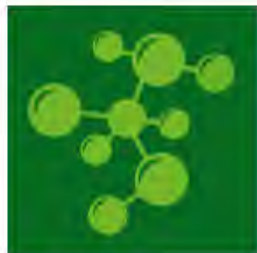




A green extraction method
for polyphenols from winter
grape pomace



biomolecules

IMPACT
FACTOR
4.694

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Natural Products Targeting Mitochondria

Guest Editor:

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Deadline for manuscript
submissions:

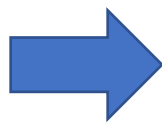
31 August 2020

Message from the Guest Editor

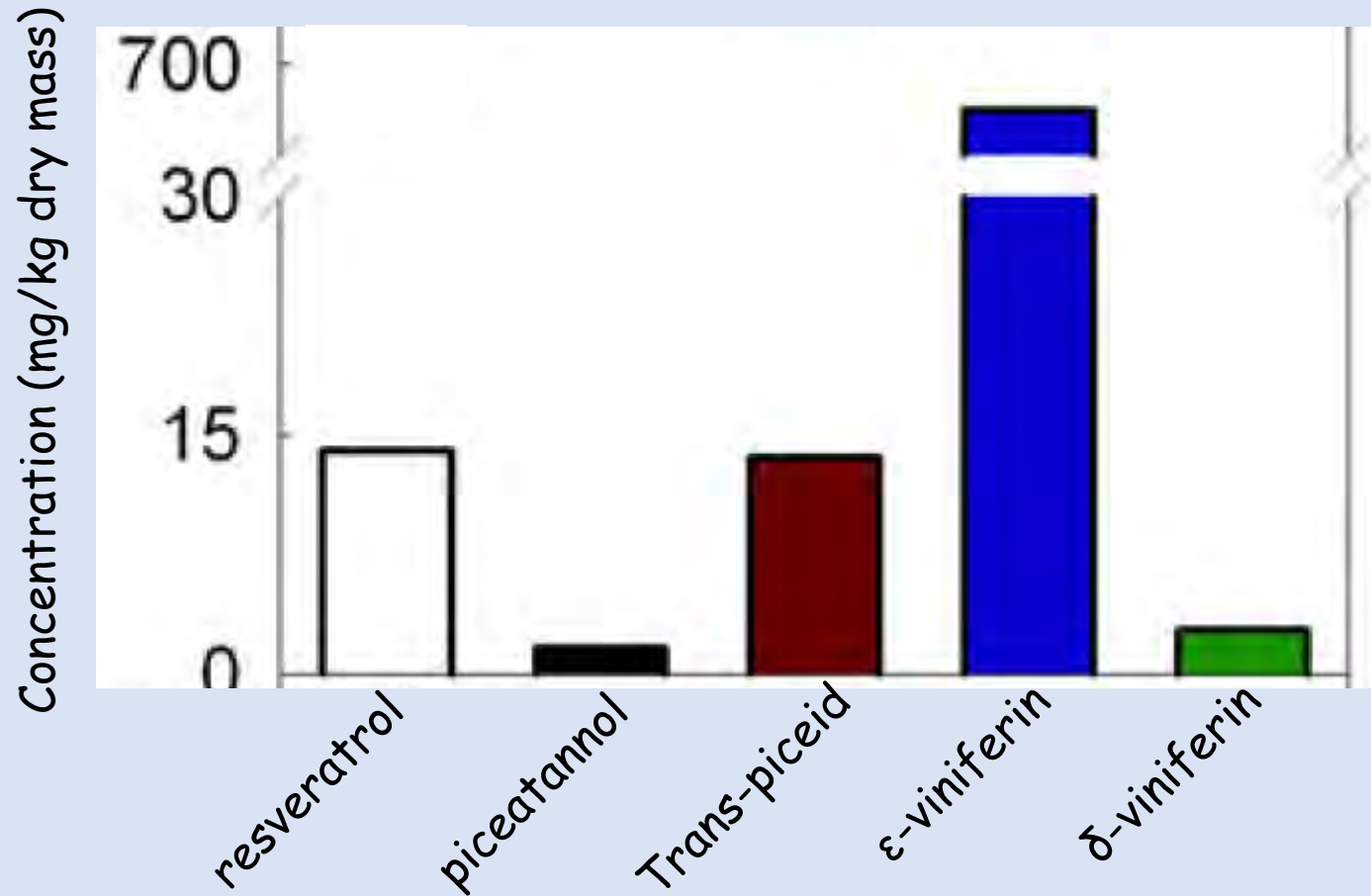
Beyond their role as ATP-generating ‘powerhouses’, mitochondria are central to the life of the cell at many levels, including their contribution to biosynthetic pathways, their participation in reactive oxygen metabolism, and their pivotal role in apoptosis. In this Special Issue, we focus specifically on natural products—those that are produced by, and can be isolated from, plants, microbes, and animals—that interact with mitochondria directly or via effects on gene expression.

