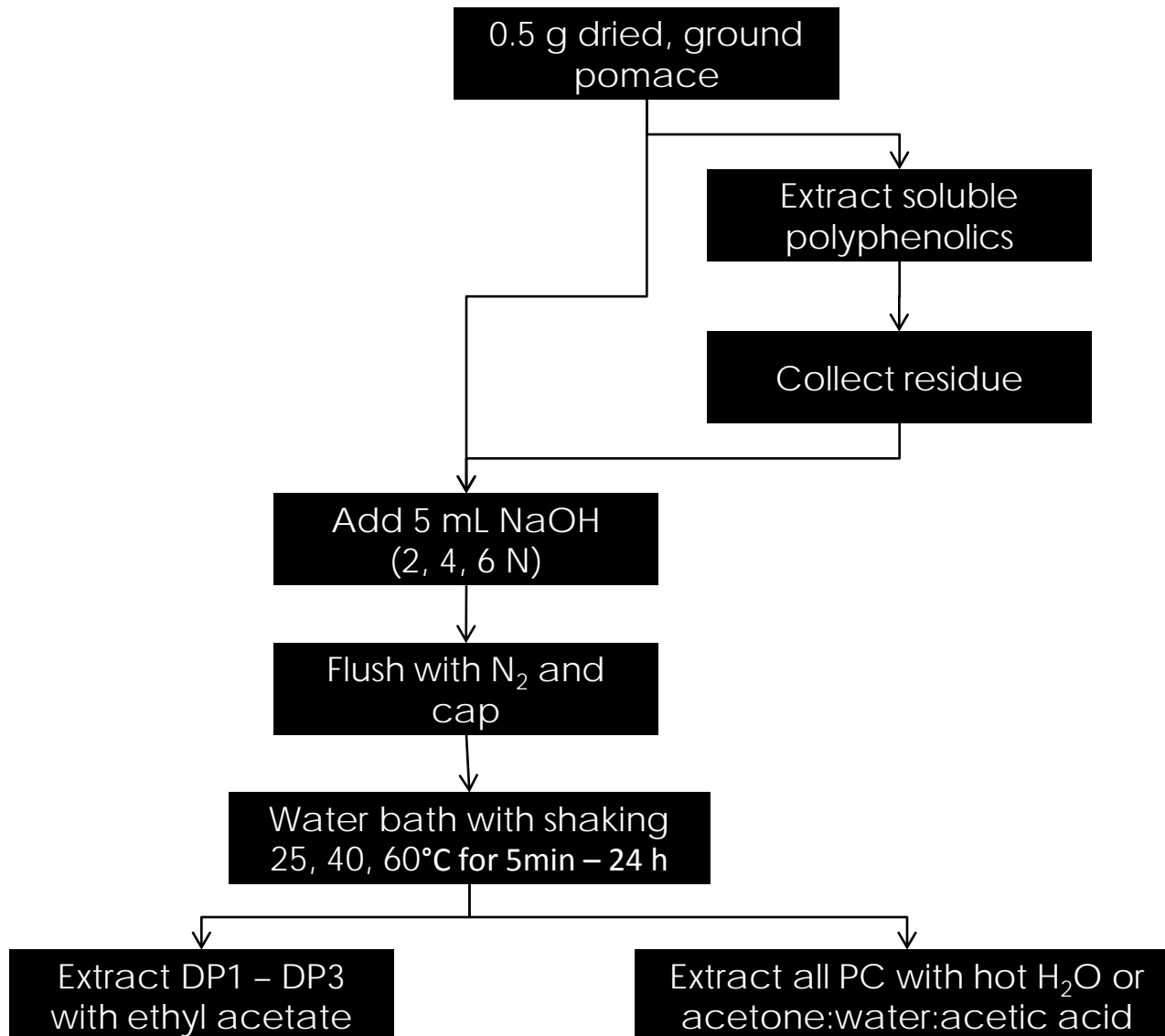
# Bound Procyanidins

- Methods to release/quantify
  - Enzymatic
    - Pectinases
    - Cellulases/Hemicellulases
    - Proteases
  - Acid catalyzed depolymerization
    - Thiolysis
    - Butanol:HCl (Porter Method)

