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Flavour quality factors and its regulation in red raspberry (*Rubus idaeus*)

By

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Declaration

This thesis is the result of the author's original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree

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Dedication & Acknowledgement

This doctoral effort and resulting thesis is in my memory of my mother, Ku Zam Zam Ku Idris.

I would like to express my sincerest gratitude to those who have provided invaluable support and assistance throughout my doctoral journey.

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List of abbreviations and chemical formulae

Standard units

^{o}C	degrees Celsius
$^{o}Brix$	degrees Brix
g	gram
x g	gravity
gml^{-1}	gram per millilitre
k	kilo
l	litre
m	milli
M	Molar
$mlmin^{-1}$	millimetre per minute
Pa	Pascal
pH	measure of acidity/ alkalinity
W	Watt
μ	micro

Textual abbreviations and chemical formulae

AFLP	Amplified fragment length polymorphism
ANOVA	analysis of variance
α	alpha
bHLH	basic helix loop helix
β	beta
C3G	cyanidin-3-glucoside
C3GR	cyanidin-3-glucosylrutinoside
C3R	cyanidin-3-rutinoside
C3S	cyanidin-3-sophoroside
cDNA	complementary DNA
CIM	composite interval mapping
4CL	4-coumarate-CoA ligase
cM	centiMorgan
cv.	cultivar
DNA	deoxyribonucleic acid
EST-SSR	expressed sequence tag-derived SSR
F3H	flavanoid-3'-hydroxylase
FW	fresh fruit weight
HCl	hydrochloric acid
HPLC	High performance liquid chromatography
HPLC/UV vis	HPLC/ Ultraviolet visible wavelength
HPLC/MS	HPLC/ Mass spectrometry
H_2SO_4	Sulphuric acid
IM	interval mapping
KW	Kruskal Wallis
KH_2PO_4	potassium diphosphate
LOD	logarithmic odds

LG	linkage group
MAB	marker assisted breeding
MIP	membrane intrinsic protein
Max	maximum value
Min	minimum value
$MgCl_2$	magnesium chloride
min	minute
MYB	myeloblastosis
NH_4	ammonium
Na_2SO_4	sodium sulphate
P3G	pelargonidin-3-glucoside
P3GR	pelargonidin-3-glucosylrutinoside
P3R	pelargonidin-3-rutinoside
P3S	pelargonidin-3-sophoroside
PCA	Principal Component Analysis
PCR	Principal Component Regression
PLSR	Partial Least Squares Regression
PLS1	Partial Least Squares regression -1
PLS2	Partial Least Squares regression -2
PSD	pooled standard deviation
QTL	quantitative trait loci
RAPD	randomly amplified polymorphic DNA
R^2	regression coefficient value
RK	Raspberry ketone
RMSEC	root mean squared error or calibration
RMSEP	root mean squared error or prediction
RPD	Ratio of prediction to deviation
SCRI	Scottish Crop Research Institute
SD	standard deviation
σ	standard deviation value
SPE	solid phase extraction
SSC	soluble solids content
TIP	tonoplast intrinsic protein
SSR	simple sequence repeats
UV	ultraviolet light
w/v	weight/ volume

Summary

Breeding in red raspberries is hampered by genetic incompatibility between varieties and a long juvenility period. Molecular breeding efforts in red raspberry have provided viable applications to incorporate disease resistance in new varieties. This approach could also be applied in developing non-GM strategies to improve flavour quality of red raspberries, successful in other related fruit crops (e.g. peach, apple), with 'good' flavour quality determined by sensory panellists. In this study, sensory data was correlated to data on flavour metabolites (e.g. sugars, organic acids and volatiles) through statistical modelling, which yielded possible causal relationships. These flavour traits data were then mapped to genetic loci and / or candidate genes on an already existing raspberry genetic linkage map and generated genomic regions most likely responsible for trait variation, potentially genetic markers for flavour quality. These markers could be used to select seedlings with propensity to develop premium flavour quality prior to planting, thus reducing time required to produce new varieties.