Industrial partnership grant with Steve Murdza and Sweet & Sticky

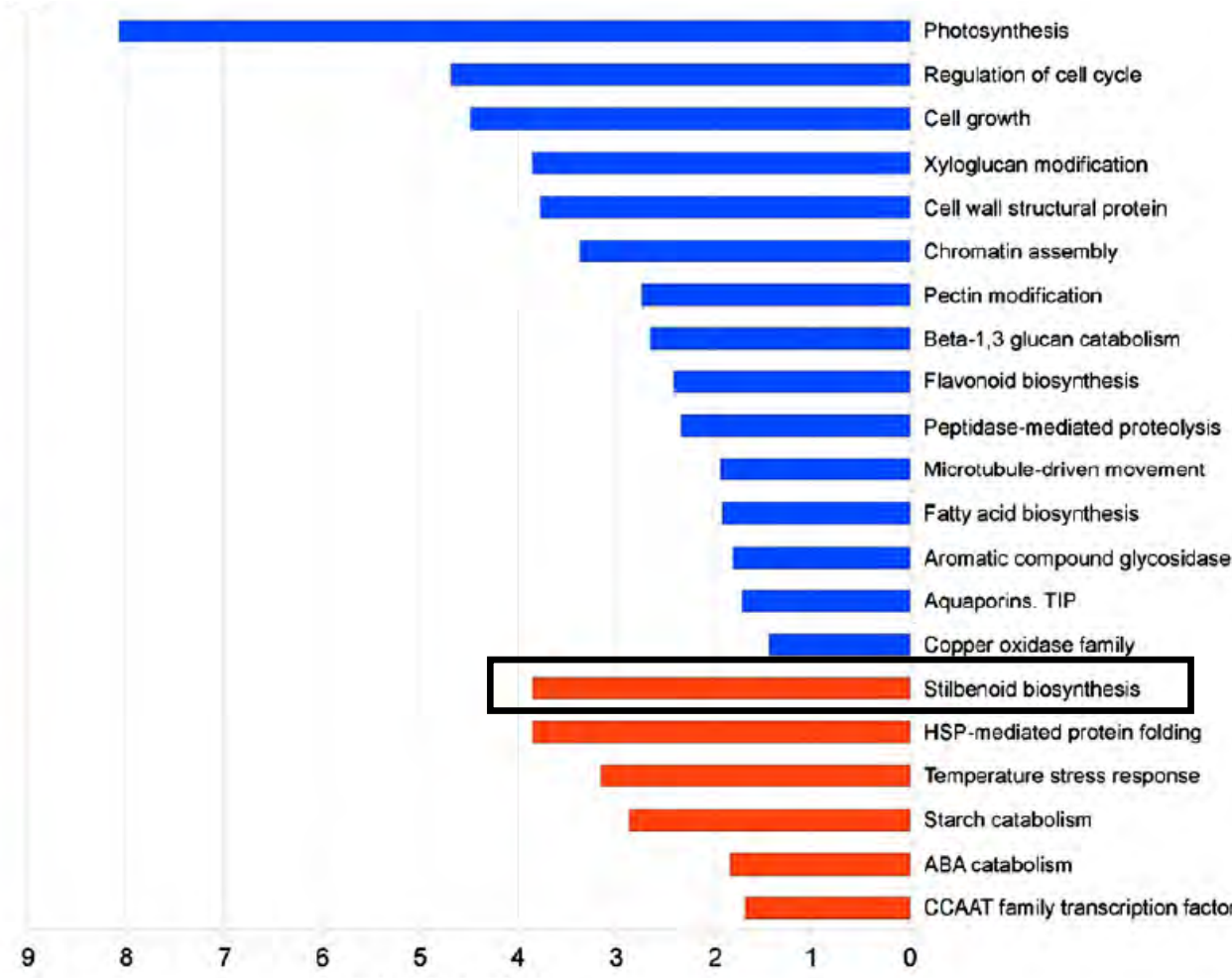
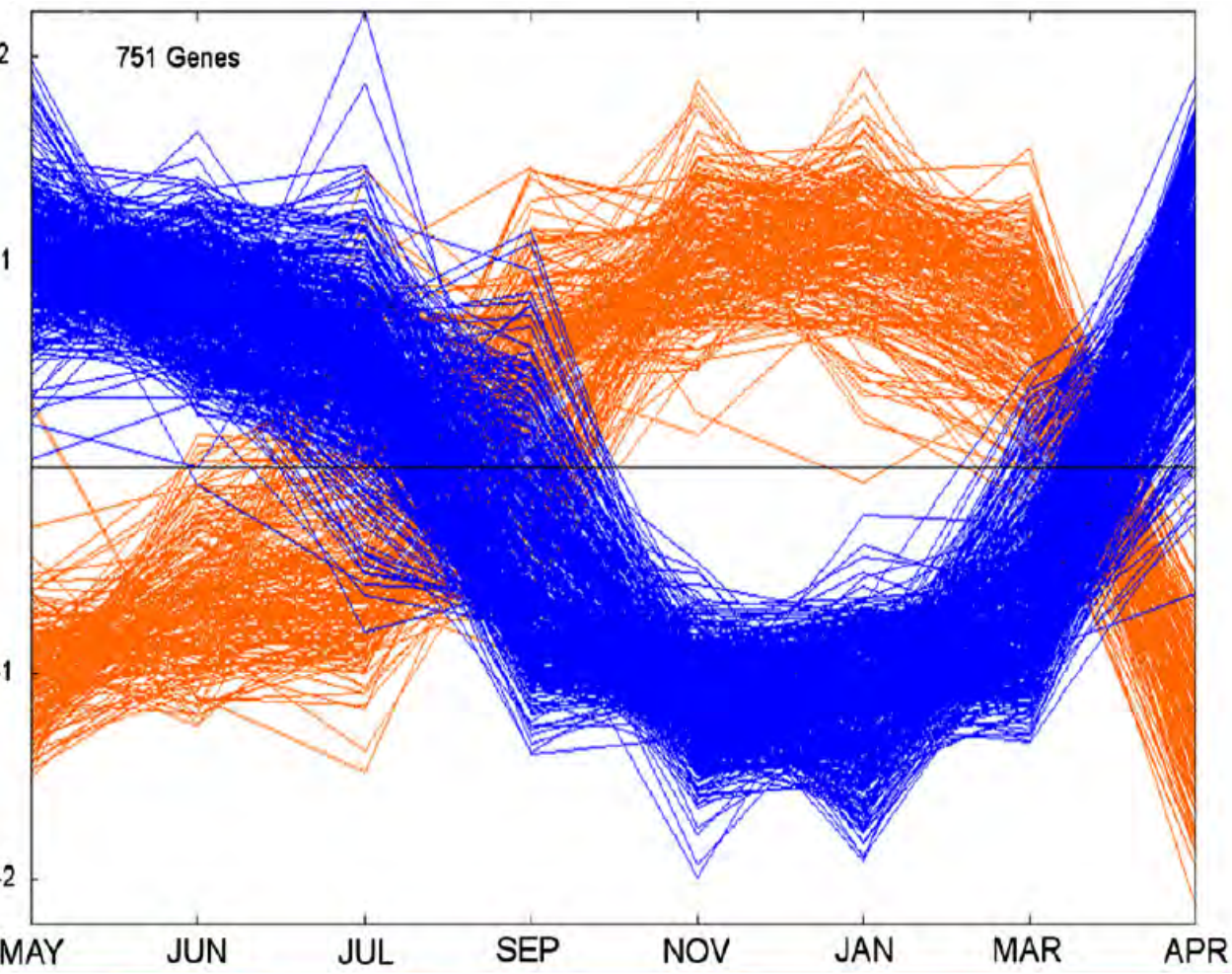