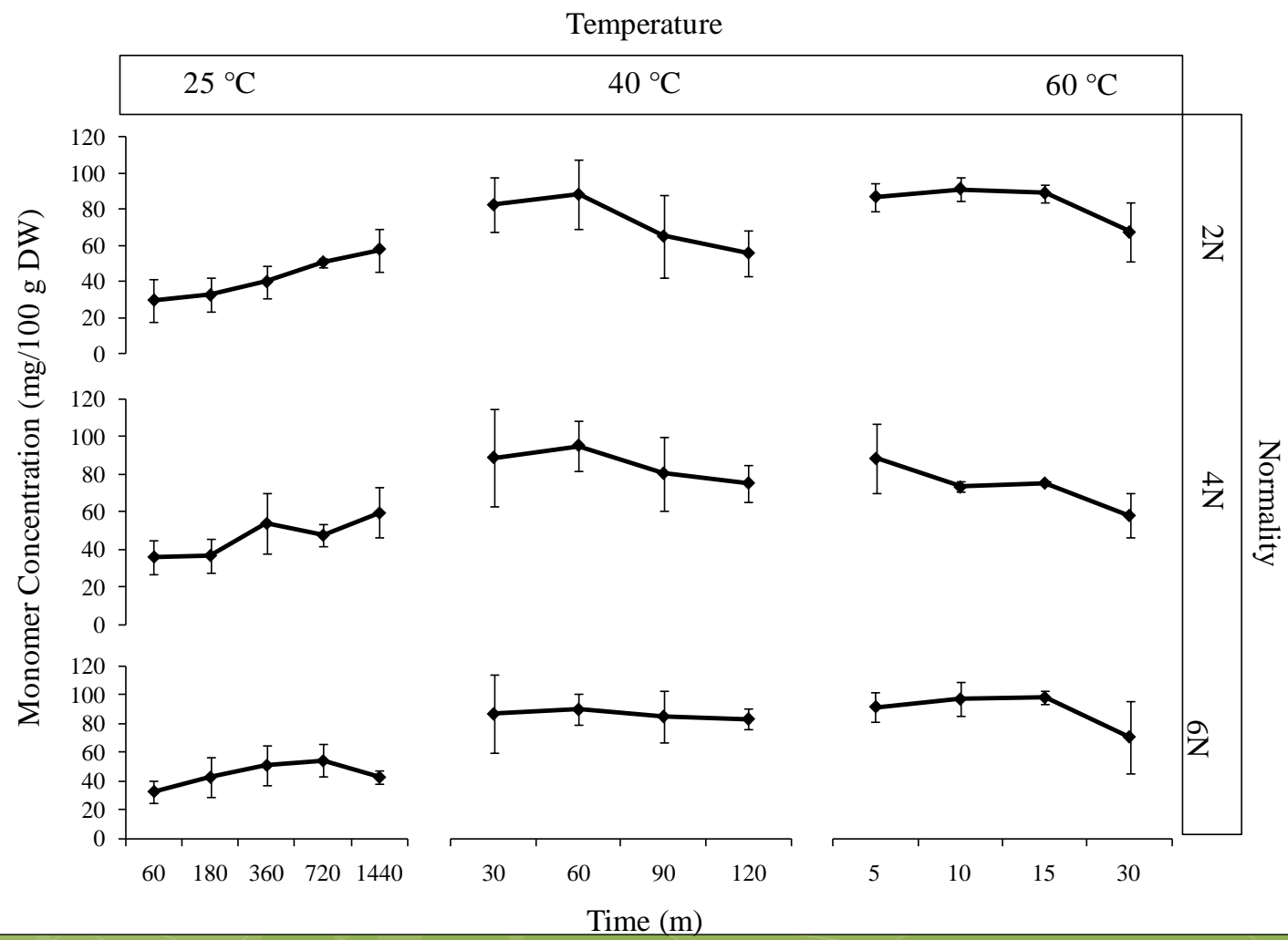
# Metabolism and Bioavailability

- Procyanidin absorption is largely dependent upon size
- Those larger than trimers (DP3) are not absorbed (Donavan *et al.*, 2002)
- May still be beneficial to gastrointestinal (GI) health
  - Fermentation Products
  - Protection against GI disorders