This study aimed to investigate flavour development in red raspberry cross population (Glen Moy x Latham) impacted by environmental (different year and cultivation methods) and genetic (different genotypes in a cross mapping population) factors. Flavour metabolites; sugars and acids contents, were quantified via chromatographic methods (HPLC-UV/Vis, HPLC-MS) and other flavour-related datasets (^{o}Brix, 10-berry weight, volatiles and pigment-related contents) were obtained from parallel researches. These datasets were correlated to sensory scorings (sweetness, sourness and flavour intensity) of progenies in the mapping populations, via statistical regression analyses (PLS-1), in order to identify factors most responsible for variances in flavour traits. Genetic explanations of flavour quality were obtained through mapping sweetness, sourness and flavour intensity and other flavour-related datasets to the red raspberry genetic linkage map (Graham et al., 2009) which yielded flavour-related quantitative trait loci (QTLs). Overall, polytunnel cultivation was most effective in producing fruits with high flavour scores and metabolites contents. Sweetness and flavour intensity, both closely linked, were significantly correlated to sugars content, with notable contributions by volatiles

content, especially hexenol. Raspberry ketone (RK) had compounded effects with other metabolites in impacting sweetness and flavour intensity but not sourness. Sourness was a complex trait poorly explained by metabolites content or any other flavour-associated variable. Linkage group (LG) 3 had most number of QTLs that co-localised to markers associated to processes affecting overall plant growth and to biosynthetic pathways of other flavour compounds (e.g. phenypropanoid pathways for volatiles). These results indicate flavour quality development as a complex trait and many factors affect it. Although genetic selection for improved flavour quality is possible, effects from genetic x environmental interactions most impact on flavour quality, with clear advantage of developing good flavour berries with increased metabolites under polytunnel cultivation.

Results from this study add to knowledge on factors affecting red raspberry flavour, which could assist breeders in developing control strategies and help focus future breeding efforts on factors that most impact on flavour quality. Furthermore, results from this study can be transferred to other *Rosaceae* species and add to existing efforts to develop modern flavour quality management strategies.

CHAPTER 1

Introduction

Chapter 1 Introduction

1.1 The red raspberry

1.1.1 *Preamble*

The raspberry is a member of the *Rosaceae* family, which has currently more than 2000 species of woody, shrub and ornamental plants. Raspberries are trailing shrubs with generally two stages of plant development, *primocane* and *floricane*. The red raspberry plant bears short-lived woody shoots on a long-lived perennial root system. In biennial fruiting cultivars (*floricane*), shoots (canes) have a two-year life cycle. In contrast, *primocane* cultivars complete the cycle of vegetative growth, flowering and fruiting in a single season. The name 'raspberries' is believed to originate either from *Raspis*, first mentioned by William Turner in 1568, or 'Kratsberre' in German meaning 'Scratchberry', both in reference to its spines or thorns (Roach, 1985). Raspberries are fruiting plants of the genus *Rubus*, which has 15 subgenera: domesticated raspberries are in *Ideobatus*. Domestication of the red raspberry began with 5 parental cultivars: Preussen, Cuthbert and Newburgh, progeny of European and North American red raspberry crosses; Lloyd George, and Pynes Royal, of pure *R. idaeus* stock (Graham et al., 2004; Finn and Hancock, 2008). Commercially important raspberry varieties today are from crosses of three raspberry species, R. *idaeus* L. subsp. *idaeus* (European), R. *idaeus* subsp. *strigosus* Michx. (North American) and *R. occidentalis* L. (black raspberries) (Ercisli et al., 2008; Graham and Woodhead, 2009). Most *Rosaceae* species have haploid chromosome numbers (x) of 7 to 9 with the exception of some of the members of *Maloideae* (x=17). A large proportion is diploid, with certain others 4x to 12x ploidy (Dirlewanger et al., 2009), and with small genome sizes compared to other angiosperms: 275 megabase pairs (Mbp) in red raspberry, 300 Mbp in peach (Dickson et al., 1992). Therefore, structural and functional genetic tool constructions are eased and there is a potential for application for certain in other *Rosaceae*.