Grape pomace contains high levels of stilbenes

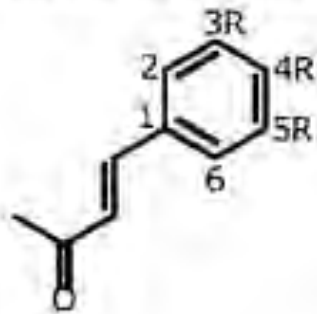


Increased stilbene synthesis gene expression in fall/winter

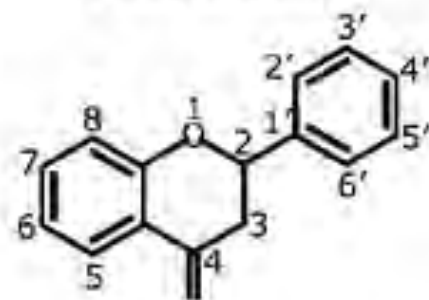


Polyphenols

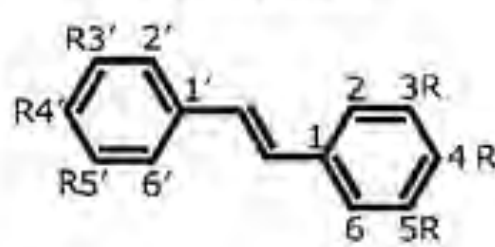
Phenolic acids



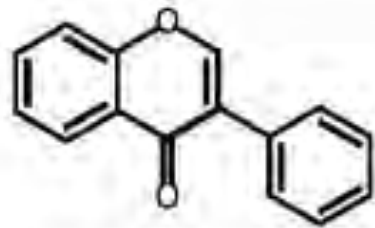
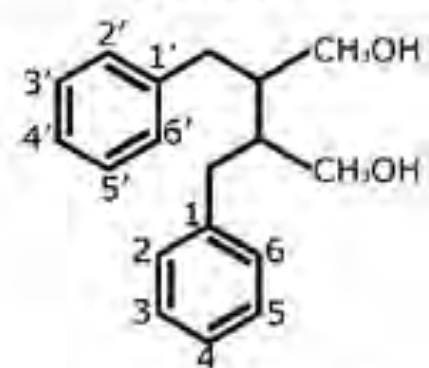
Flavonoids