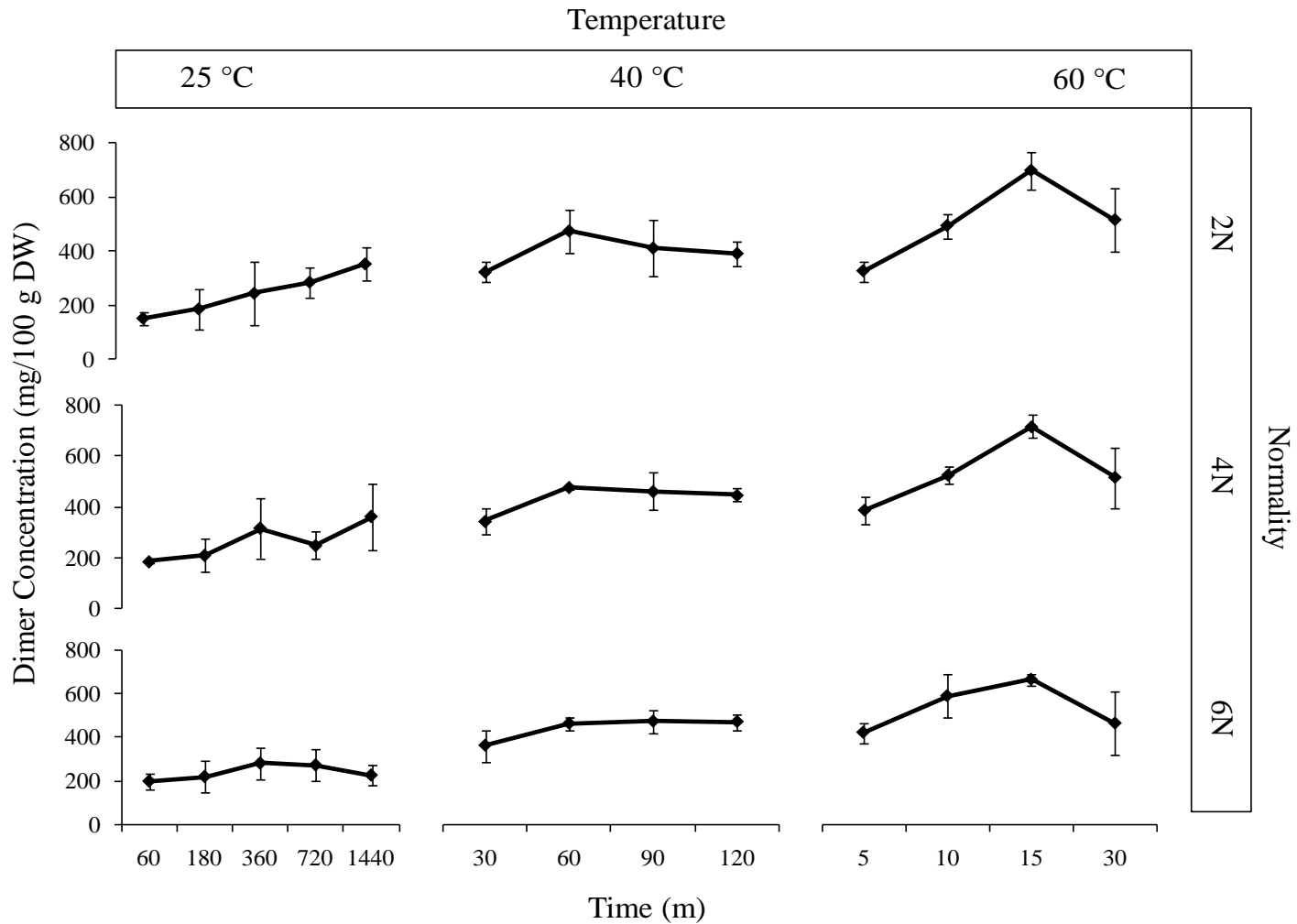




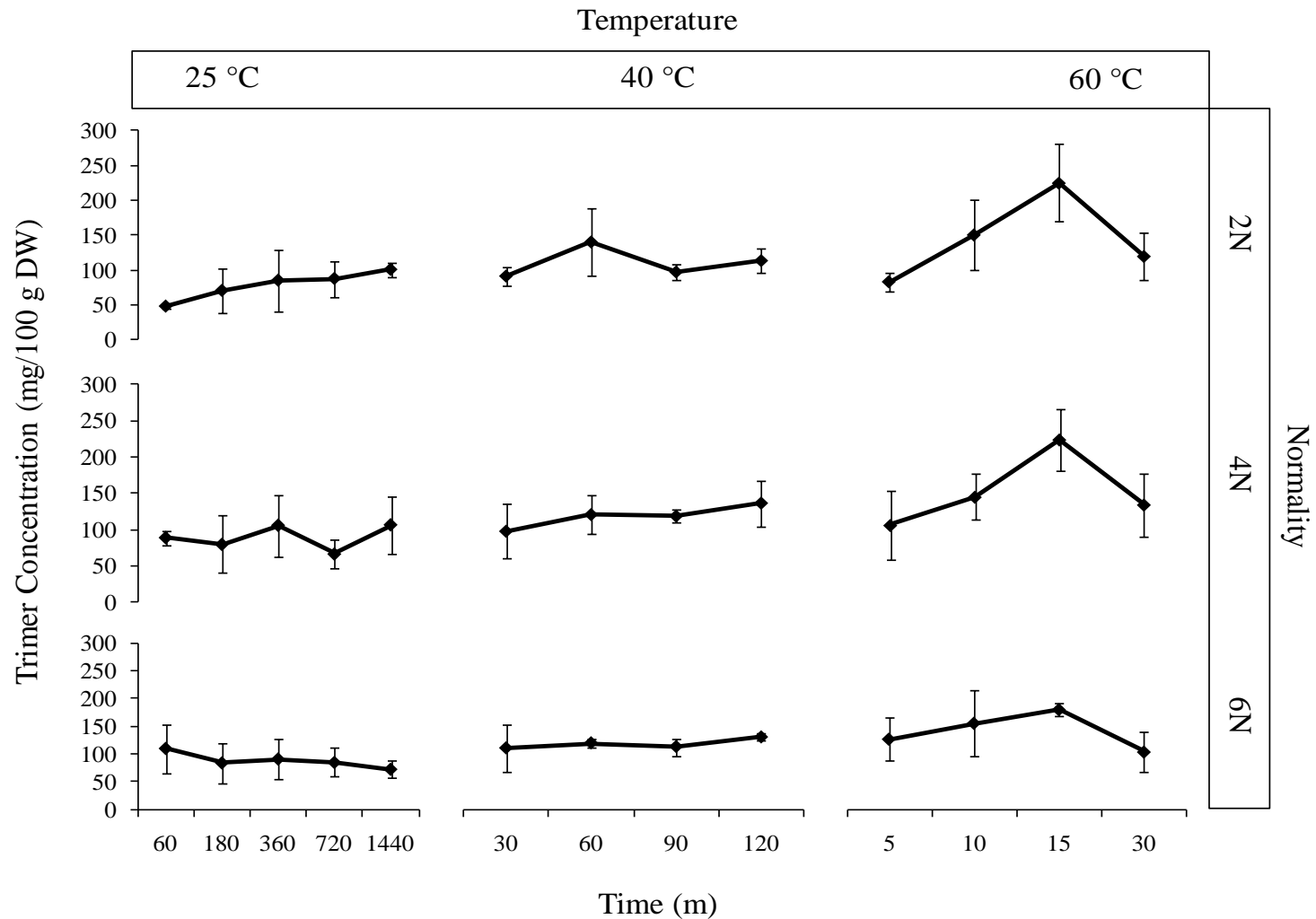
# Changes in procyanidin monomer (DP1) content in cranberry pomace treated