The role of ethene and cell-wall modifying enzyme activities in the determination of raspberry (*Rubus idaeus* L.) fruit firmness

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Introduction

Though the United Kingdom produces 7.4% of World Raspberry production it is a net importer of raspberries. This is due mainly to limited shelf-life, caused by tissue softening. The work presented here addresses the relationship between respiration, ethene evolution and softening to allow a targeted approach for molecular science and the improvement of fruit quality.

Plant material

Raspberry genotypes were chosen from two different genetic backgrounds of SCRI and HRIEM. From SCRI these were Glen Prosen (firm) and Glen Clova (soft) and from HRIEM, EM 4997 (firm) and EM 5007 (soft). Ten different classes of ripening raspberry fruits (based on colour and circa fresh weight (g)) were used for analysis from field-grown crops of each genotype.

Ripeness class	Fruit description
0	**Flower buds just opened**
1	**Petals fallen, fruits very small and green; 0.25-1g**
2	**Small and green; 0.75-1.5g**
3	**Small and pale green; 1-2g**
4	**Expanded and white in appearance; 2-3g**
5	**Entirely pink; 3-4g**
6	**Light red; 3-4g**
7	**Entirely red; 4-5g**
8	**Very dark red; 4-5g**
9	**Ripeness class 7 harvested and stored at 20°C for 24h**

Ripening rates

Time to ripen (50 for each genotype) from ripening class 4 to 7 did not vary between genotypes. From flower opening to the green fruit stage there were significant differences in ripening time with firmer genotypes taking longer to complete this stage.

Genotype source	SCRI		HRIEM	
Raspberry genotype	Glen Prosen	Glen Clova	4007	5007
Fruit firmness Character at harvest	**Firm**	**Soft**	**Firm**	**Soft**
Ripeness class	Ethene production (mg hr^{-1} g fw)			
5	7.93 ± 6.87^{c}	16.94 ± 6.03^{b}	12.84 ± 0.48^{b}	30.40 ± 7.63^{a}
7	23.35 ± 2.53^{c}	34.34 ± 5.03^{b}	20.01 ± 7.04^{c}	55.28 ± 5.82^{a}
Ripening class	Time (days)			
0 to 2	36.25 ± 2.81^{c}	30.34 ± 2.40^{b}	35.27 ± 2.10^{b}	32.45 ± 1.65^{a}
2 to 4	24.49 ± 1.85^{c}	24.48 ± 1.42^{b}	22.80 ± 1.42^{c}	18.53 ± 1.68^{a}
4 to 5 ^{NS}	3.01 ± 0.78	2.04 ± 0.44	2.64 ± 0.43	2.18 ± 0.33
5 to 7 ^{NS}	1.32 ± 0.77	1.22 ± 0.43	0.69 ± 0.51	1.01 ± 0.41

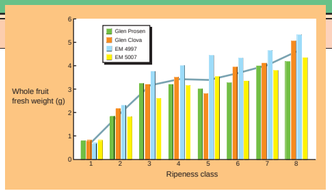
a,b denotes anova categories for significant differences where p < 0.05

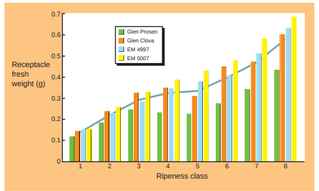
Ethene evolution

Ethene production for whole fruits (berry plus receptacle) from ripening classes 5 and 7 (averages of 9 berries) shows that higher ethene levels are associated with fruit which is relatively soft at harvest. Rate of ethene production increased as fruit matured and correlated with penetrometry data (n=9).

Fruit growth character

These bar charts show the average fresh weight (g) for whole fruits and receptacle only repectively. Average values for all four genotypes (where n = 50 for each genotype) at each ripening class illustrate the growth pattern of maturing red raspberry fruits and receptacles (—). This is: growth from ripening classes 1 to 3, a plateau in development from 3 to 5 and continued growth from 5 to 8. This pattern was also true of dry weight data which is not presented here. There was no significant difference in whole fruit fresh or dry weights between either SCRI variety and EM 5007; EM 4997 was significantly larger than the other three genotypes.