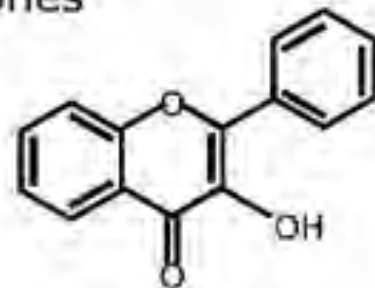
Stilbenes



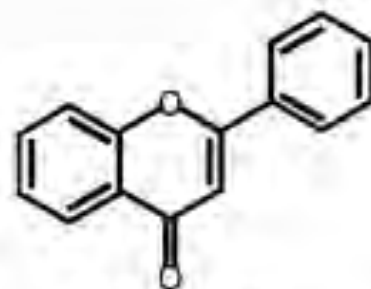
Lignans



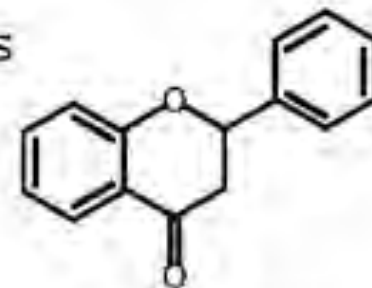
Isoflavones



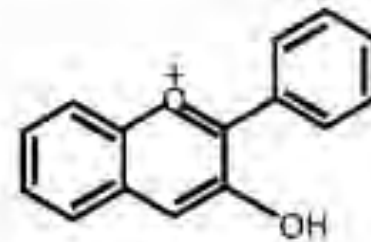
Flavonols



Flavones



Flavanones

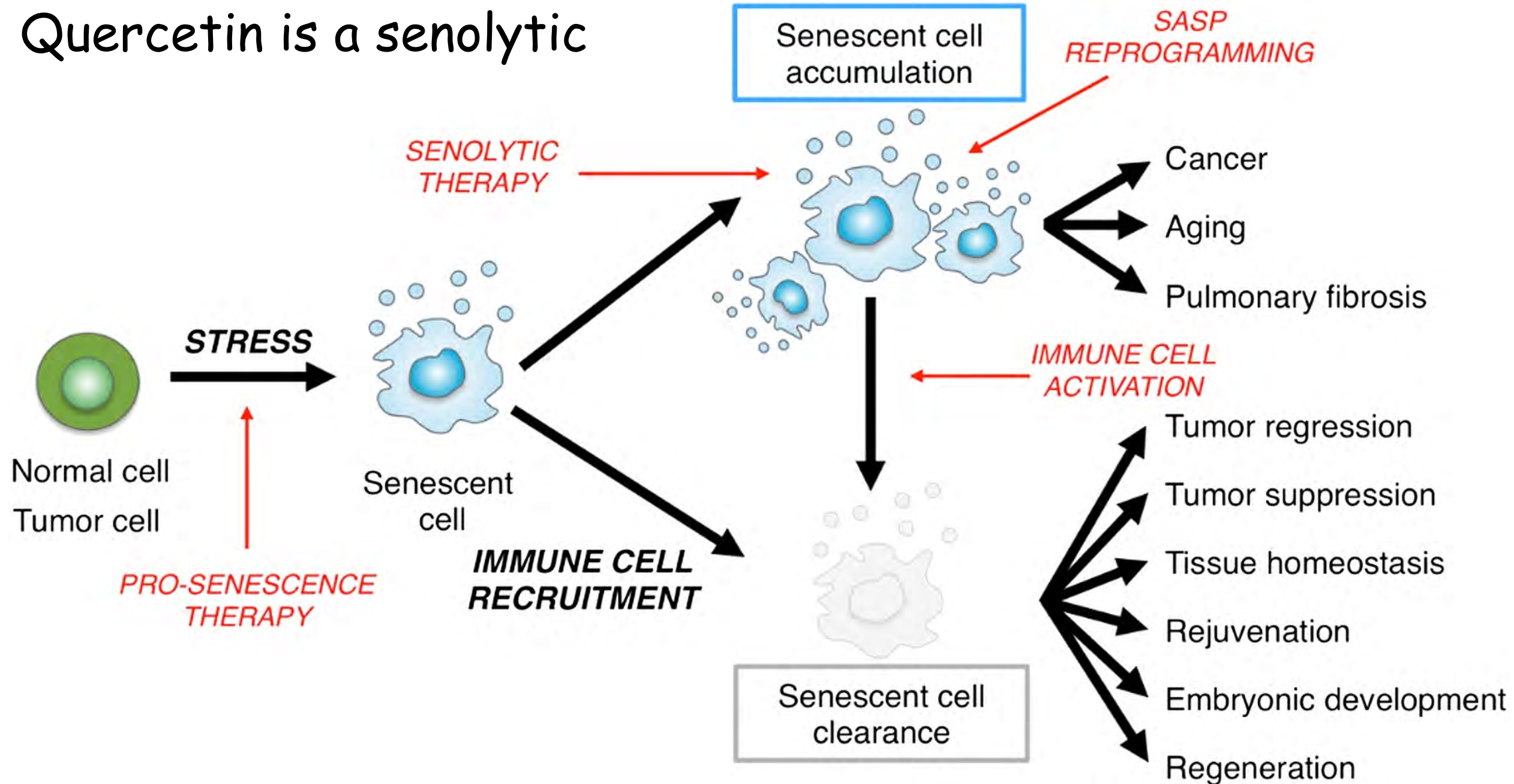


Anthocyanidines

Several grape pomace polyphenols are thought to have health benefits

Polyphenol	Cancer	Diabetes	Aging
Resveratrol	✓	✓	✓
Polydatin	✓	✓	
Delphinidin	✓	✓	
Kaempferol	✓	✓	
Quercetin	✓	✓	✓

