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

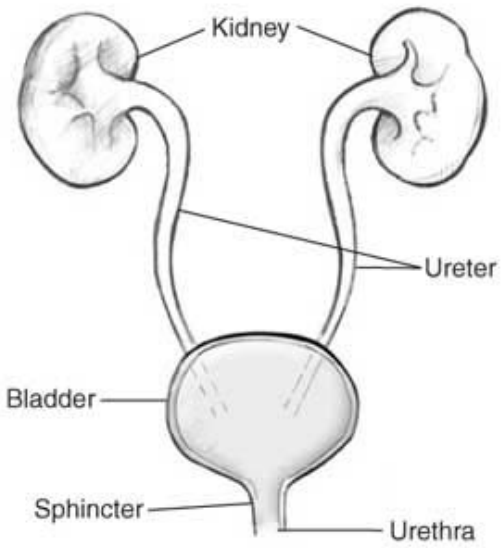
- Rich source of anthocyanins, flavonols, procyandins
- Procyanidins may contain A or B-type linkages

B-type dimer

A-type dimer

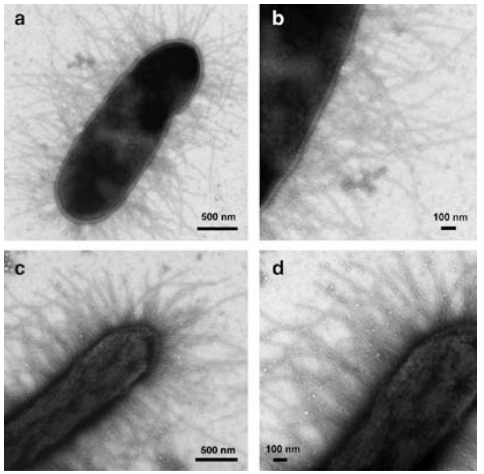
Urinary Tract Infections (UTI)

- 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

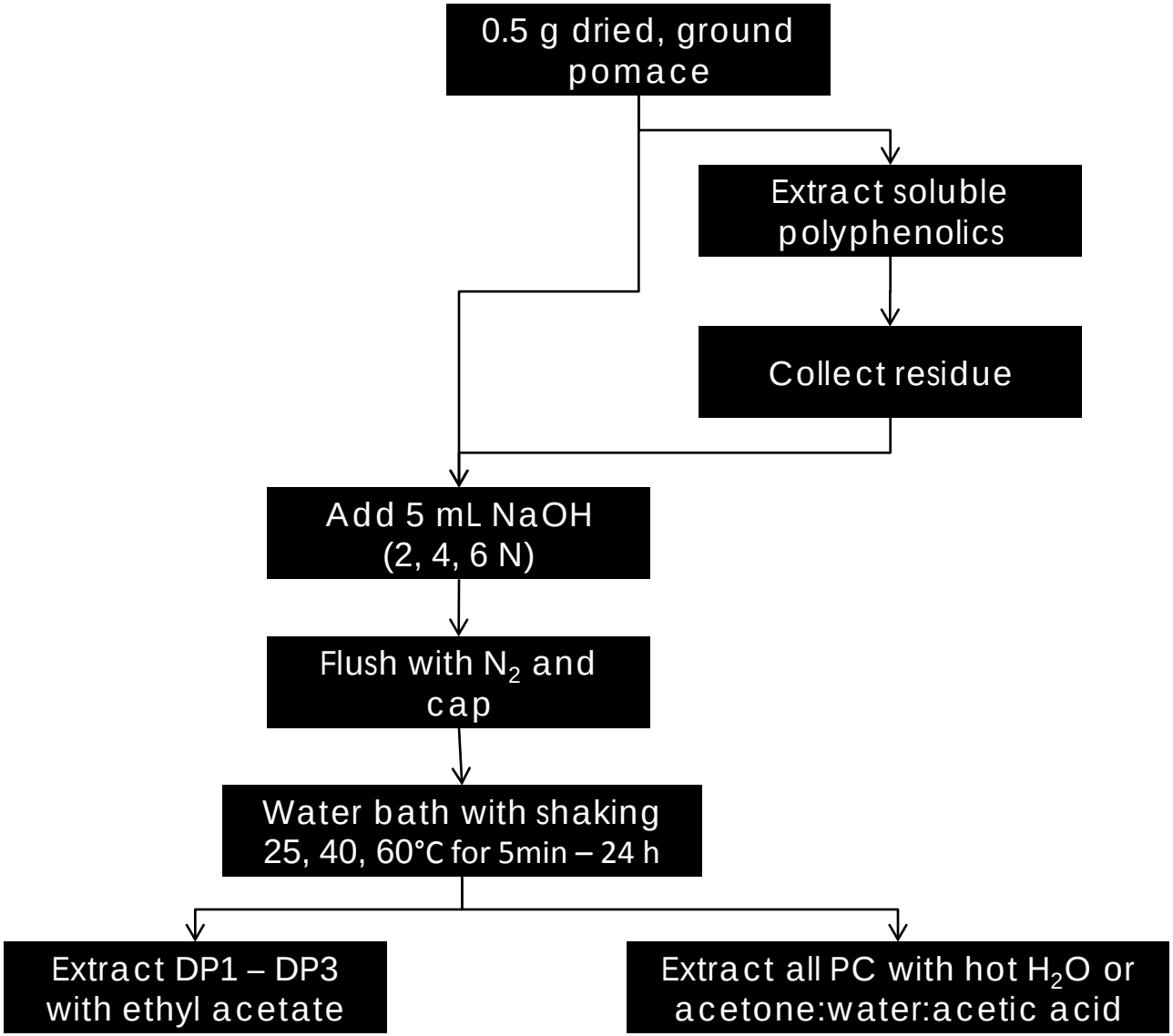
- Procyanidin levels decrease drastically during fruit ripening
  - Metabolized
  - Bound to other cell components
- Bind to cell wall material
  - Hydrophobic interactions causing phenols to reside in pockets
  - Hydrogen bonding between hydroxyl groups of phenols and oxygen present in polysaccharides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