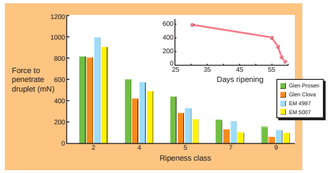


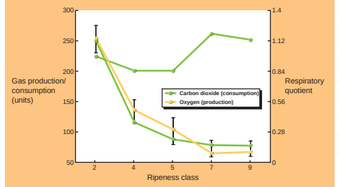
At ripening class 7 to show that druplet firmness relates to other fruit parameters, namely; ethene evolution; time to ripen; and receptacle weight. The ripe fruit of EM 5007 which produces the highest ethene levels, ripen in the shortest time and is amoung the softest fruit. This is in contrast to Glen Prosen which takes longest to ripen, has the lowest ethene levels and produces firmer ripe fruit. These differences corresponds with receptacle size: compare Glen Prosen which has a receptacle around circa. 0.5 times as large as EM 5007.

Genotype	Relative fruit-firmness	Druplet firmness (mN)	Ethene evolution (mg hr^{-1} g fw^{-1})	Time to ripen (days)	Receptacle fresh weight (g)
EM 5007	**Soft**	133^{a}	55.28^{a}	54.17^{c}	0.58^{a}
Glen Clova	**Soft**	121^{a}	34.34^{b}	$58.08^{b,c}$	0.47^{b}
EM4997	**Firm**	191^{b}	20.01^{c}	$61.40^{a,b}$	0.51^{b}
Glen Prosen	**Firm**	210.3^{b}	23.35^{c}	65.00^{a}	0.34^{c}

a,b,c denotes anova categories for significant differences where p < 0.05

The force taken in milli Newtons (mN) to penetrate the skin of developing red raspberry druplets (n=20). For all four genotypes firmness declined in a linear fashion as fruit matured. Also, fruit found to be characteristically firm at harvest is also firmer throughout fruit development.

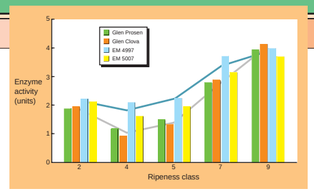




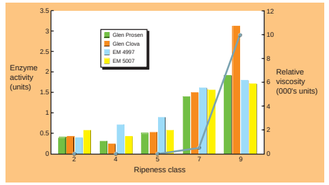
Between genotypes there was no significant variation in respiration which declined as ripening proceeded. Illustrated are Glen Clova berries (where n=3; and each replicate is the average of 3

Cell wall modifying enzyme activity

PG activity (µ moles of glucose released per gram cell-wall dry weight per hour at 37oC; where n = 3, and each replicate is the average of 10 berries) throughout ripening did not differ with genetic background nor did it correlate with relative fruit firmness.The average values for SCRI varieties (—) and EM genotypes (—) show that the activity pattern during ripening corresponds to the growth curve of developing fruits. That is, increased activity during ripening class 2 (green) when fruit expands and becomes more pale to reach ripening class 4 (white) when activity is suppressed during the growth plateau (ripening classes 4(white) to 5(pink)) and a rapid increase in activity to maximum levels from class 5 (red) and after harvest.



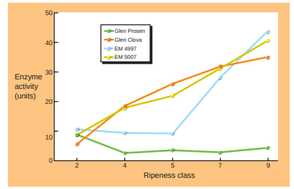
Cx activity showed no relationship with ripe fruit firmness. Viscometery data (relative viscosity per gram cell-wall dry weight per hour) is also shown (—) for developing Glen Clova fruits.