Quercetin is a senolytic



Increase in research on 'polyphenols' published in PubMed indexed journals

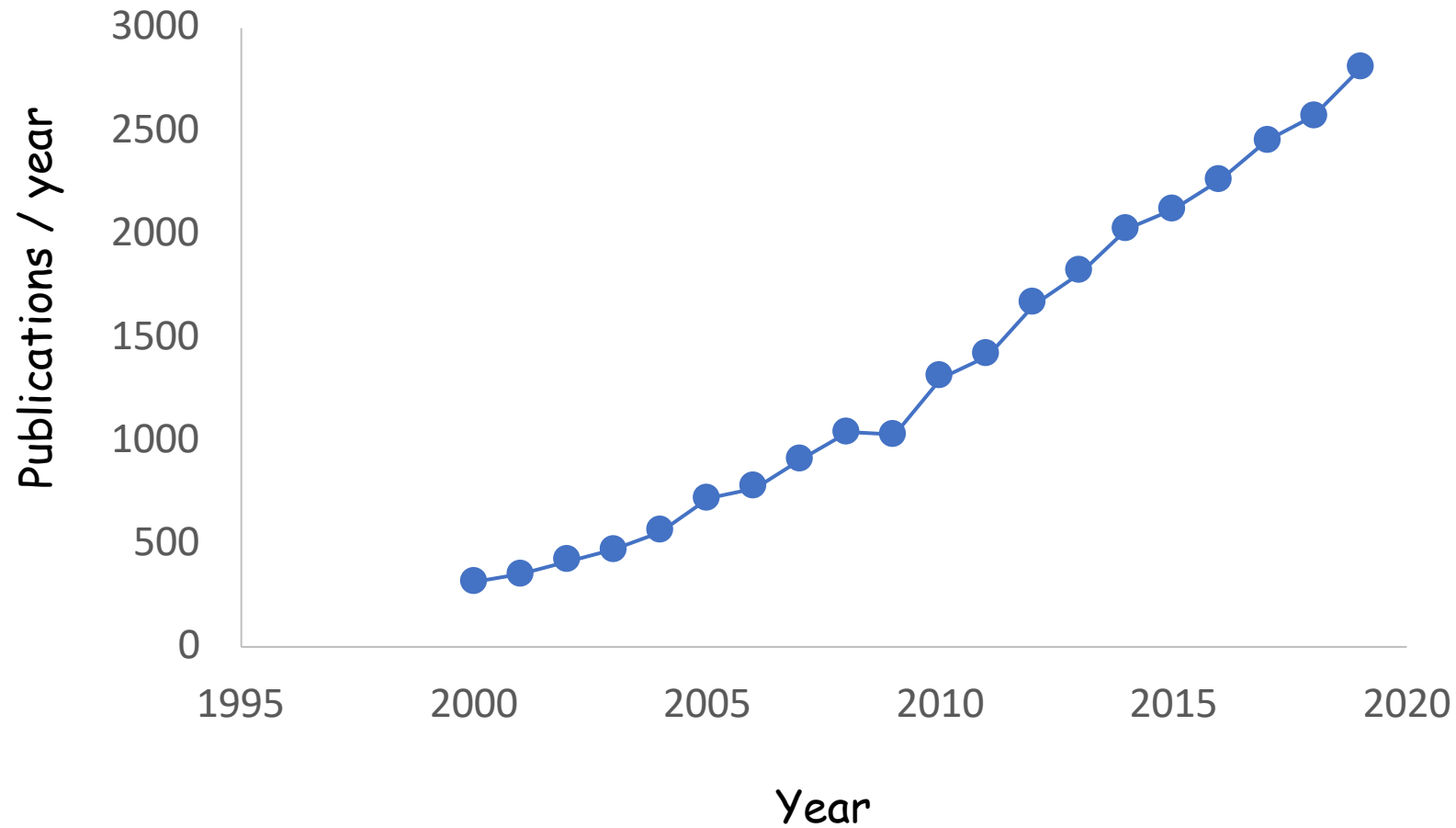


Table 12. Market potential of Ontario red pomace skins using Bioflavia as a reference

	2007	2008	2009	2010	2011
Grapes produced (t)	56315	60780	47595	53747	64495
Pomace produced(t)	14078.75	15195	11898.75	13436.75	16123.75
Red wine pomace (t)	5490.71	5926.05	4640.51	5240.33	6288.26
Red pomace skin (t)	4281.66	4621.13	3618.67	4086.41	4903.59
Market Potential (\$)	435,949,814.3	470,514,600.2	368,445,909.8	416,070,224.1	499,273,431.1


How to 'extract' value from waste pomace?

How to 'extract' value from waste pomace?

- Extract polyphenols in edible form
- Low solubility in water
- Avoid costly organic solvents
- Minimize investment in infrastructure

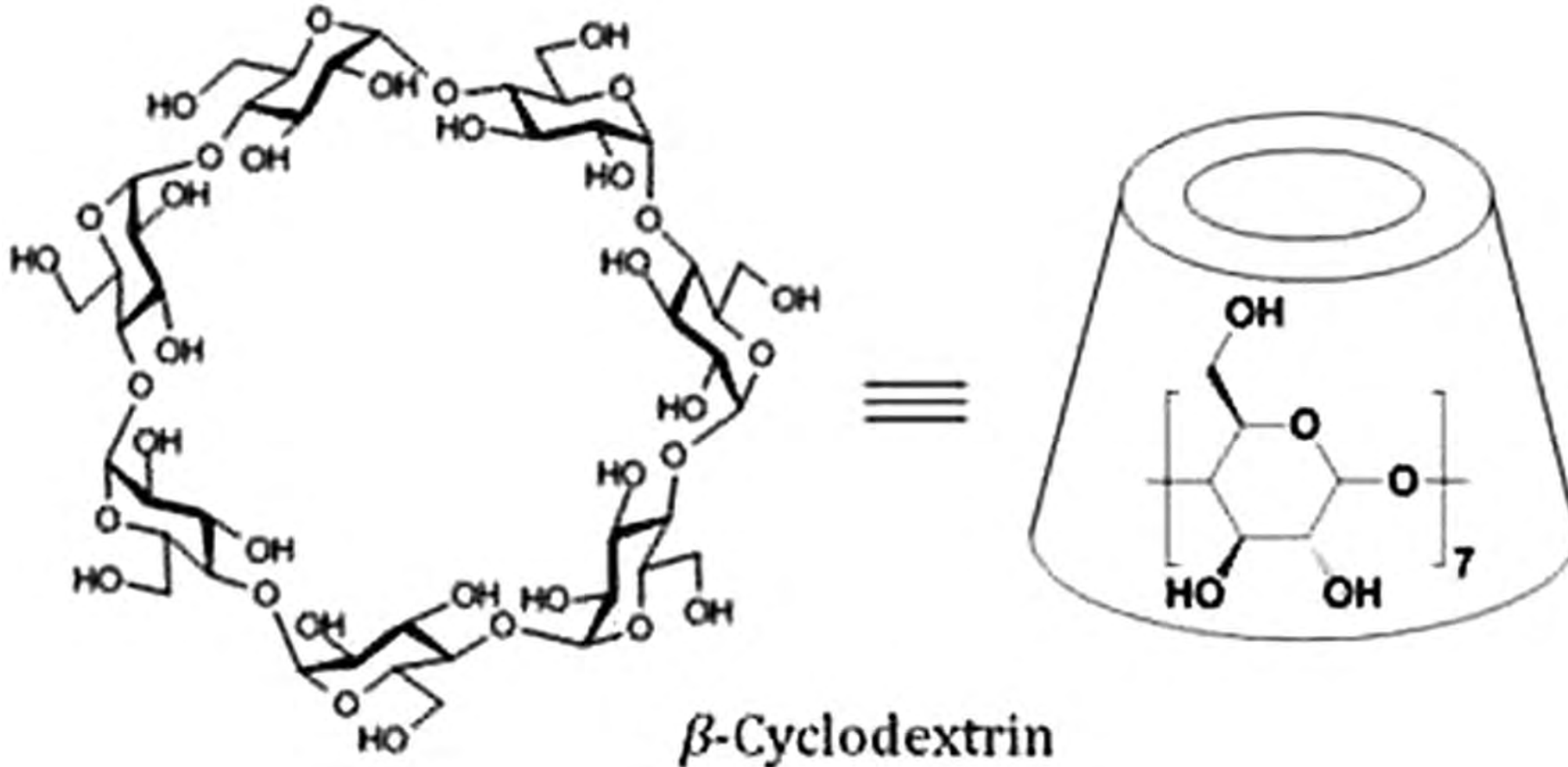
Journal of Food Measurement and Characterization