Bound Procyanidins

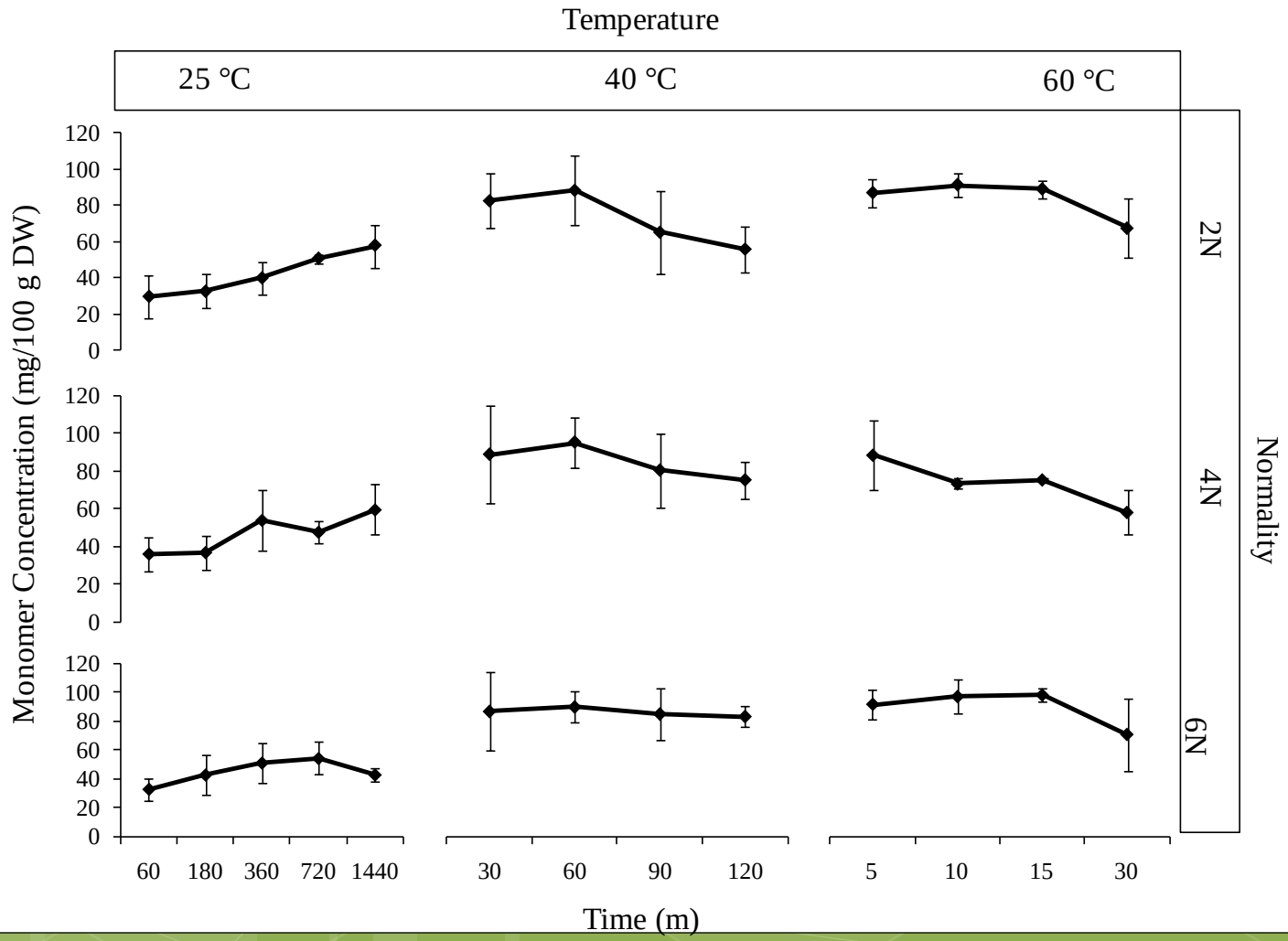
- Methods to release/quantify
  - Enzymatic
    - Pectinases
    - Cellulases/Hemicellulases
    - Proteases
  - Acid catalyzed depolymerization
    - Thiolysis
    - Butanol:HCl (Porter Method)

Metabolism and Bioavailability

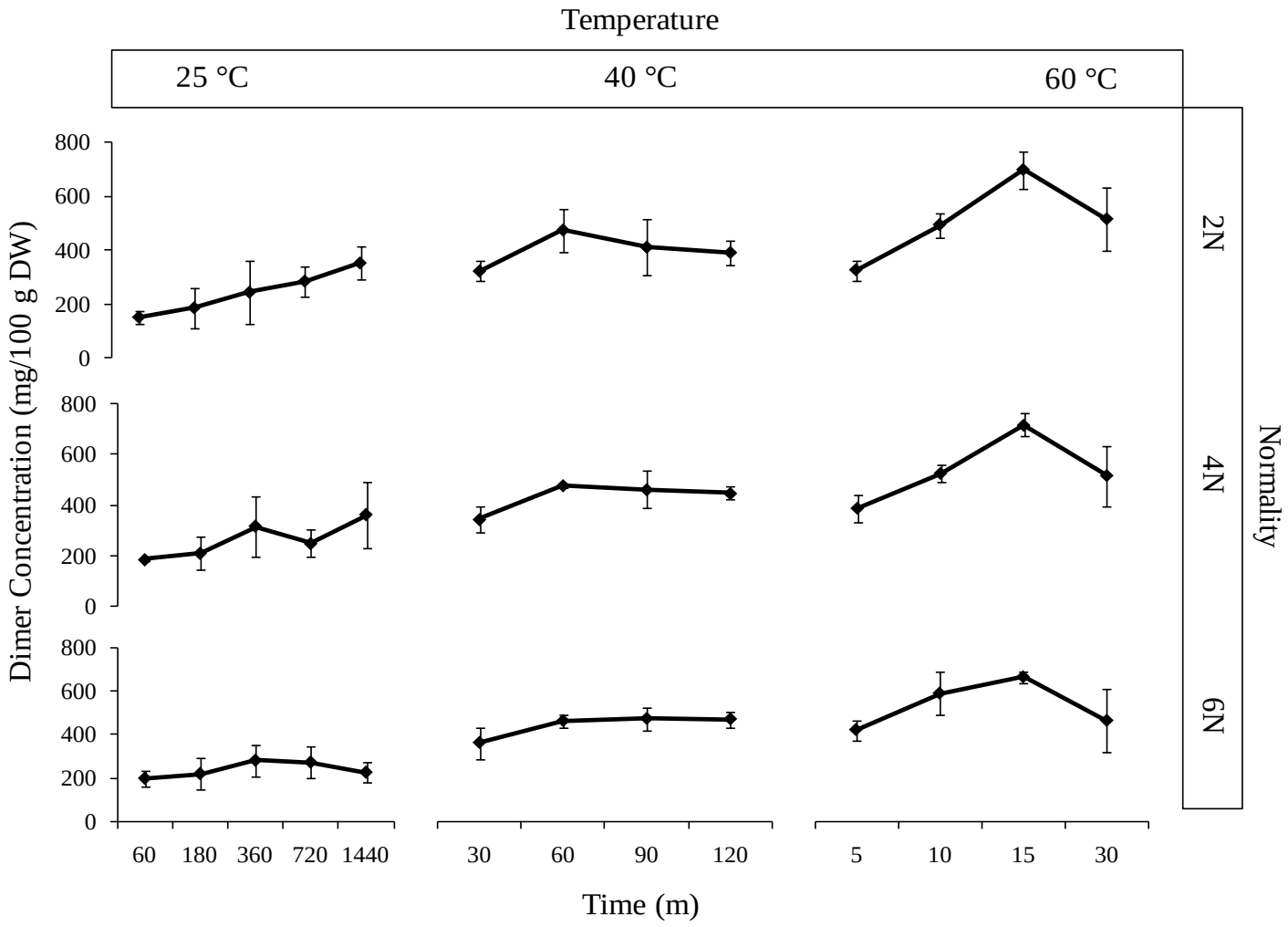
- Procyanidin absorption is largely dependent upon size
- Those larger than trimers (DP3) are not absorbed (Donavan *et al*., 2002)
- May still be beneficial to gastrointestinal (GI) health
  - Fermentation Products
  - Protection against GI disorders