with sodium hydroxide



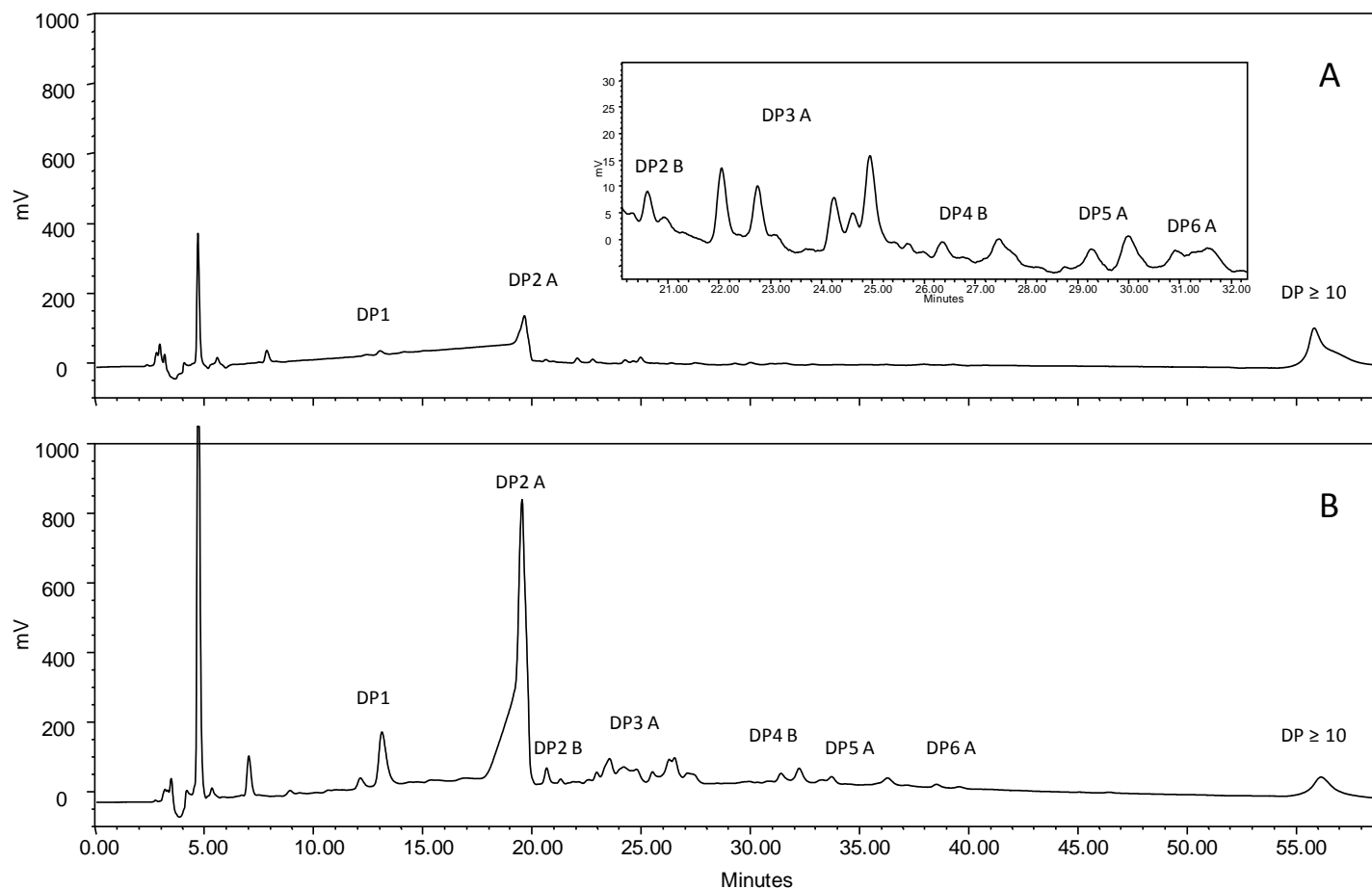
# Changes in procyanidin dimer (DP2) content in cranberry pomace treated with sodium hydroxide



