An update on the quantification of flavonoids in different soft fruits using hyphenated mass spectrometry

Future-proofing berryfruit CLIMAFRUIT

The Interreg IVB North Sea Region Programme

The James Hutton Institute

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Chemical Analysis

- **Separation**: HPLC/ UPLC – Reversed Phase Chromatography (long vs. short gradient): A= 0.1% FA; B=50:50:0.1% ACN:H_2O:FA

- Optimization of transitions for different compound ions, using different fragmentor voltages and collision energies → MRM Method

Compound optimisation: RB Standards

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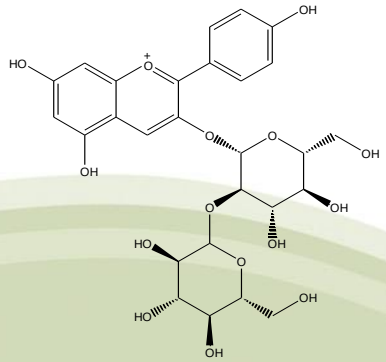
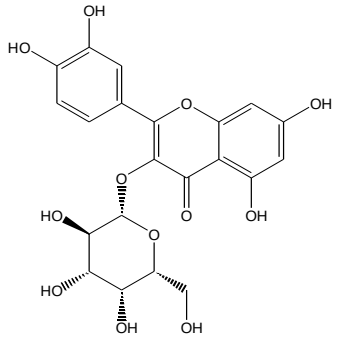
Table 1 Optimized compound parameters used in Multiple Reaction Monitoring (MRM) mode for 11 compounds

Compound Name	Type	Precursor Ion	Product Ion	Dwell	Fragmentor	Collision Energy	Cell Accel. Voltage	Polarity
Cyanin	Standard	611.2	287	10	166	38	7	Positive
Cyanidin-3-O-sophoroside	Standard	611.2	287	10	132	34	7	Positive
Cyanidin-3-O-rutinoside	Standard	595.2	287	10	166	34	7	Positive
Cyanidin-3-O-sambubioside*	Standard	581.2	287	10	132	38	7	Positive
Cyanidin 3-glucoside	Standard	449.1	287	10	132	60	7	Positive
Hyperoside	Standard	463.1	300	10	166	26	7	Negative
Quercetin-3-O-glucuronide	Standard	477.1	301	10	132	30	7	Negative
Morin	Standard	301	125	10	138	15	7	Negative
Cyanidin 3-(2glucosyl)rutinoside	*Std equivalent	757.2	287	10	166	40	7	Positive
Cyanidin 3-(2-xylosyl)rutinoside	*Std equivalent	727.2	287	10	200	60	7	Positive
Pelargonidin 3-(glucosyl)rutinoside	*Std equivalent	741.2	271	10	200	60	7	Positive
Pelargonidin 3-sophoroside	*Std equivalent	595.2	271	10	166	60	7	Positive



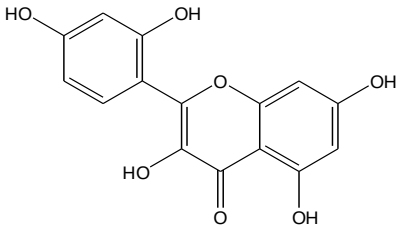
Pelargonidin-3-O-Sophoroside



Hyperoside

Quality Control

- *Rubus idaeus* ‘Glen Ample’, *Rubus fruticosus* ‘Karaka Black’, *Ribes nigrum* ‘Ben Alder’
- Extraction Efficiency – 3 Extraction Replicates
- Instrument Stability – 3 Injection Replicates
- Instrument Stability – Internal Standard Morin
- Randomisation of samples



Colourimetric Experiment

- Five different maturation level of *Rubus idaeus* ‘Glen Ample’
- Storage: Fresh vs Freezer (1 week)
- 1 Sample per treatment
- How do absolute amounts of organic acids, sugars & flavonoids correlate within different maturation levels and storage conditions?
- How are quality traits affected by storage?