0.5 g dried, ground pomace
Extract soluble polyphenolics
Collect residue
Add 5 mL NaOH (2, 4, 6 N)
Flush with N_2 and cap
Water bath with shaking 25, 40, 60°C for 5min – 24 h
Extract DP1 – DP3 with ethyl acetate
Extract all PC with hot H_2O or acetone:water:acetic acid

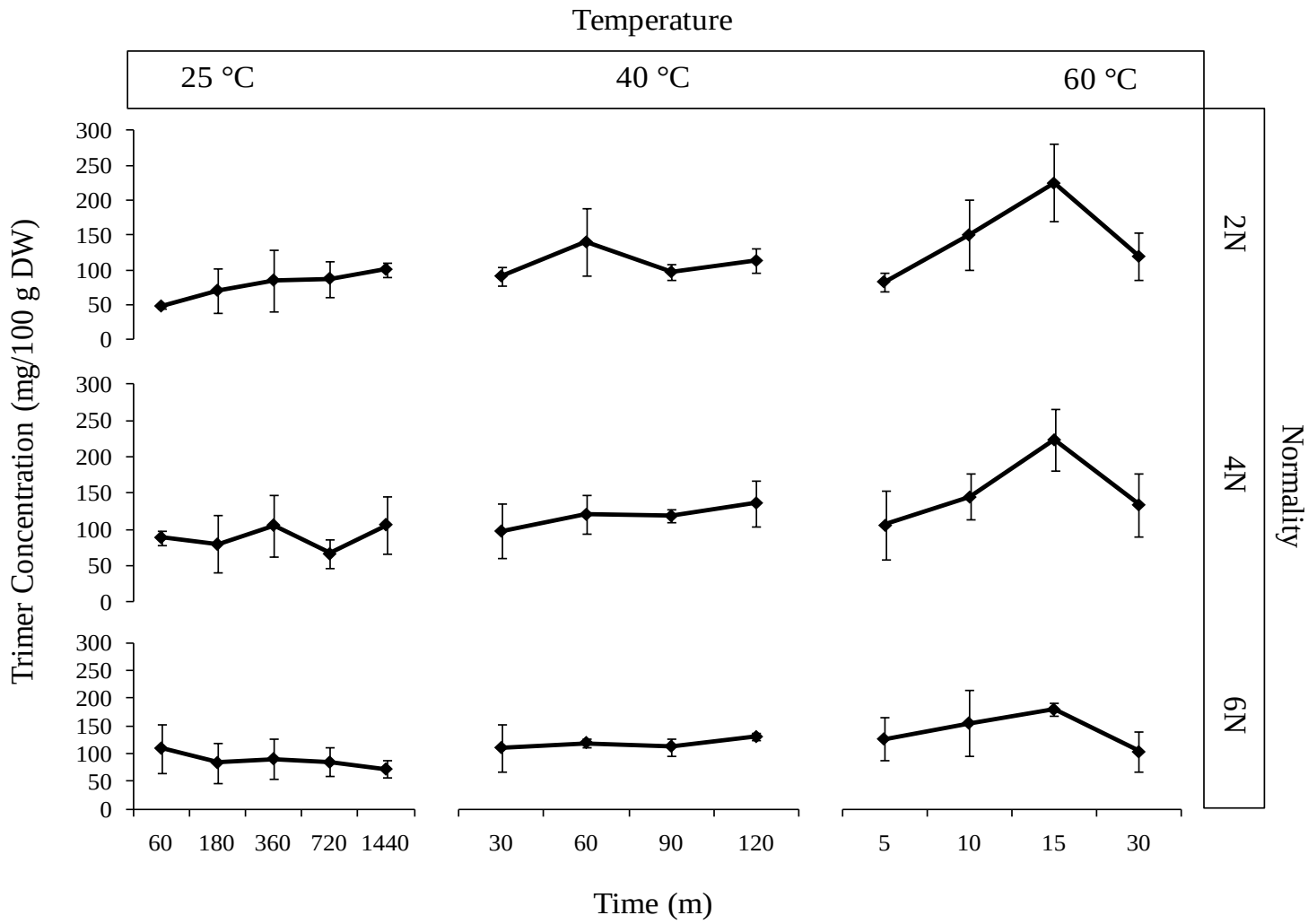
Changes in procyanidin monomer (DP1) content in cranberry pomace treated with sodium hydroxide



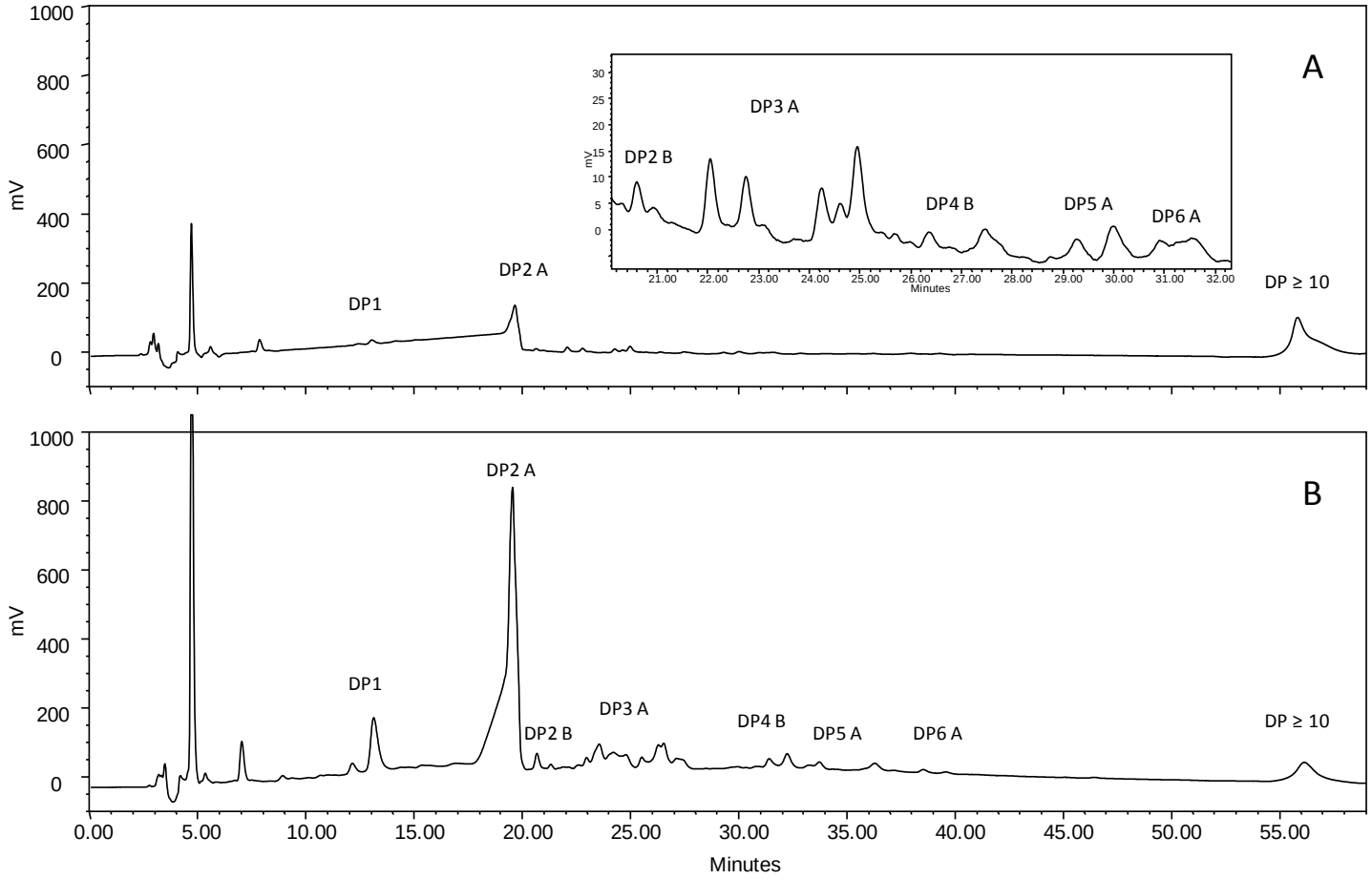
Changes in procyanidin dimer (DP2) content in cranberry pomace treated with sodium hydroxide