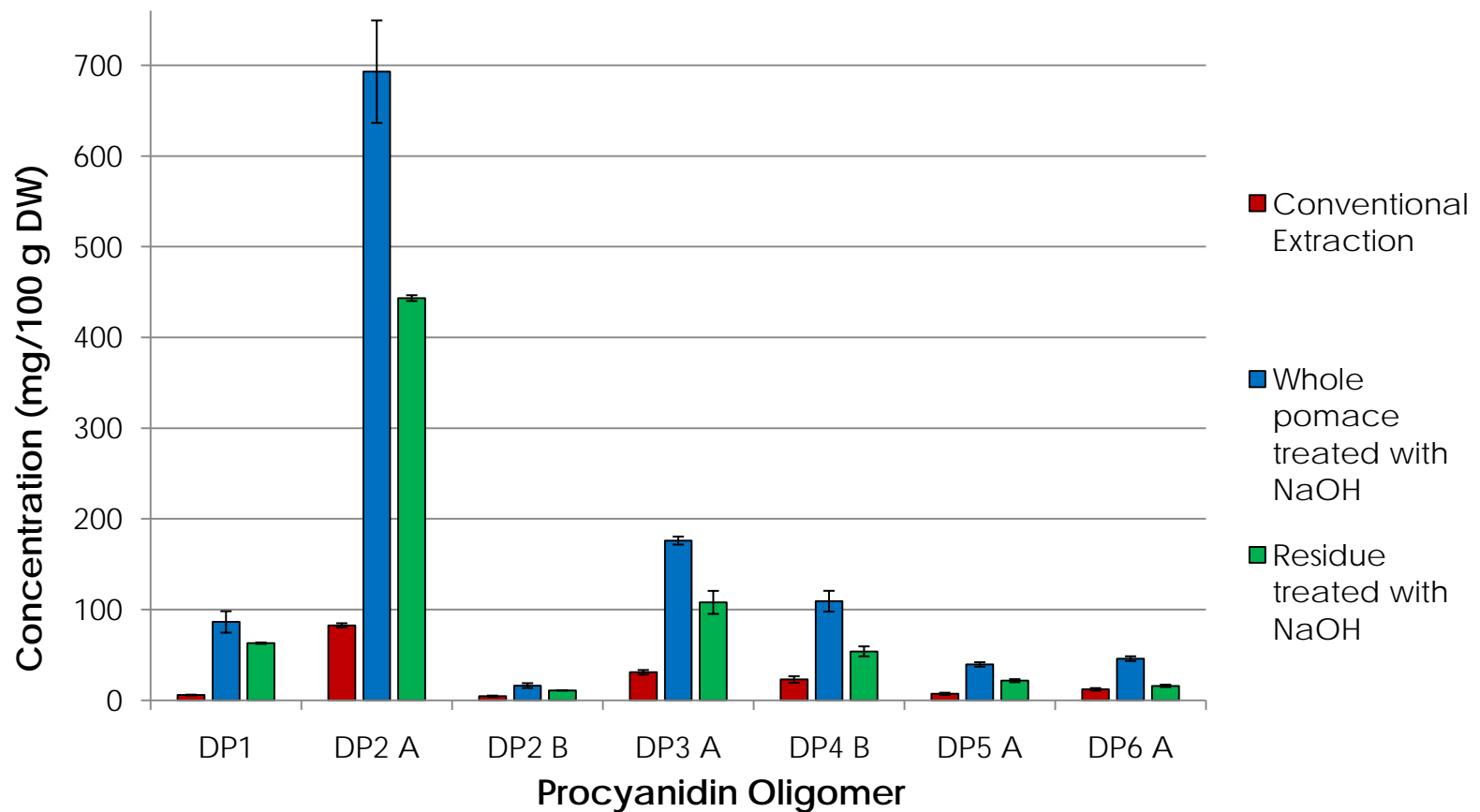
# Changes in procyanidin trimer (DP3) content in cranberry pomace treated with sodium hydroxide



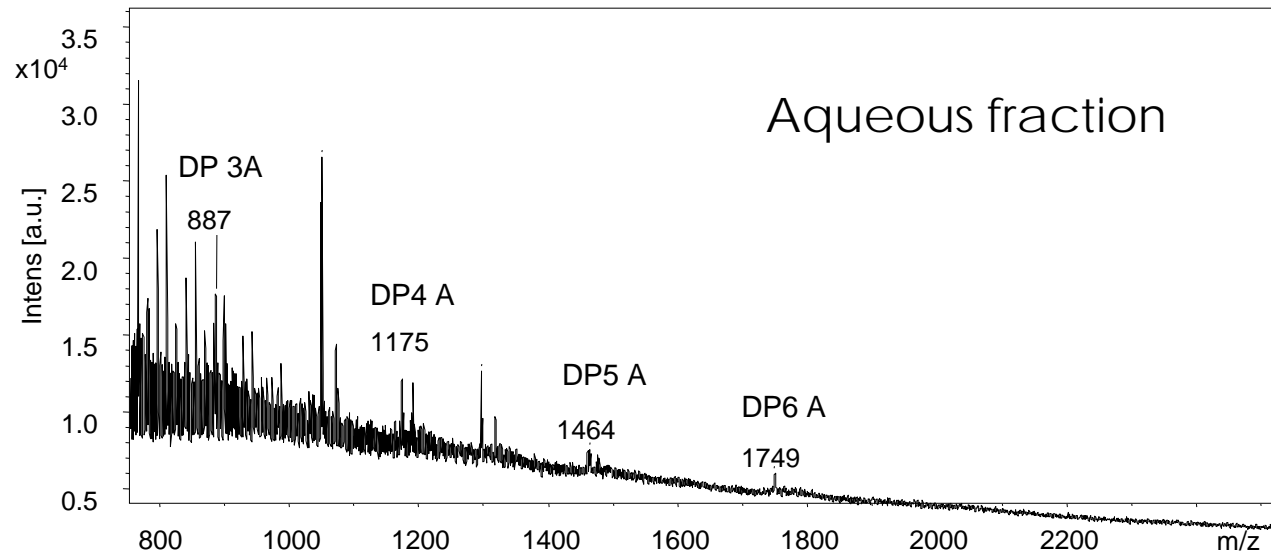
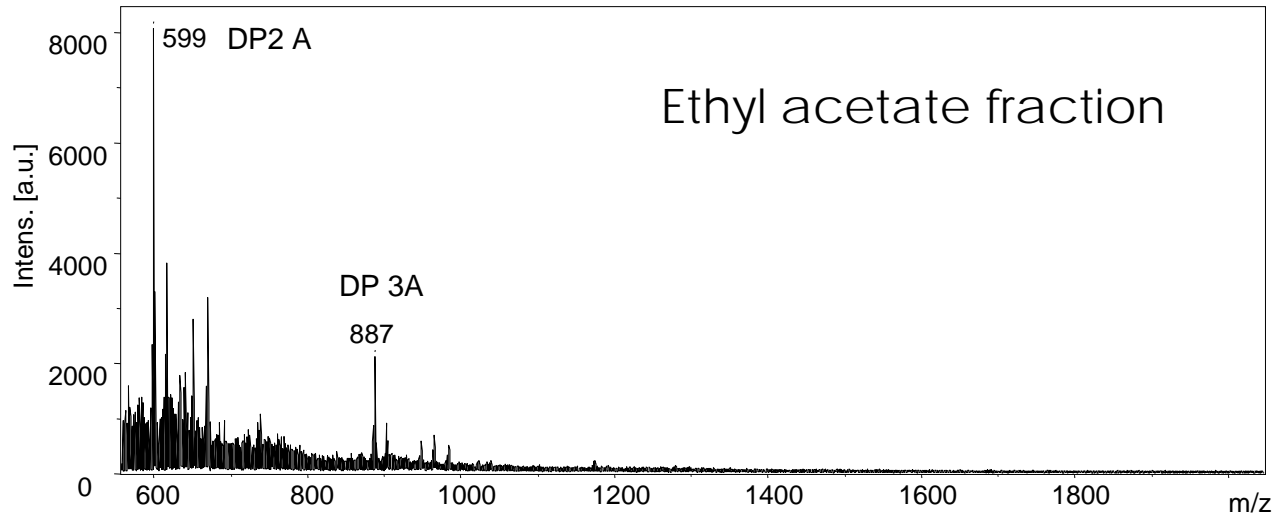
# HPLC chromatograms of procyanidins in cranberry pomace before (A) and after (B) treatment with sodium hydroxide