A limited metabolomics analysis validates sonication-assisted extraction of Ice Wine grape pomace polyphenols and demonstrates their seasonal variation

Jeffrey A. Stuart¹  · Shehab Selim¹ · Sam McGowan¹

Beta-cyclodextrin:

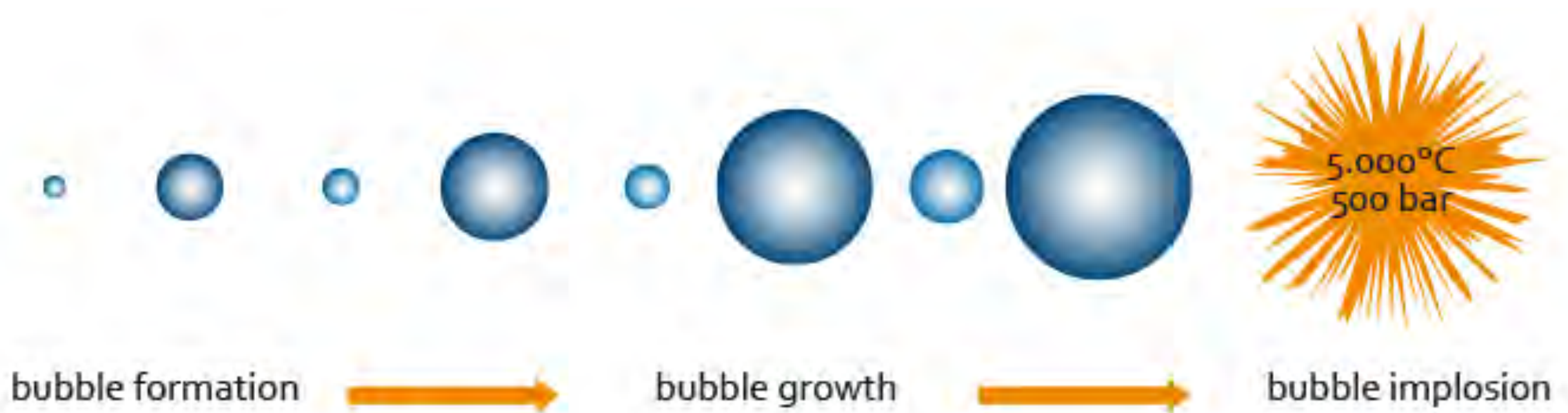
- Very water soluble (up to 1.8% mass/volume) in water
- Hydrophobic core accepts hydrophobic molecules like polyphenols



Beta-cyclodextrin:

- Edible (Generally Recognized as Safe; GRAS)
- Digested by bacteria in large intestine
- Cargo molecules protected during transit in upper GI tract