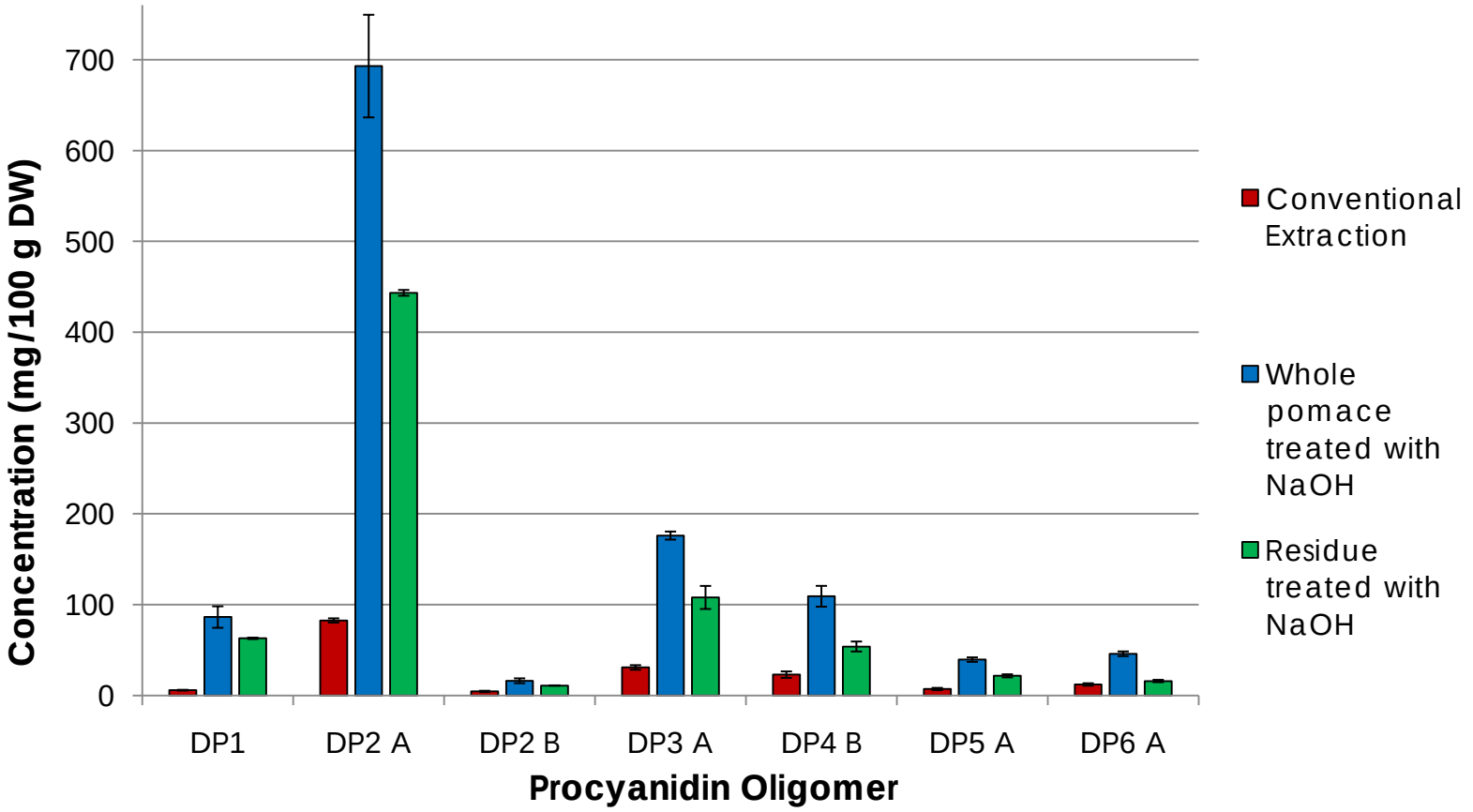
Changes in procyanidin trimer (DP3) content in cranberry pomace treated with sodium hydroxide