## Procyanidin oligomer (DP1 – DP6) composition of cranberry pomace before and after treatment with sodium hydroxide

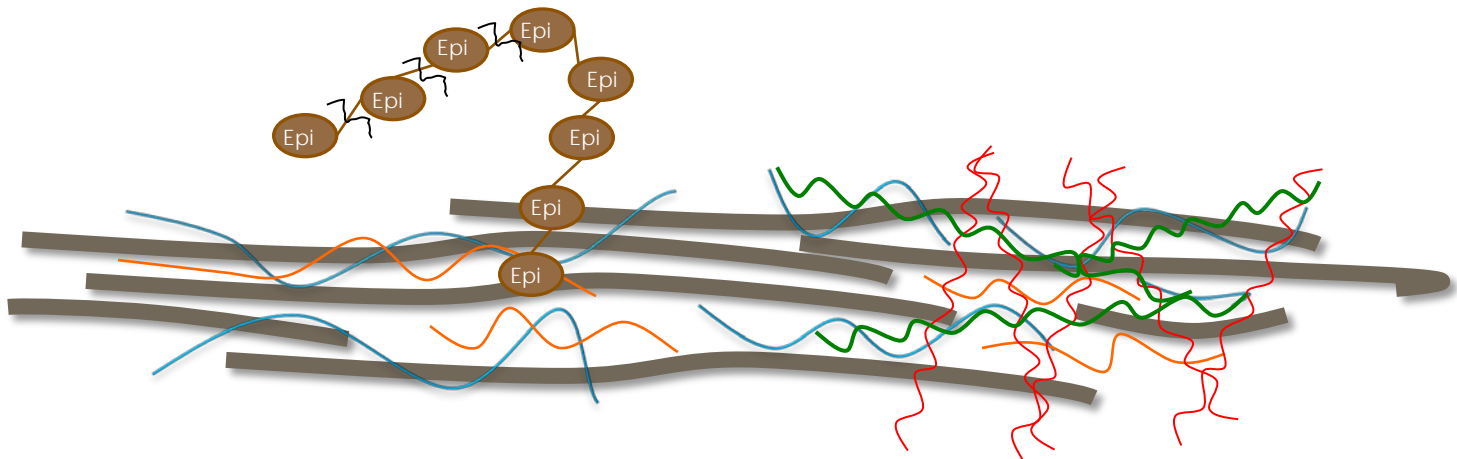


# MALDI-TOF-MS



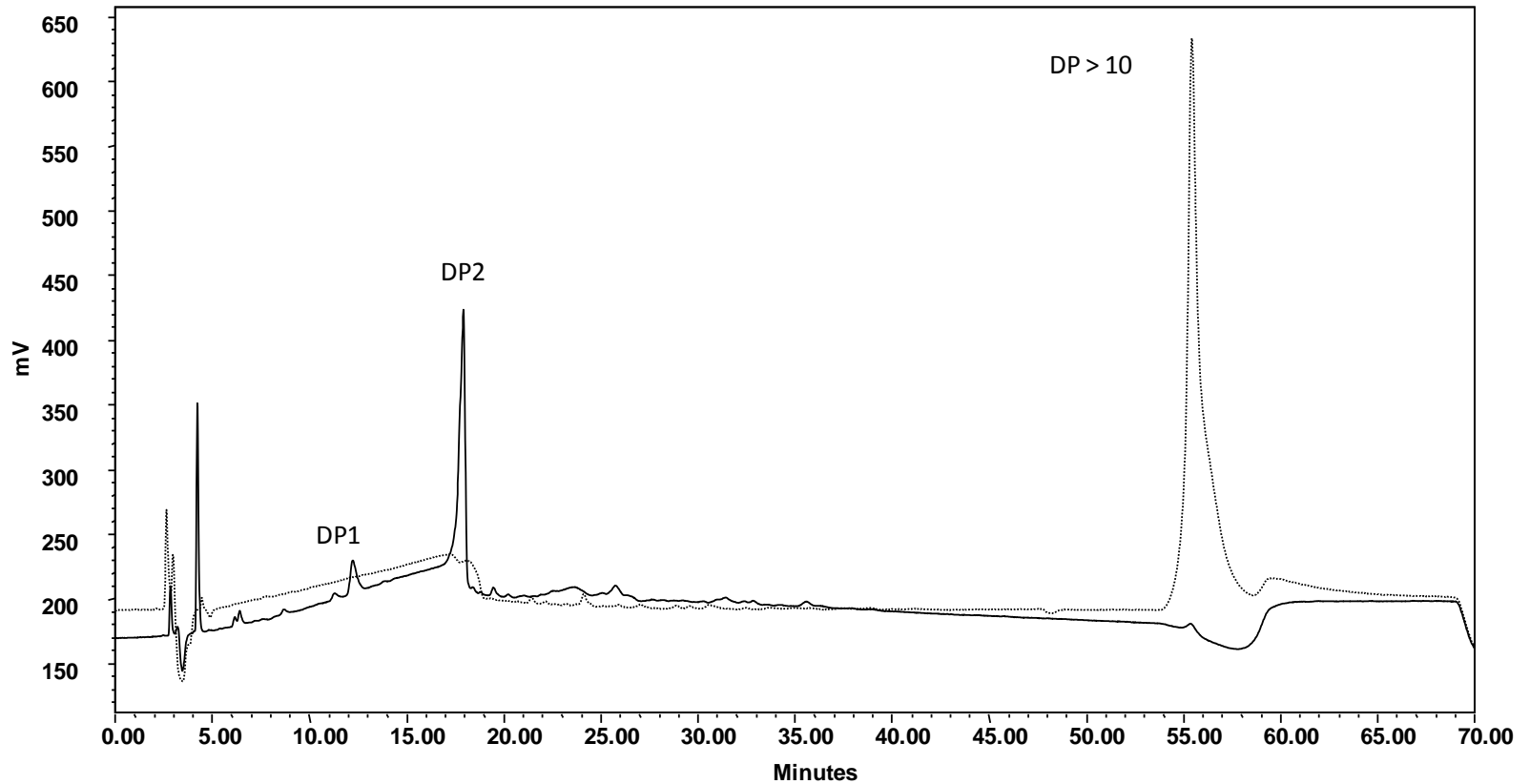
# Mechanism

- Polymeric procyanidins bound to cell wall
- Depolymerization
- Solubilization of Cell Wall Material
  - Hemicellulose soluble in alkali

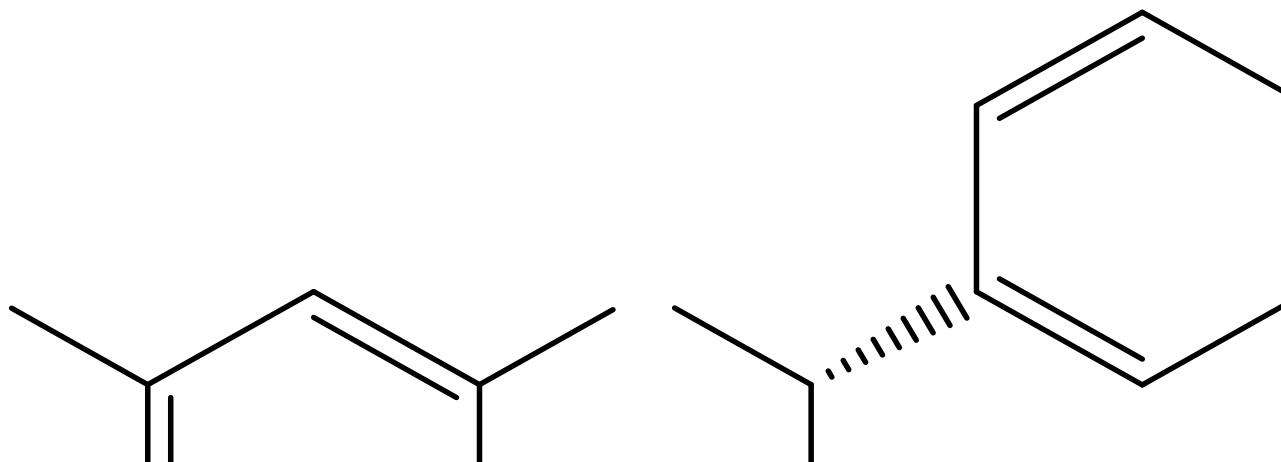




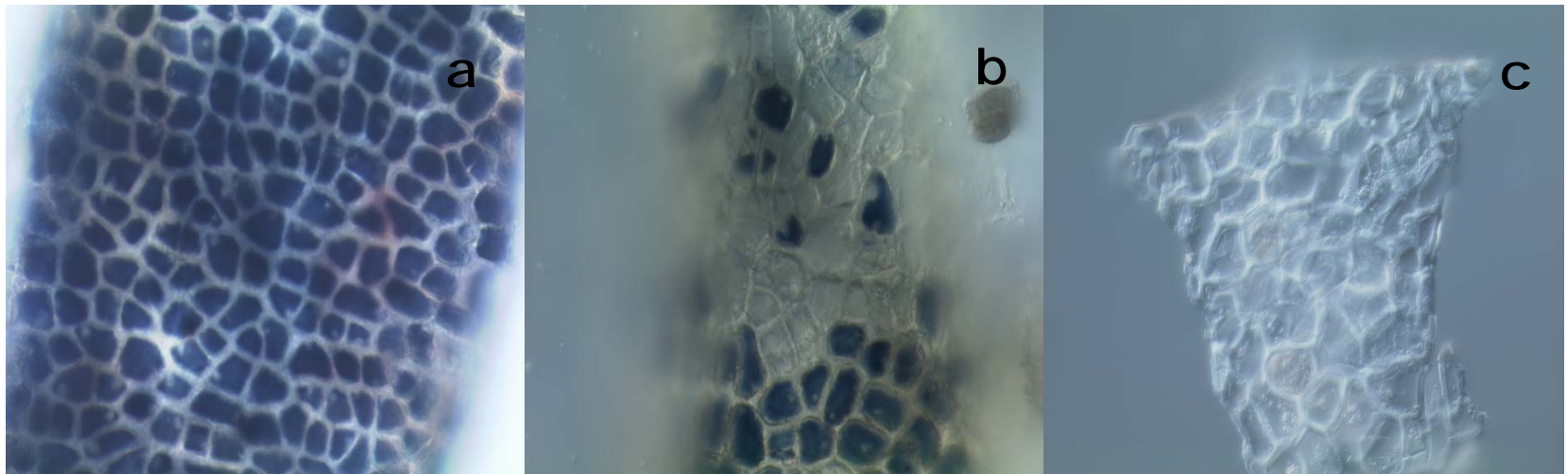
# HPLC chromatograms of purified polymeric procyanidins from cranberry pomace before (dotted) and after Alkaline Hydrolysis (solid)