Modern domesticated and commercial *Rosaceae* fruit varieties are relatively inbred, resulting in plants more susceptible to diseases that affect plant vigour and fruit quality. This is because genes responsible for disease susceptibility are not out-

crossed and remain in gene pool. Introduction of novel and functional DNA sequences into genomes of cultivated species is a potential solution to this problem. Suitable genetic material could originate from wild plants or other domesticated berries which have advantageous phenotypic traits. Key traits important in raspberry varieties as assessed by Finn and Hancock (2008) are listed in Table 1.1. Responses to growing conditions are generally more influential than genetic determinants of plant and fruit trait diversity in cultivated berries. However, genetic variation between species and subgenera is more pronounced with increasing genetic distance. In the genestock of *R. idaeus* (European red raspberry) and *R. strigosus* (North American red raspberry), genetic similarity, assessed from study of DNA polymorphisms, was greater (60%) than either to *R. occidentalis* (black raspberry) (40%) or *R. rubus* (blackberry) (22%) (Graham et al., 1997; Stafne et al., 2005). Genetic similarity with wild plants is even less (<10%) due to the previously mentioned narrow genetic base from breeding strategies. One study found little genetic similarity between wild raspberries and the cultivated cv. Glen Clova or between wild plants within a small sampling area (Graham et al., 1997). In Korean black raspberry (*R. coreanus*), cultivation on mainland or island locations correlated with genetic divergence (Dong et al., 2009). Divergence is greater in raspberries from different continents and separated by large distances (Zhang et al., 2009). Genetic dissimilarity provides a germplasm pool that can be utilised in breeding.

1.1.2 Raspberry breeding and its objectives

Breeding is hampered by genetic obstacles such as apomixes, pollen incompatibility and poor seedling germination. Use of heterozygous germplasm necessitates screening of large seedling populations. Breeding is the general improvement in stock through crossing inter-mating individuals showing promise of progenies with enhanced phenotype profiles: quantified as response to selection (Graham and Jennings, 2009).

Table 1.1 Comparison of desirable phenotypic traits of commercially important *Rubus* species worldwide (Finn and Hancock 2008).

Subgenus	Species	Ploidy	Origin	Important traits
Ideobatus	R. *idaeus strigosus* Michx.	2x	North America	As a potential source of untapped diversity for many traits.
Ideobatus	R. *occidentalis* L.	2x	Eastern North America	Progenitor species for black raspberry and for red improvement: tolerance to heat and humidity; resistance to aphids, bud moths, leaf rollers, cane beetles and two-spotted spider mite; in fruit susceptibility to rot and firmness; late-ripening floricane fruit.
Ideobatus	R. *idaeus idaeus*	2x	Europe	A primary parent for red raspberry.
Ideobatus	R. *coreanus* Miq.	2x	China	Early ripening, vigour, and fruit colours (orange-black). Resistance to: aphids; cane blight; midge blight; spur blight; cane Botrytis; anthracnose; European raspberry beetle; mildew; leaf spot; and root rot.
Ideobatus	R. *glaucus* Benth.	4x	South America	Low chilling requirement; vigour; fruit aroma and size; small seeds and drupelets; extended fruiting season; root rot resistance.
Ideobatus	R. *parvifolius* L.	2x, 4x	Japan, China, Australia	Low chilling requirement; resistance to drought, high temperature and humidity; resistance to: leaf and cane spot, spider mite and root rot; some tolerance of fluctuating winter temperatures; productive; fruit size.
Chamaemorus	R. *chamaemorus* L.	8x	Circumpolar/ Sub-arctic	Aromatic flavour; ascorbic acid content; thornlessness; winter hardiness

A major breeding target is for fruit flavour quality (Graham and Jennings, 2009): primarily taste and aroma but also texture and mouthfeel. Desirable attributes are fruity, floral sweet berries with some acidity and no bitterness (Harrison et al., 1998). Also important to multiple retailers of fresh fruit are appearance and long shelf life whereas colour, brightness, size and shape of berries are desirable for processing markets. Large berries are important to consumers, and thus to retailers, and for farmers, it is cost effective for manual harvesting.

Desirable fruit flavour characters can be obtained by classical crosses but this is time consuming because of long juvenility. To produce a new commercial variety for a market takes 8 - 15 years. Backcrosses, plant trials and fruit evaluations are necessary to select out progeny with a genetic predisposition to develop undesirable traits. Seedling selection based on genetic criteria would reduce the timescale. A prerequisite is a genetic linkage map, populated by polymorphic molecular markers useful in identifying chromosomal regions, quantitative trait loci (QTL), determining character intensity. Within QTL, candidate genes are likely determinants of trait intensity. Screening seedlings for genotype before plant maturity could accelerate breeding of varieties of premium fruit quality.