Ultrasonic cavitation



Sonication method development

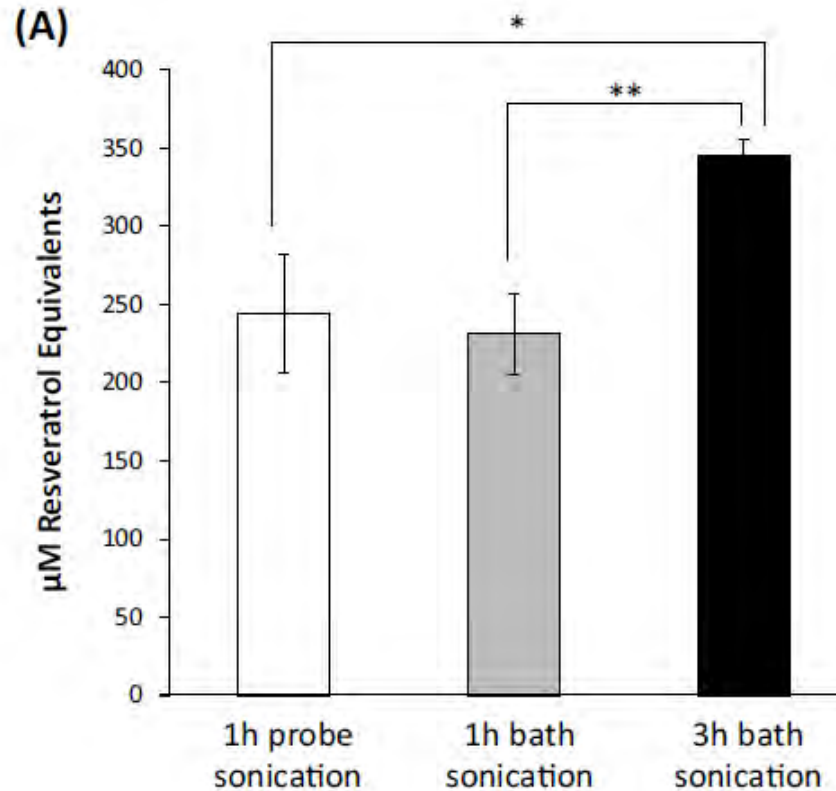


VS

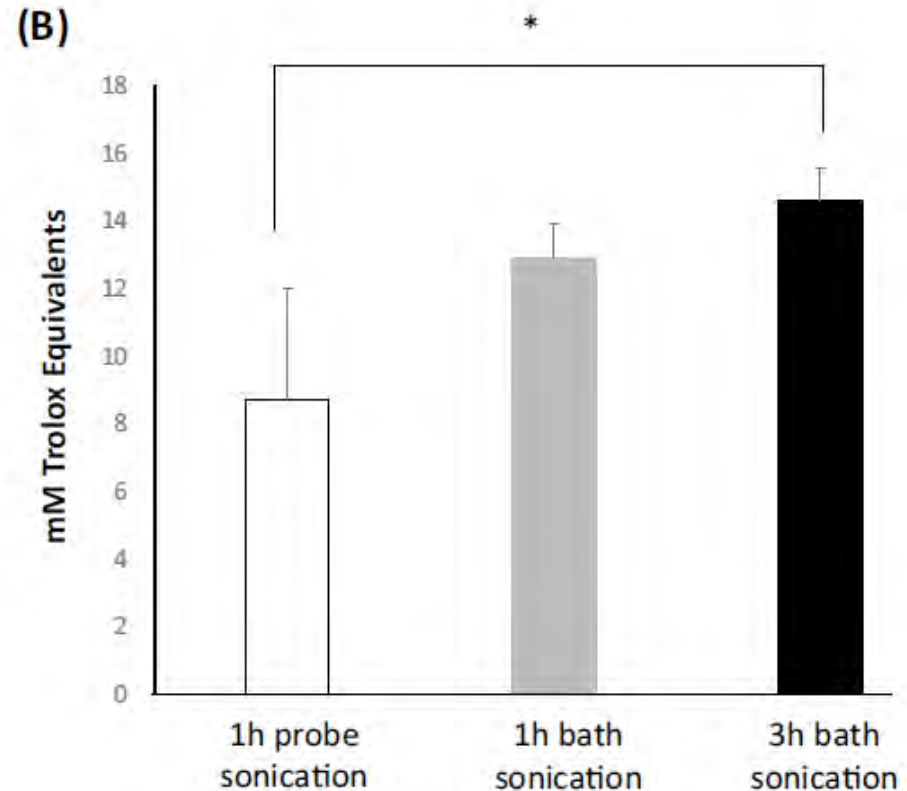


Probe and bath sonication equally capable of extracting (apparent) polyphenols

Folin & Ciocalteu's Phenol Reagent (FCR) assay



Oxygen Radical Absorbance Capacity (ORAC) assay



Grape collection:

1. Cabernet Franc and Vidal grapes collected in:

November, December, January of 2015, 2016, 2017

2. Pressed and pomace stored in -80°C freezer for up to three years until processing

Extraction Method:

1. Pomace thawed and food-processed to mince
2. Washed to remove water-soluble molecules
3. Centrifuged to pellet material (supernatant discarded)
4. Ultrasound-assisted extraction into a 1.5% aqueous solution of beta-cyclodextrin

Extraction Method:

5. Centrifuged and remaining solid material discarded
6. Freeze-dry product
7. Send to 'The Metabolomic Innovation Centre (TMIC)' at University of Alberta for analysis



Table 2 Polyphenol concentrations in aqueous BCD extracts from pomace of Vidal grapes harvested in November 2015

Polyphenol	Yield (µg/mL extract)
Gallic acid	7.717 ±0.515
Protocatechuic acid	ND
Catechin	33.727 ±4.084
Chlorogenic acid	6.997 ±0.490
Caffeic acid	43.197 ±3.991
Vanillic acid	ND
Syringic acid	ND
Coumaric acid	ND
Benzoic acid	ND
Ferulic acid	ND
Sinapic acid	2.948 ±0.103
Kaempferol	ND
Resveratrol	ND
Dihydrobenzoic acid	ND
Isorhamnetin	ND
Apigenin	ND
Luteolin	ND
Rutin	15.230 ± 1.008
Myricetin	ND
Quercetin	ND

Limited polyphenolics
profile in white wine
grape extracts

Polyphenol	November
Gallic acid	1.48 ± 0.15
Protocatechuic acid	1.67 ± 0.26
Chlorogenic acid	4.27 ± 0.58
Vanillic acid	3.87 ± 0.48
Syringic acid	3.67 ± 0.11
Benzoic acid	5.25 ± 0.50
Coumaric acid	2.87 ± 0.50
Caffeic acid	6.83 ± 1.02
Ferulic acid	1.54 ± 0.22
Sinapic acid	2.85 ± 0.34
Resveratrol	3.18 ± 0.54
Polydatin	ND
Pterostilbene	ND
Kaempferol	ND
Catechin	10.06 ± 1.19
Epicatechin	ND
Rutin	8.71 ± 0.82
Quercetin	4.06 ± 0.25
Delphinidin	11.78 ± 3.94

Reasonable yield of polyphenols
from red wine grapes

Table 4 Polyphenol levels of January extracts compared to a typical red wine.