# Depolymerization Mechanism



# Light microscopy



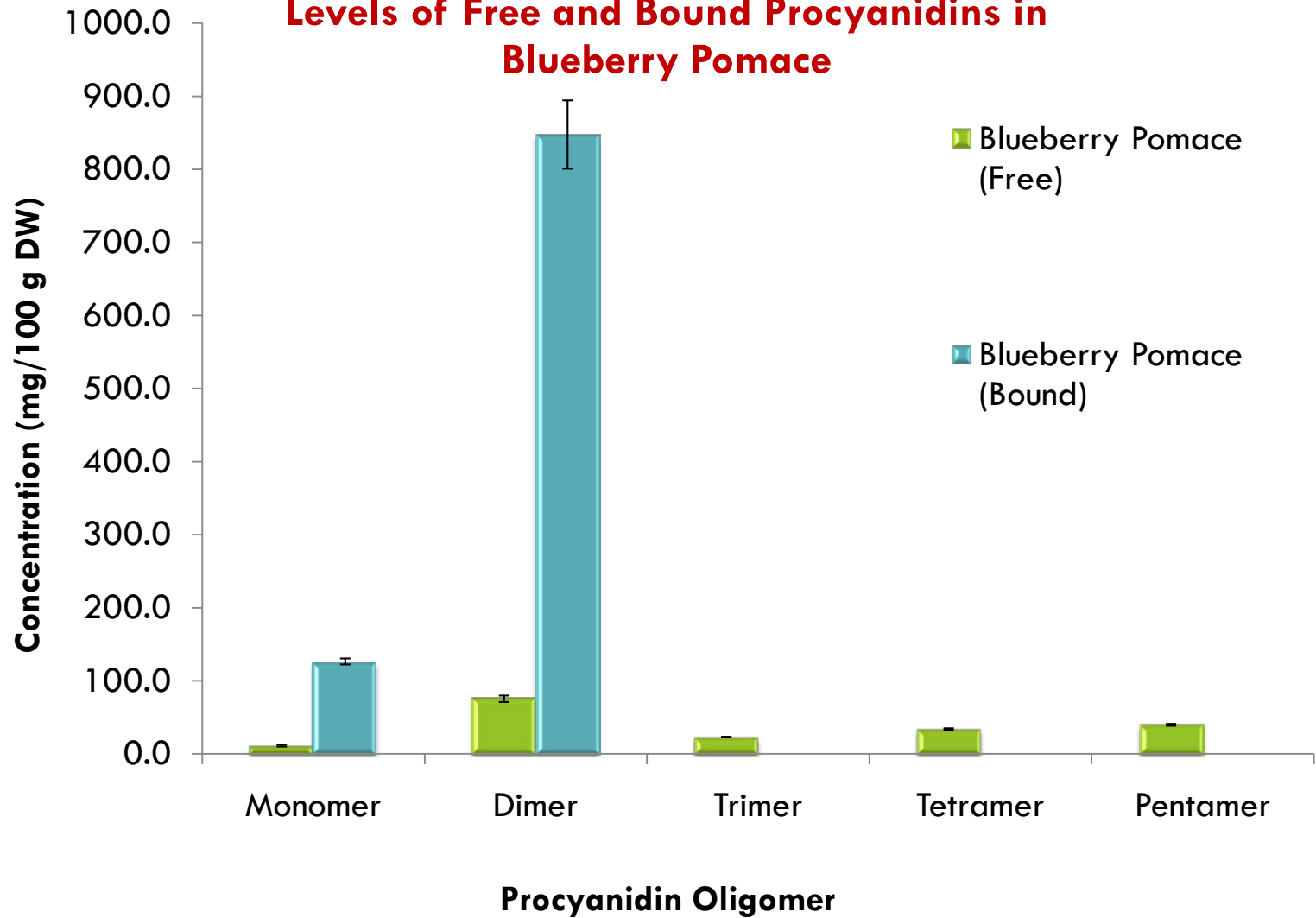
Differential interference contrast (DIC) microscopy images of:  
(a) ground cranberry pomace  
(b) ground cranberry pomace after conventional extraction  
(c) ground cranberry pomace after alkaline hydrolysis.

All were stained with dimethylaminocinnamaldehyde (DMACA)