This genetic strategy for seedling selection on the basis of DNA polymorphisms as allelic markers, is termed marker assisted breeding (MAB). This has potential to overcome lengthy plant trial evaluations and ensure planted seedlings have genetic predisposition to develop high overall quality (Graham et al., 2004).

1.1.3 Breeding strategy: Identification of QTLs

As QTLs for flavour traits have potential for marker assisted breeding programmes for fruit quality, it is necessary to understand inheritance from parent by progeny. Traits in eukaryotes are categorised as discrete or continuous. Discrete traits are Mendelian, in that alternate phenotypes are observed (e.g. yellow vs. green seed), whereas continuous traits show a normal distribution of values (e.g. height, weight, flowering time) (Hartl and Jones, 2002). For both, the statistical probability of inheritance in progeny can be calculated based on Mendel's Law of Inheritance.

Continuous trait expression is not independent of environmental factors, such as growing conditions, and there is thus an interaction as is observed in horticulturally important traits (e.g. fruit weight, metabolites contents). Usefulness of QTLs for seedling selections is therefore dependent on a degree of environmental influence. Minimal influence on trait development would reflect a robust or true QTL, normally achieved through monitoring trait intensities over a few generations to identify marker alleles of high probability of impact on continuous trait values. Reproducibility over generations is the first indicator of true QTLs with underlying candidate gene/s that have putative influence on traits. These genes can be cloned and expressed in other plants to determine the impact on trait expression. Initial QTL mapping in *Rosaceae* species began with genetic markers of indeterminate functions verified by expression studies and also study of candidate genes of other related species (Viruel et al., 1995; Joobeur et al., 1998; Maliepaard et al., 2002; Stafne et al., 2005). To facilitate work in red raspberry, an important genetic linkage map was developed at the James Hutton Institute by Graham and her co-workers (Graham et al., 2004): this initially segregated 273 polymorphic DNA markers into 7 linkage groups and was subsequently developed and refined (Woodhead et al., 2008; Graham et al., 2009; McCallum et al., 2010).

Another genetic selection strategy is based on study of candidate genes, of either related or non-related species. Use of QTLs and other genetic markers in *Rubus* breeding was extensively reviewed by Antonius-Klemola (1999), Hokanson (2001) and Skirvin and others (Skirvin et al., 2005).

1.1.4 Breeding for other plant characteristics

Other potential objectives of MAB are plant disease resistance and physical traits. Substantial losses in European raspberry cultivation from pest infestation and diseases could be reduced by marker assisted breeding for genetic resistance to *Phytophthora* root rot and *Botrytis* grey mould, the two most common diseases of great impact (FruitGateway: *www.fruitdisease.co.uk*). Recently, resistances to other diseases afflicting raspberries (cane *Botrytis* and spur blight), were linked to gene *H* (genotypes HH or Hh) mapped on linkage group 2 (Graham et al., 2006). This gene

determines the presence of cane root hair, possibly co-segregating with disease resistance. A gene for red stele root rot resistance (Rpf1) in *Fragaria* species was identified from a cross of susceptible and resistant genotypes (Haymes et al., 1997). Studies into root rot resistance have been reported in: soybean (Weng et al., 2001); pepper (Ogundiwin et al., 2005); tomato (Zhang et al., 2002) and also previously raspberry (Pattison et al., 2007).

Factors related to flowering impact on fruit density and ripening. Major flowering QTLs have been identified: CRY2 in *Arabidopsis*, affected externally by day length of which down-regulation promoted flowering on shortened days (El-Assal et al., 2001). Over-expression of CRY2 in tomato delayed flowering in both short and long day environments with anthocyanin accumulation in leaves and increase in lycopene content in fruit (Giliberto et al., 2005), impacting on its visual sensory attributes.

1.1.5 Fruit quality and breeding strategies

A trait important to raspberries is berry yield. In primocane this is influenced by amount of branching and extent of lateral development; in floricane this is also influenced by number and height of young canes, consistency of bud break and internodes. Erect, spineless canes are desirable for ease of picking.