Results: Correlation Matrix

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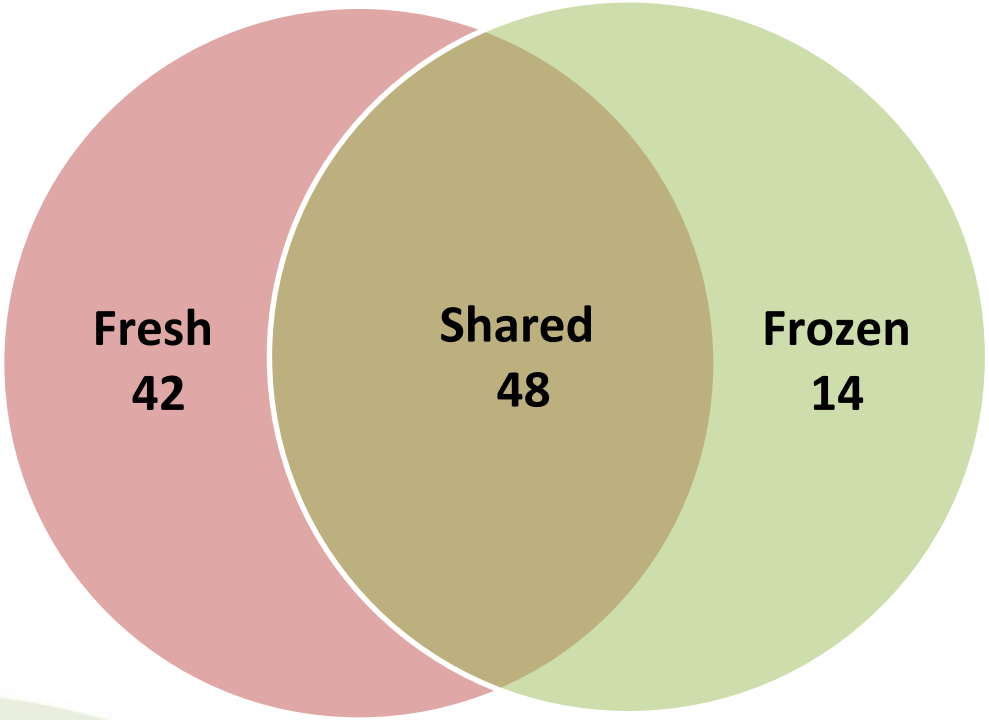
	Fresh	OA		Sugars			Anthocyanins									Fl		
		Citrate	Malate	Frc	Glc	Suc	Cyanin	Cy-3-O-Soph	Cy-3-O-Samb	Cy-Gluc-Rut	Cy-Gluc	Pelar-Soph	Cyan Xyl Rut	Pelar Gluc Rut	Cyan Rut	Hyperoside	Querc Gluc	
OA	Citrate		0.88	0.20	0.04	-0.07	-0.58	-0.25	-0.40	-0.58	-0.33	-0.55	-0.61	-0.54	-0.47	0.29	-0.19	-1.00
	Malate	0.88		-0.22	-0.41	-0.08	-0.75	-0.49	-0.70	-0.87	-0.70	-0.81	-0.88	-0.85	-0.81	0.07	-0.33	-0.75
Sugars	Frc	0.20	-0.22		0.97	0.50	0.52	0.67	0.75	0.67	0.79	0.69	0.65	0.68	0.72	0.17	-0.17	-0.50
	Glc	0.04	-0.41	0.97		0.35	0.65	0.75	0.85	0.79	0.91	0.80	0.77	0.81	0.85	0.28	0.04	-0.25
	Suc	-0.07	-0.08	0.50	0.35		0.27	0.26	0.33	0.33	0.22	0.38	0.35	0.26	0.23	-0.68	-0.88	0.00
Anthocyanins	Cyanin	-0.58	-0.75	0.52	0.65	0.27		0.93	0.95	0.86	0.88	0.94	0.86	0.89	0.89	0.22	0.17	0.25
	Cy-3-O-Soph	-0.25	-0.49	0.67	0.75	0.26	0.93		0.94	0.73	0.88	0.85	0.72	0.80	0.83	0.42	0.14	0.50
	Cy-3-O-Samb	-0.40	-0.70	0.75	0.85	0.33	0.95	0.94		0.92	0.98	0.98	0.91	0.95	0.96	0.26	0.14	0.75
	Cy-Gluc-Rut	-0.58	-0.87	0.67	0.79	0.33	0.86	0.73	0.92		0.93	0.98	1.00	0.99	0.98	0.03	0.15	1.00
	Cy-Gluc	-0.33	-0.70	0.79	0.91	0.22	0.88	0.88	0.98	0.93		0.95	0.92	0.96	0.98	0.33	0.25	
	Pelar-Soph	-0.55	-0.81	0.69	0.80	0.38	0.94	0.85	0.98	0.98	0.95		0.98	0.98	0.98	0.09	0.10	
	Cyan Xyl Rut	-0.61	-0.88	0.65	0.77	0.35	0.86	0.72	0.91	1.00	0.92	0.98		0.99	0.97	-0.01	0.13	
	Pelar Gluc Rut	-0.54	-0.85	0.68	0.81	0.26	0.89	0.80	0.95	0.99	0.96	0.98	0.99		1.00	0.15	0.22	
	Cyan Rut	-0.47	-0.81	0.72	0.85	0.23	0.89	0.83	0.96	0.98	0.98	0.98	0.97	1.00		0.22	0.25	
Fl	Hyperoside	0.29	0.07	0.17	0.28	-0.68	0.22	0.42	0.26	0.03	0.33	0.09	-0.01	0.15	0.22		0.76	
	Querc Gluc	-0.19	-0.33	-0.17	0.04	-0.88	0.17	0.14	0.14	0.15	0.25	0.10	0.13	0.22	0.25	0.76		