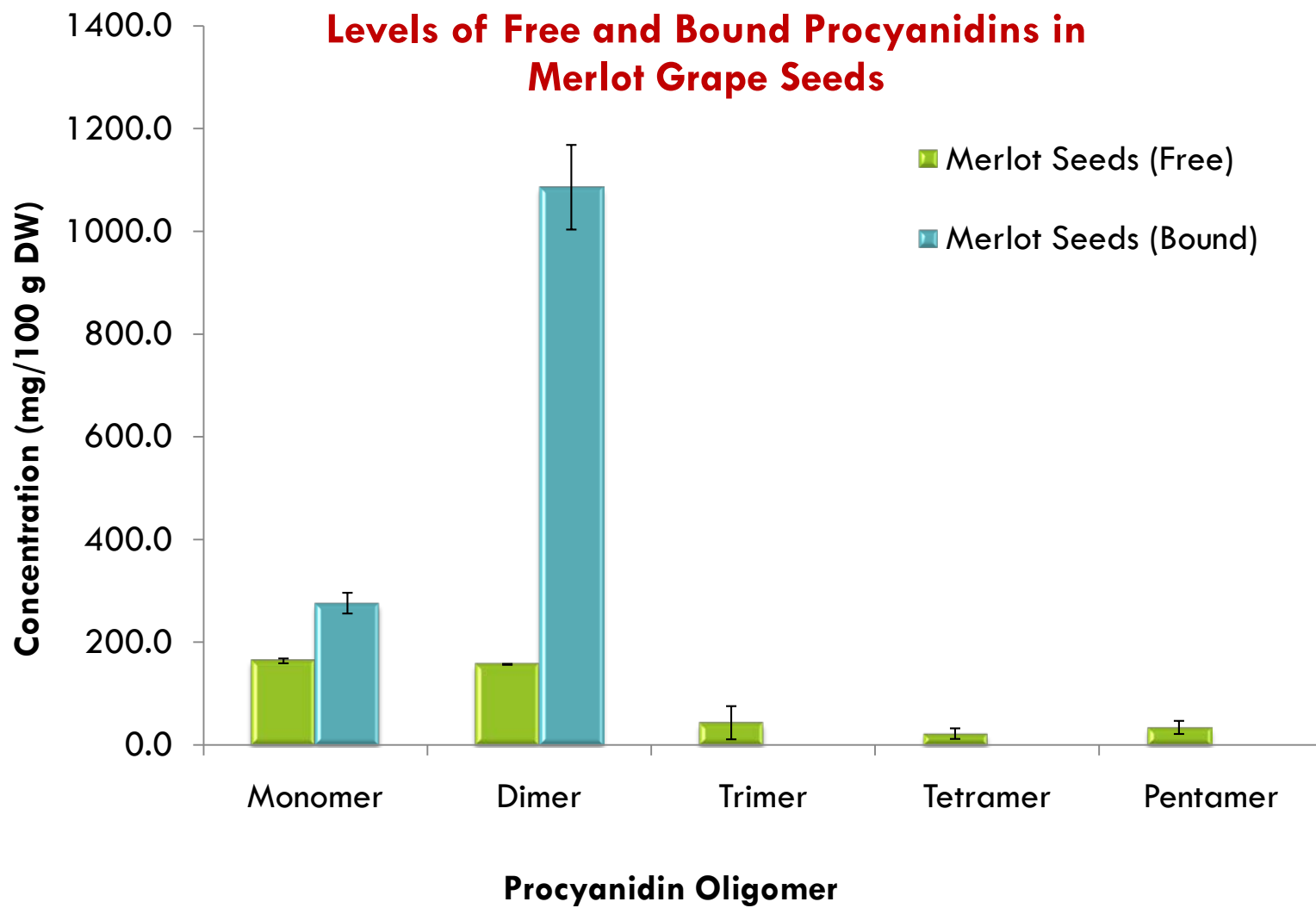
# Anti-adhesion

Anti-adhesion Properties of Cranberry Pomace		
Sample	Amount of Procyanidins (mg/100mg) <sup>a</sup>	% Anti-adherence <sup>b</sup>
Untreated	0.95	17.37
Alkaline DP1 – DP3	0.83	13.15
Alkaline DP ≥ 4	0.80	31.19
Alkaline All Procyanidins	1.7	36.15
<sup>a</sup> Procyanidins were obtained from 100 mg cranberry pomace		
<sup>b</sup> % Anti-adherence based on 0.8 mg/mL of whole pomace		

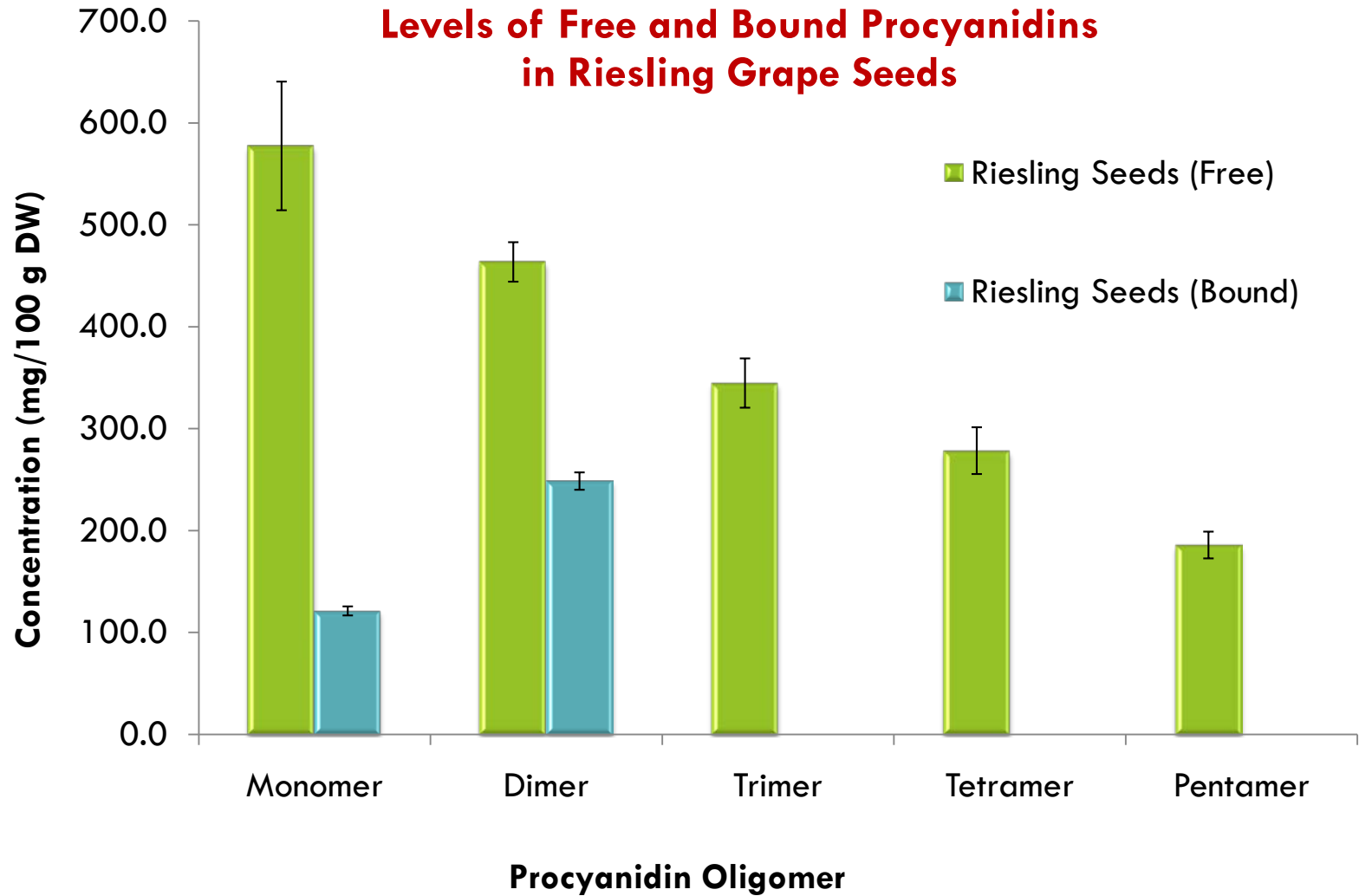
## Levels of Free and Bound Procyanidins in Blueberry Pomace



## Levels of Free and Bound Procyanidins in Merlot Grape Seeds



## Levels of Free and Bound Procyanidins in Riesling Grape Seeds



# Summary

- Alkaline hydrolysis increased the total amount of procyanidins extracted from cranberry pomace, indicating the presence of “bound” procyanidins
- Procyanidins released are recoverable
- Increase was likely due to a combination of depolymerization and solubilization of cell wall material
- Procyanidins extracted by alkaline hydrolysis had greater anti-adhesion ability than those extracted conventionally



# Conclusions and Future Work

- Alkaline conditions can be used to recover procyanidins from fruit waste material.
- Resulting compounds may be more bioavailable due to their lower molecular weight.
- More work needs to be done to understand the contributions of depolymerization, enhanced extraction, degradation