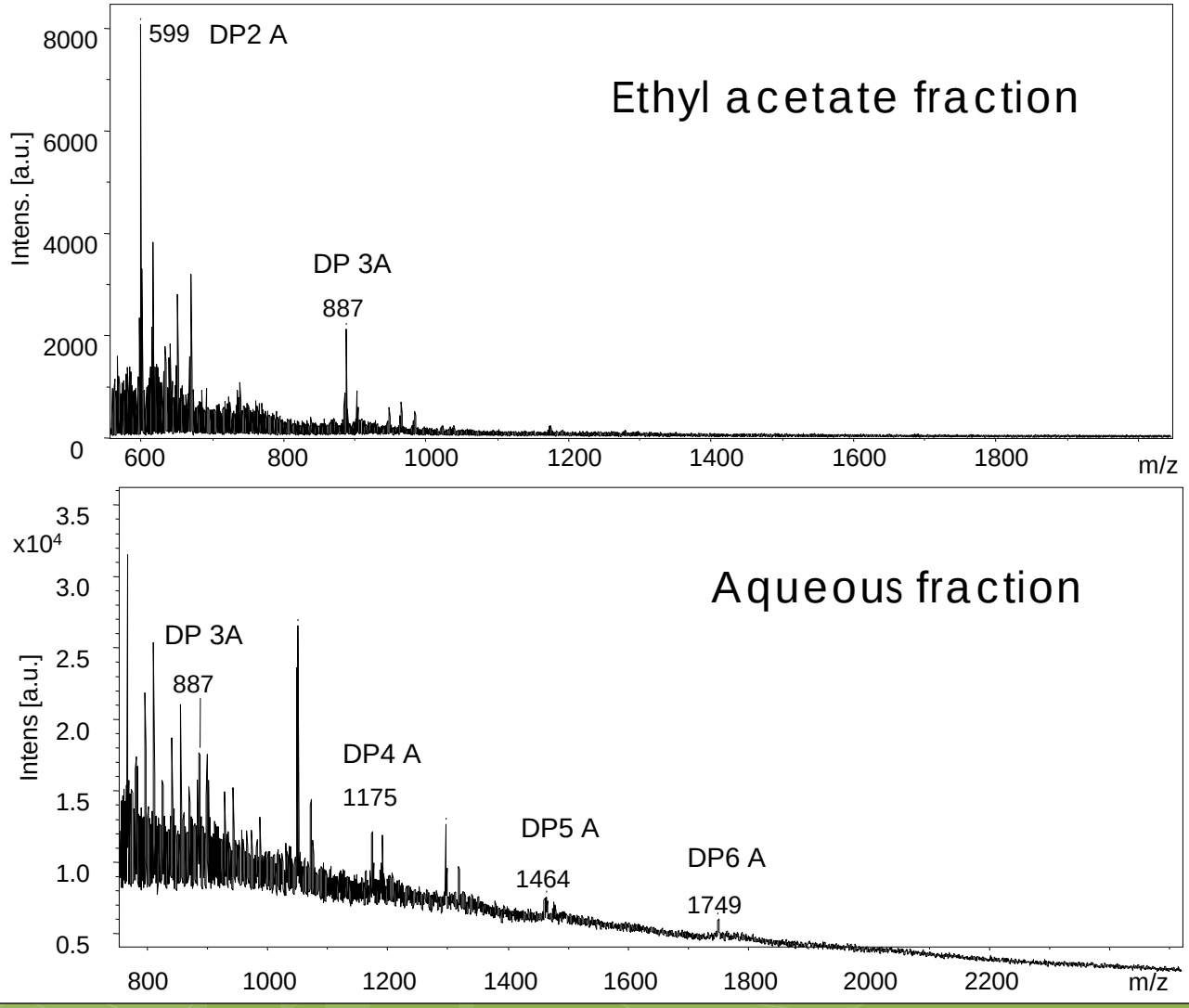
HPLC chromatograms of procyanidins in cranberry pomace before (A) and after (B) treatment with sodium hydroxide