Results: Venn Diagram



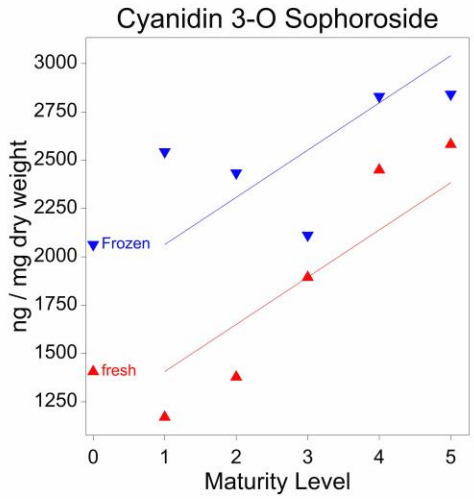
→cut off: > +/- r= 0.75

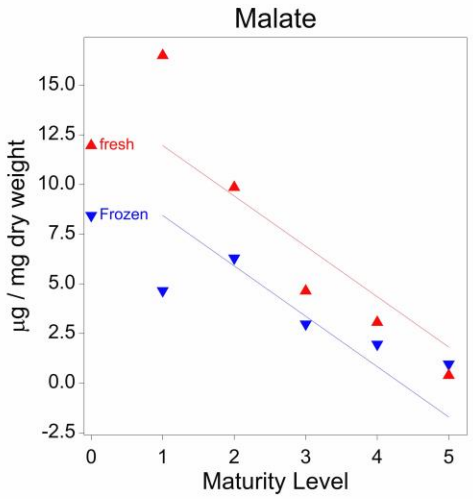
Results: Regression analysis

Table 2 Regression analysis of organic acids, sugars, anthocyanins ad flavonols in frozen and fresh raspberries

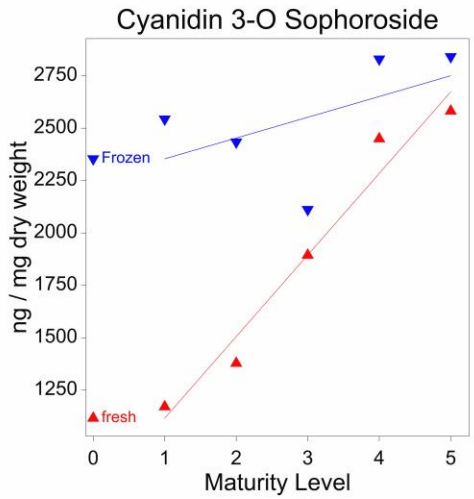
	Colour	Type	Colour.Type
Malate*	<.001	0.01	0.01
Cyanidin-3-O-sambubioside	<.001	0.02	0.06
Cyanidin-3-2-xylosyl-rutinoside	<.001	0.63	0.38
Cyanidin-3-O-sophoroside*	0.00	0.00	0.03
Pelargonidin-3-O-sophoroside	0.00	0.02	0.69
Oxalate	0.02	0.01	0.13
Pelargonidin-3-glucosyl-rutinoside	0.00	0.33	0.89
Cyanidin-3-O-rutinoside	0.00	0.94	0.47
Cyanidin-3-O-glucoside	0.00	0.54	0.21
Cyanin	0.00	0.23	0.08
Citrate	0.02	0.64	0.33
Glucose	0.08	0.10	0.22
Sucrose	0.13	0.07	0.61
Hyperoside	0.17	0.97	0.12
Quercetin-3-O-glucuronide	0.19	0.83	0.25
Fructose	0.30	0.05	0.20