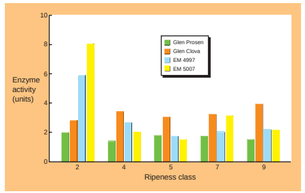
PAGE IEF or Rotofor TM IEF of Glen Clova red fruit extracts revealed numerous isoforms for all four cell-wall modifying enzymes. For each cell-wall modifying enzyme activity the number of isozymes detected and the IEF point (pI) of each is shown below. Major isozyme activity is identified by pI values in red.

Enzyme activity	Number isozymes	pI
Poly galaturonase	8	**3.3**, 3.6, 4.4, **8.6**, 8.7, 8.9, 9.1, **10.1**
Pectin methyl esterase	5	6.9, **7.2**, **8.5**, **8.7**, **8.8**
Cellulase	4	**2.4**, 2.8, **3.2**, **3.5**
ß-galactosidase	8	5.9, 6.1, **6.3**, **6.5**, **6.8**, 6.9, 7.1, 7.3

PME activity as for PG for one unit of *Aspergillus niger* PG did correspond to relative fruit firmness at harvest. This was particularly evident for Glen Clova throughout ripening but also for EM 4997 in the final ripening classes.



For each SCRI variety ß-gal activity (µ moles of p-nitrophenol released per gram cell-wall dry weight per hour at 20oC) was unaltered throughout ripening and was significantly higher ($p < 0.05$) in Glen Clova (softer) fruits. For EM's 5007 and 4997 activity correlated with fruit firmness only at ripening classes 2 and 7 ($p < 0.05$). Therefore, a correlation exists between ß-gal activity and fruit firmness (at harvest) though ß-gal activity is high early in fruit maturation.



Ethene application

Long term exposure (48 hr) of green fruit to 21 vpm ethene led to enhanced carbon dioxide production, decreased druplet firmness and induced red pigmentation of druplets (data are averages of three berries). This suggests a causative role for ethene in raspberry fruit ripening.

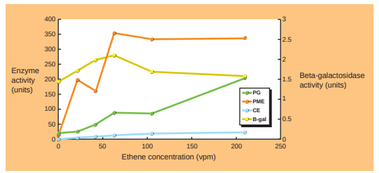
Cultivar	Ripeness class	Ethene exposure	% CO_2 (after 48 hours)	Firmness (mN)	Optical density (515nm)
Glen Prosen	2	**Yes**	**2.3**	**714**	**1.0 ± 0.3**
		No	**1.9**	**806**	**0.2 ± 0.0**
Glen Clova	2	**Yes**	**1.8**	**228**	**1.0 ± 0.3**
		No	**1.1**	**344**	**0.2 ± 0.0**
Anova					
Cultivar		***	*	NS	
Ethene exposure		***	***	***	

NS Not significantly different

* Significantly different at p < 0.05

*** Significantly different at p < 0.01

At 21 vpm ethene (as for Table 4 data) the activities of all four enzymes tested increased significantly (Enzyme units are as defined for Figures 5 to 8; here n = 3, and each replicate is the average of 5 berries). With increasing ethene levels PME and ß-gal increased rapidly and was maximal by 63 and 42 vpm ethene respectively. PG and Cx activities appeared less reponsive and increased in a linear fashion with increasing ethene concentration.



Summary

- Raspberry is an ethene-responsive non-climacteric fruit and this is the first record of significant cell-wall modifying enzyme activity in druplets. In ripe red fruit all enzyme activities increased significantly and contribute to the "soft-fruit" character of raspberries.
- Fruit firmness differences at harvest exist over the whole developmental time course and fruit firmness at harvest may be determined early in the course of fruit development.
- ß-gal is a factor which may determine firmness differences during early fruit development. In addition, rate of ethene evolution relates directly to fruit receptacle size and together with PME activity may determine differences in firmness between raspberry fruit genotypes.
- These findings allow a targeted molecular approach to enhance the disease resistance of ripe Rubus species.

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