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

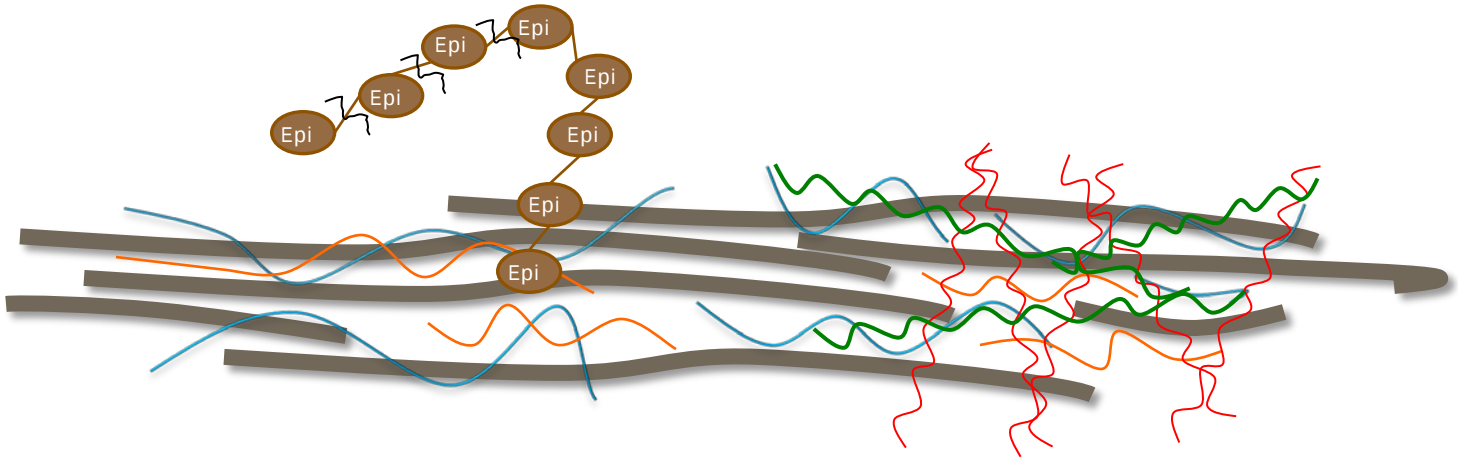


MALDI-TOF-MS

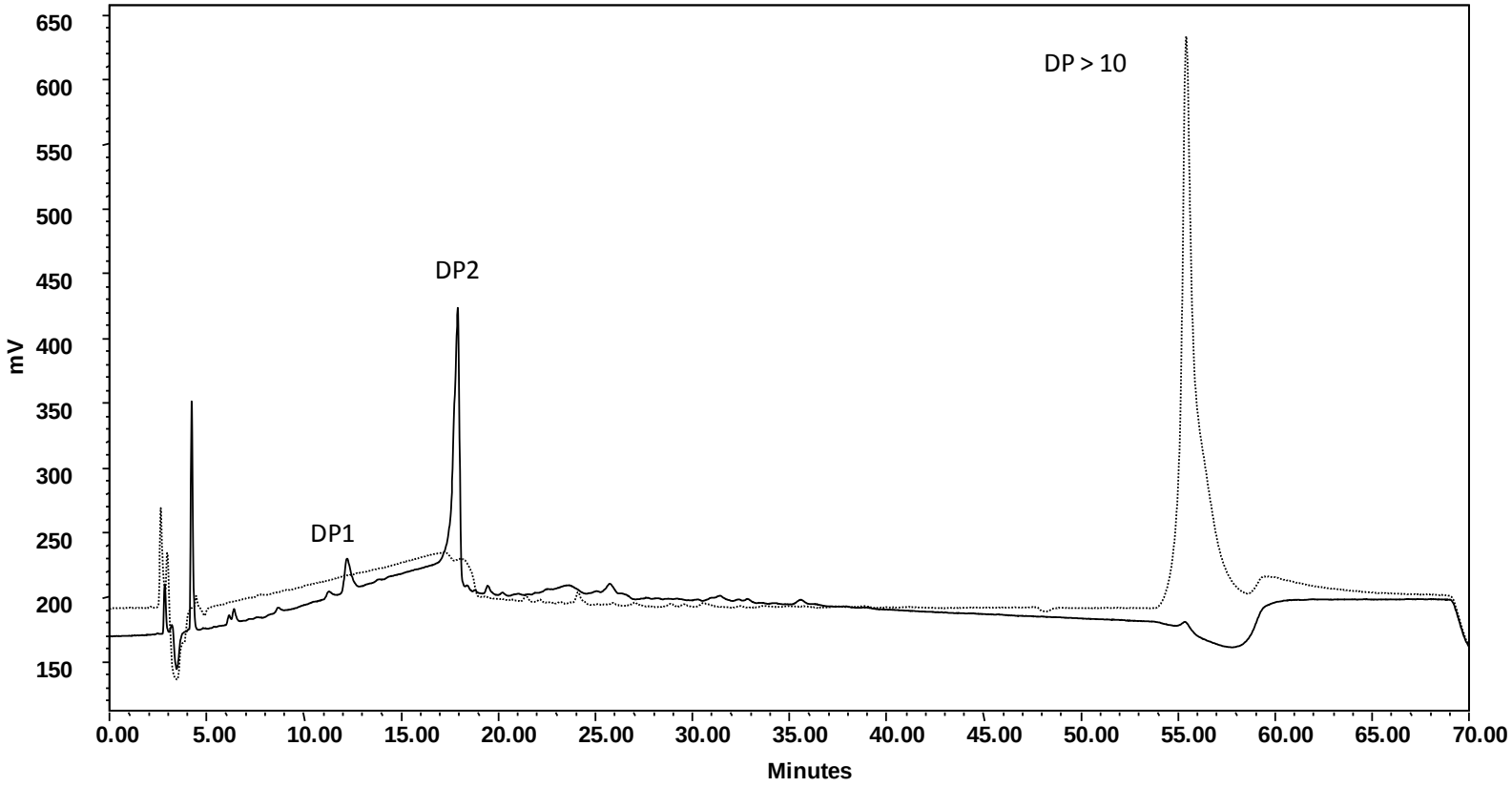

Ethyl acetate fraction
599 DP2 A
DP 3A
887
Intens. [a.u.]
8000
6000
4000
2000
0
600
800
1000
1200
1400
1600
1800
m/z
Aqueous fraction
x10^4
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Intens [a.u.]
DP 3A
887
DP4 A
1175
DP5 A
1464
DP6 A
1749
800
1000
1200
1400
1600
1800
2000
2200
m/z

Mechanism

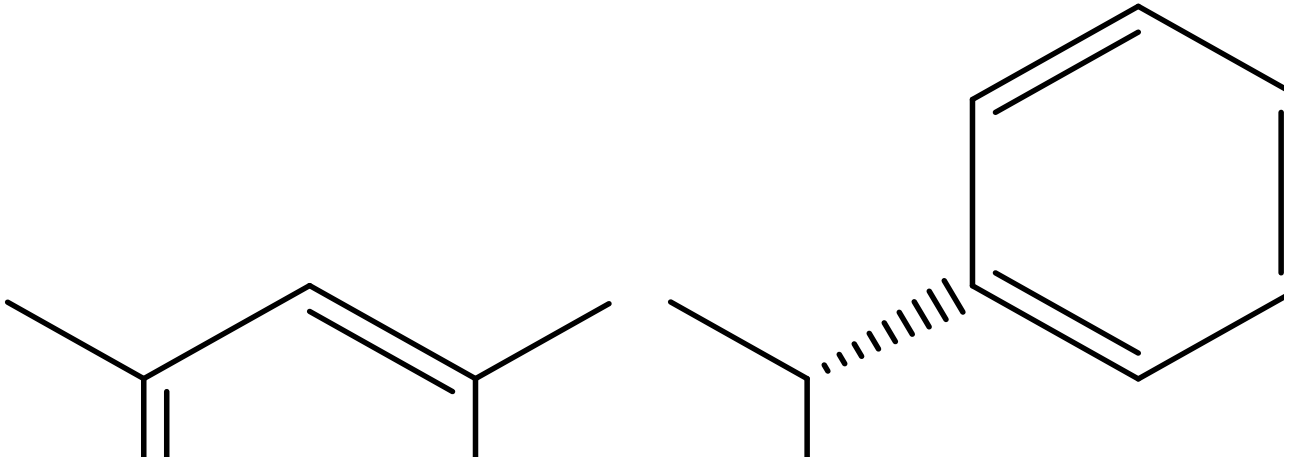
- Polymeric procyanidins bound to cell wall
- Depolymerization
- Solubilization of Cell Wall Material
  - Hemicellulose soluble in alkali



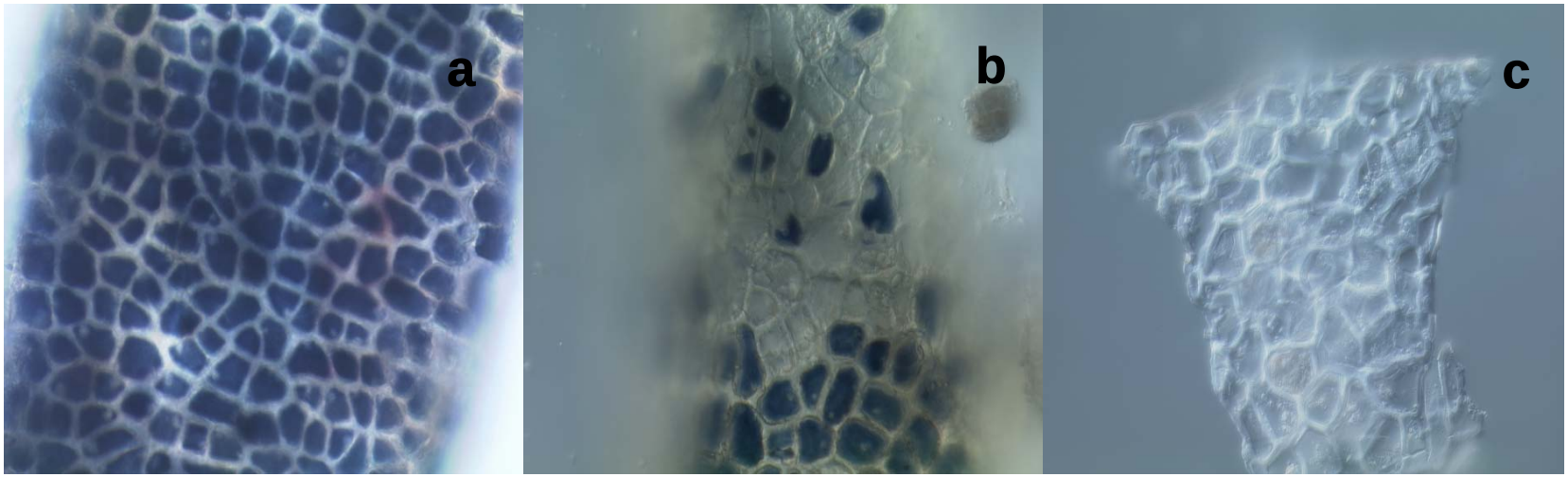
HPLC chromatograms of purified polymeric procyanidins from cranberry pomace before (dotted) and after Alkaline Hydrolysis (solid)