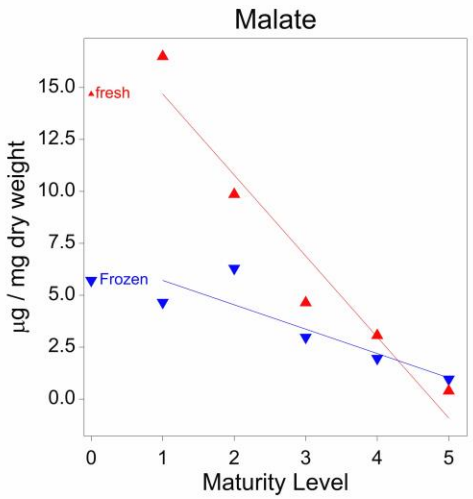
Results: Regression Example





Significant different intercepts





Significant different slopes between different treatments

Graphical Illustration

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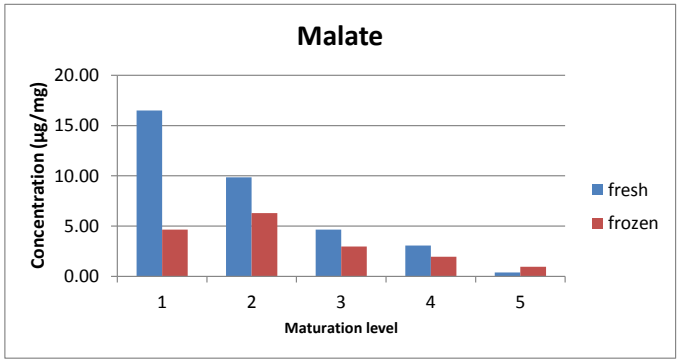

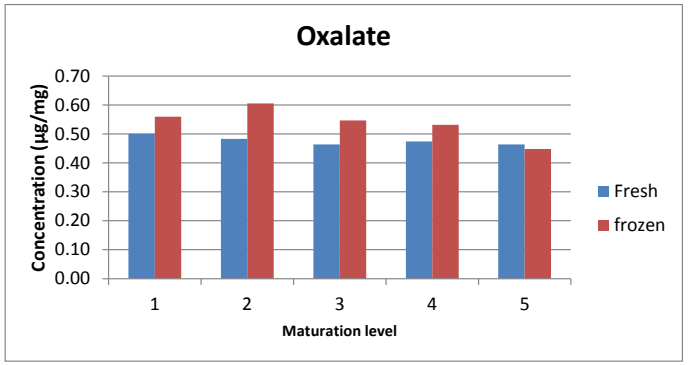

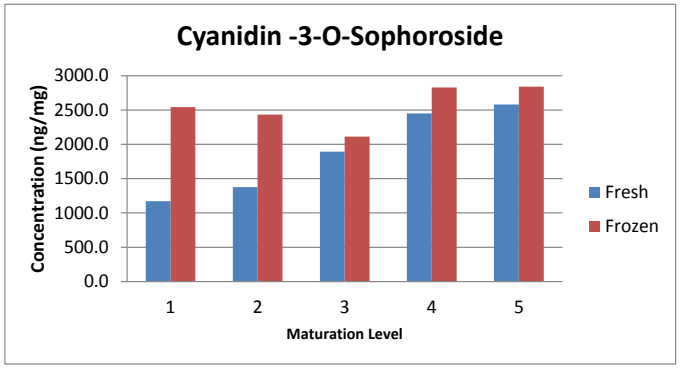

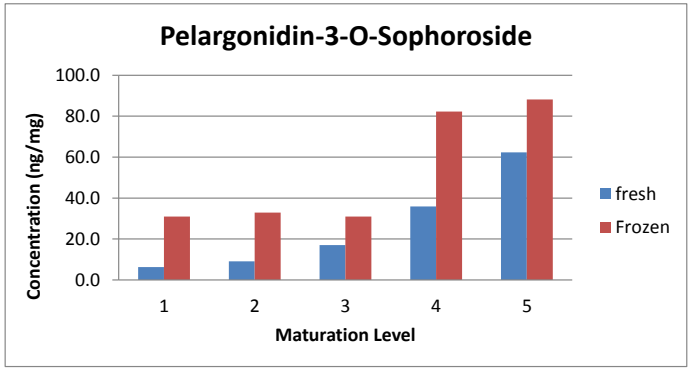

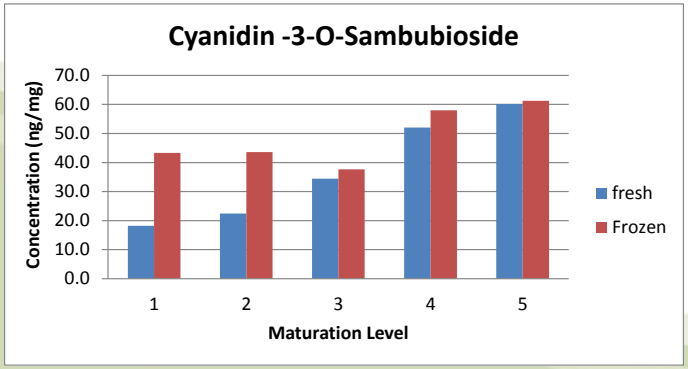

Summary Colourimetric Experiment

- Organic acids decrease with higher maturity level.
- Sugars, anthocyanins and flavonols increase with increasing maturity level.
- Regression intercept for Malate significantly higher in fresh raspberries, while oxalic acid, cyanidin-3-O-sambubioside, cyanidin-3-O-sophoroside and pelargonidin-3-O-sophoroside significantly lower in fresh raspberries.
- Significant different slopes for malate and cyanidin-3-O-sophoroside.