Polyphenol	Amount (mg) in 175 mL red wine ^a	Mass of extract required for equivalent amount of polyphenol (mg)
Gallic acid	6.28	3670
Protocatechuic acid	0.30	270
Chlorogenic acid	7.14	760
Vanillic acid	0.56	129
Syringic acid	0.47	104
Benzoic acid	NA	
Coumaric acid	0.96	1655
Caffeic acid	3.29	404
Ferulic acid	0.14	1000
Sinapic acid	0.12	71
Resveratrol	0.47	326
Polydatin	1.09	452
Catechin	11.92	1145
Rutin	1.42	840
Quercetin	1.45	502
Delphinidin	0.19	8

<http://phenol-explorer.eu/>

Polyphenol	November	December	January
Gallic acid	1.48 ± 0.15	1.12 ± 0.11	1.71 ± 0.12
Protocatechuic acid	1.67 ± 0.26	0.15 ± 0.02 ^a	1.11 ± 0.22 ^b
Chlorogenic acid	4.27 ± 0.58	18.62 ± 2.25	9.40 ± 0.24 ^{a,b}
Vanillic acid	3.87 ± 0.48	4.06 ± 0.17	4.35 ± 0.27
Syringic acid	3.67 ± 0.11	4.75 ± 0.08 ^a	4.54 ± 0.30 ^a
Benzoic acid	5.25 ± 0.50	4.55 ± 0.17	1.98 ± 0.09 ^b
Coumaric acid	2.87 ± 0.50	2.21 ± 0.24	0.58 ± 0.06 ^{a,b}
Caffeic acid	6.83 ± 1.02	6.73 ± 0.26	8.14 ± 0.25 ^b
Ferulic acid	1.54 ± 0.22	1.28 ± 0.52	0.14 ± 0.01 ^{a,b}
Sinapic acid	2.85 ± 0.34	2.74 ± 0.28	1.70 ± 0.20 ^{a,b}
Resveratrol	3.18 ± 0.54	1.30 ± 0.11 ^a	1.44 ± 0.08 ^a
Polydatin	ND	ND	2.41 ± 0.18
Pterostilbene	ND	ND	ND
Kaempferol	ND	ND	ND
Catechin	10.06 ± 1.19	8.41 ± 0.40	10.41 ± 1.05
Epicatechin	ND	ND	ND
Rutin	8.71 ± 0.82	0.16 ± 0.02 ^a	1.69 ± 0.40 ^{a,b}
Quercetin	4.06 ± 0.25	4.10 ± 0.12	2.89 ± 0.09 ^{a,b}
Delphinidin	11.78 ± 3.94	23.76 ± 0.51 ^a	24.66 ± 1.75 ^a

Time of year changes the polyphenol profile of Cabernet Franc pomace extracts

Averages ± SEM of 3 years

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Gallic acid	1.48 ± 0.15	1.12 ± 0.11	1.71 ± 0.12
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Conclusions:

Ultrasound-assisted extraction of red wine grape pomace into an aqueous BCD solution produces an edible product with appreciable polyphenol yield

Some polyphenols present at levels comparable to red wine

Key polyphenols of interest (e.g. resveratrol, polydatin, quercetin) are present in winter-harvested Cabernet Franc grapes through to at least January

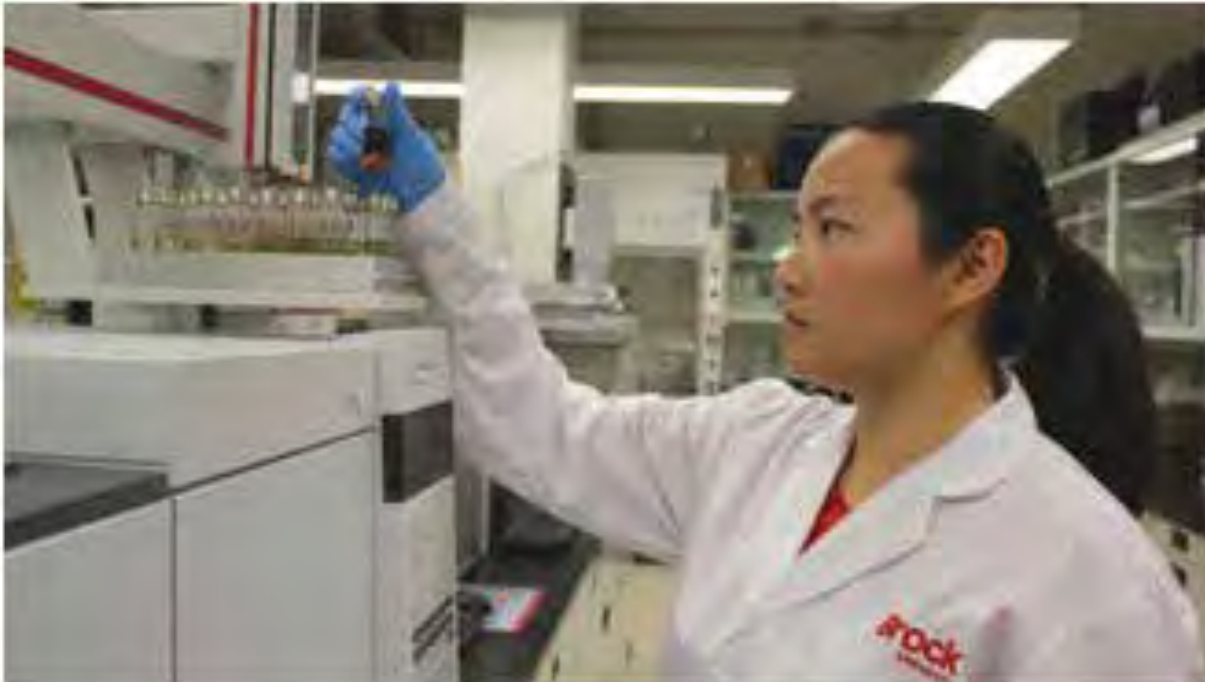
Next steps (Funded by recent FedDev grant awarded to Brock):

1. Increase efficiency of sonication-assisted extraction using high-volume temperature-controlled flow-through probe sonication



Next steps (Funded by recent FedDev grant awarded to Brock):

2. Perform metabolomics here at CCOVI using new mass spec instruments



Acknowledgements:

- Elaine Corbett and Dennis McCormick - Ontario Genomics
- Rupasri Mandal and Jen Reid - TMIC (The Metabolomics Innovation Centre)



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BSc Biotechnology



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Medical School
U of Ottawa