Depolymerization Mechanism



Light microscopy

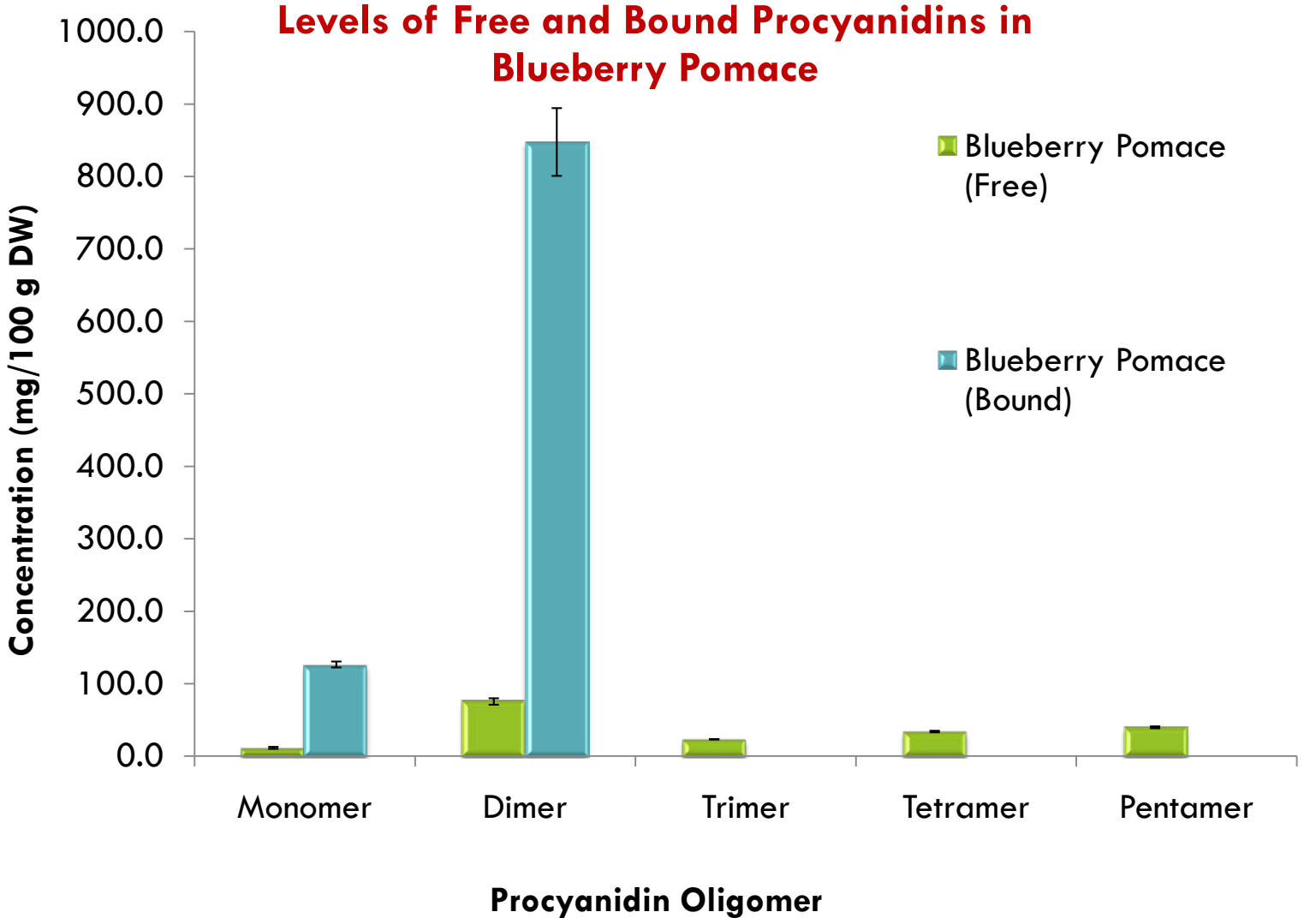


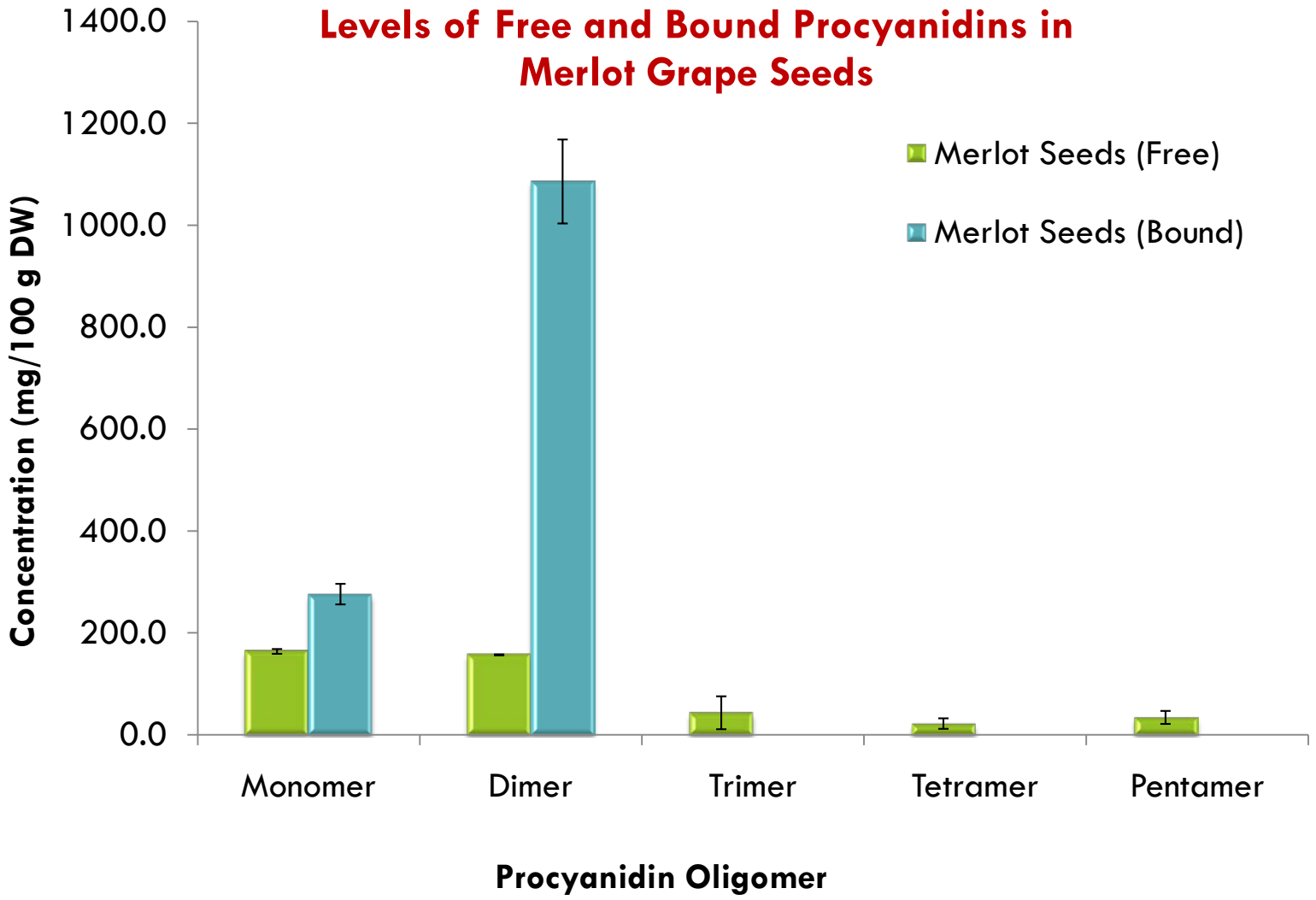
Differential interference contrast (DIC) microscopy images of:
(a) ground cranberry pomace
(b) ground cranberry pomace after conventional extraction
(c) ground cranberry pomace after alkaline hydrolysis.

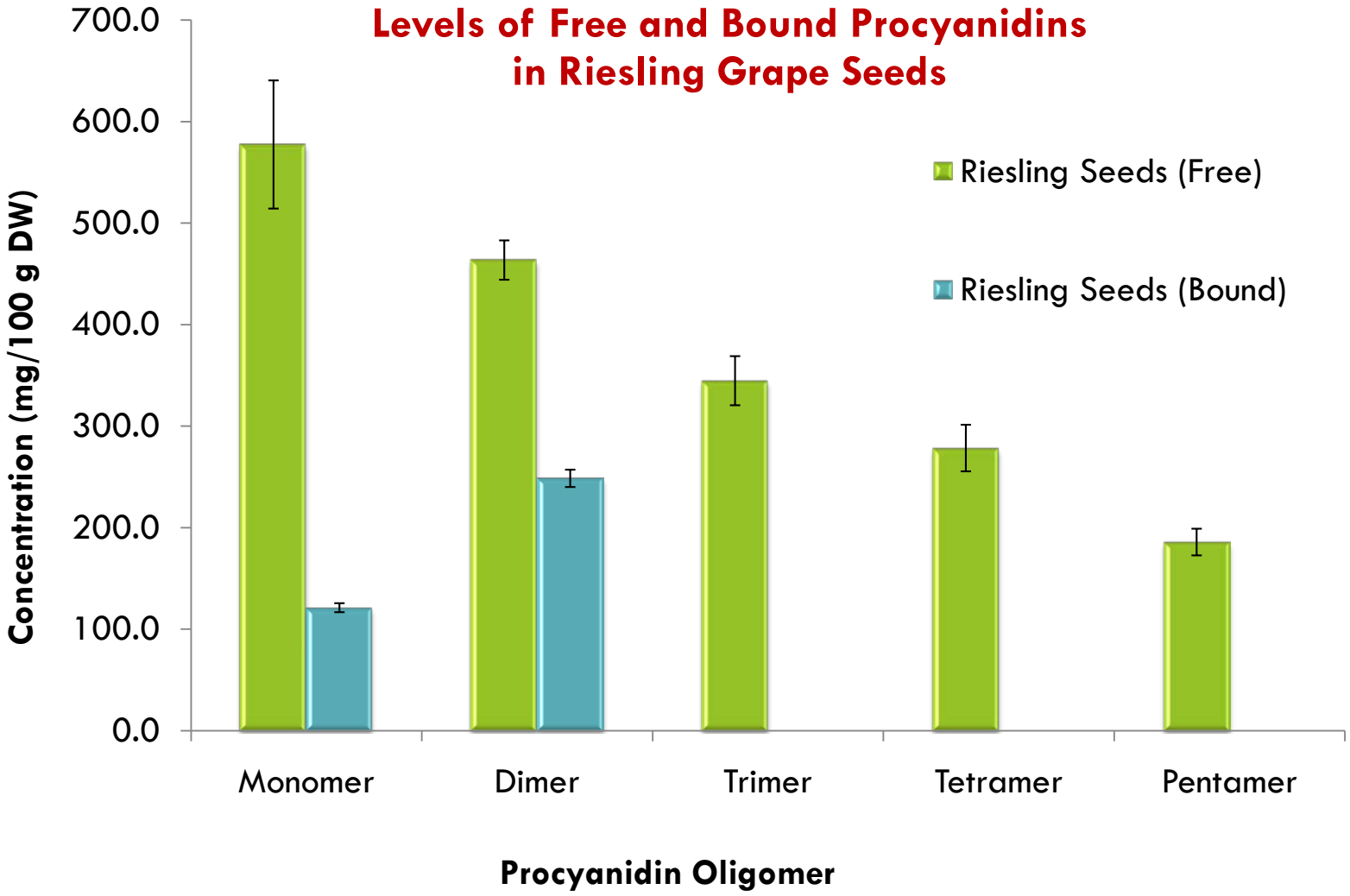
All were stained with dimethylaminocinnamaldehyde (DMACA)

Anti-adhesion

Anti-adhesion Properties of Cranberry Pomace		
Sample	Amount of Procyanidins (mg/00mg)[a]	% Anti-adherence [b]
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
[a] Procyanidins were obtained from 100 mg cranberry pomace		
[b] % Anti-adherence based on 0.8 mg/mL of whole pomace		


Levels of Free and Bound Procyanidins in Blueberry Pomace
1000.0
900.0
800.0
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
Concentration (mg/100 g DW)
Blueberry Pomace (Free)
Blueberry Pomace (Bound)
Monomer
Dimer
Trimer
Tetramer
Pentamer
Procyanidin Oligomer


Levels of Free and Bound Procyanidins in Merlot Grape Seeds
Concentration (mg/100 g DW)
1400.0
1200.0
1000.0
800.0
600.0
400.0
200.0
0.0
Merlot Seeds (Free)
Merlot Seeds (Bound)
Monomer
Dimer
Trimer
Tetramer
Pentamer
Procyanidin Oligomer


Levels of Free and Bound Procyanidins in Riesling Grape Seeds
Concentration (mg/100 g DW)
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
Riesling Seeds (Free)
Riesling Seeds (Bound)
Monomer
Dimer
Trimer
Tetramer
Pentamer
Procyanidin Oligomer

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