Flavour is an important quality index for fruit (Liem et al., 2004a, 2004b; Péneau et al., 2006; Brug et al., 2008), particularly taste, specifically sweetness and sourness. An appropriate balance makes fruit attractive and palatable. Metabolites that contribute to these sensory characters are therefore important. Scoring of apple varieties by Danish children correlated preference with high sugar content and sugars/acids ratios (Kühn and Thybo, 2001). In American consumers, high soluble solids content (SSC) was a determining factor in consumer acceptance, although firmness was most important (Harker et al., 2008). Acceptance was directly correlated to postharvest dry matter and sugar content in kiwifruit (Harker et al., 2009). Palatability and preference are important to ensure repeat consumption of fruits, essential components for a healthy and balanced diet. Taste is however only one parameter in overall fruit quality. Consumers use visual and olfactory cues as retail purchase criteria and consequently both are important to the multiple retailers who determine cultivar success.

Responses of fruit to postharvest handling, storage and other shelf life characters, are important to multiple retailers and many consumers. Such characters (fruit firmness, chilling adaptability and spoiling rate) are likely determinants of variety market success. Such factors are inherited as continuous traits (Oraguzie et al., 2004; Lurie and Crisosto, 2007; García-Gago et al., 2009; Quesada et al., 2009). Variance analysis of both quantitative trait data and DNA polymorphisms over a few progeny generations can yield loci important for flavour character (Tieman et al., 2006; Fernie et al., 2006): QTLs that correlate with specific structural and regulatory – "candidate" – genes.

Study of continuous traits in fruit quality, both genetic regulation and impact on sensory characters, has been most active in tomato, due to commercial interests and availability of diploid parents and also in melon (Appendix 1). QTLs identified in *Rosaceae* species have colinearity with QTLs in model fruit plant systems (notably tomato).

As shown in Table 1.2, many researchers relate fruit physical and chemical attributes, quantified instrumentally, to quality, without parallel sensory assessments. This can be a potential source of QTL bias. Instrumental measurements are popular, being time and cost efficient whereas sensory data are not highly regarded by reductionist scientists, even when using trained panels. However, high concentrations of metabolites do not necessarily translate to good flavour characters and intensity. This is due to the human brain integrating stimuli from non-volatile and volatile compounds on taste and aroma detectors in gustatory (palate and tongue) and olfactometric (orthonasal and retronasal) sensory systems. Thus for example, peach cultivars with the highest fructose and low acids content were not correlated with high scoring of 'sweetness' or 'sourness' but with 'fruity flavour', from principal component analysis (PCA) (Esti et al., 1997). Instrumental measurements of quality, when correlated to sensory data, would yield fruit quality information data both robust and objective.

1.2 Ripening effects on flavour quality

1.2.1 Preamble

Generally, in ripening of commercial fruits (peach, strawberry and melon), biochemical and physiological changes alter composition (*e.g*. starch, sugars, organic acids, volatiles and pigment content), structure (*e.g.* cell wall breakdown and flesh softening) and appearance (*i.e*. peel and flesh colour) (Zerbini, 2008a, b). Completion of ripening makes fruit palatable. Important for final fruit quality are sugars and acids accumulation, linked to sweet and sour taste intensities. Other factors affect final quality - volatiles biosynthesis linked to aroma development and chlorophyll breakdown and pigment production for colour. Fruit firmness is important for flavour, influencing oral release of flavour compounds on ingestion and mastication and also postharvest processing quality. Fruit ripening processes thus influence flavour both directly and indirectly.

1.2.2 Cell wall degradation

Cell wall structure determines flesh firmness and thus juice release when chewing. Pectins are important in cell wall integrity, as are cellulose microfibers embedded within pectin-hemicellulose matrices. Degradation of cell walls begins with pectin depolymerisation, which varies in rate between different varieties. For example, accelerated cell wall degradation was found in the softer red raspberry variety Glen Clova compared with machine harvestable Glen Prosen (Stewart et al., 2001). Pectin reductions are also observed in mango (Muda et al., 1995), strawberry (Rosli et al., 2004) and peach (Brummell et al., 2004). Fruits differ in galactose and arabinose content reductions, due to varying activities of polygalacturonases. Key effects of pectin reduction on fruit quality are on texture, but correlations between instrumental and sensory assessment are scarce (Harker et al., 2002; Mehinagic et al., 2003; Esti et al., 2002).

Table 1.2 Genetic regulations of traits in fruit quality in important *Rosaceae* fruit crops: QTLs and genes

Fruit/Cross	**Trait**	**Gene/QTL, LG**	**Associated sensory traits**	**Gene/QTL,LG**	**Reference**
PEACH *P. davidiana* (P1908) x P. *persica* cv. Summergrand	Soluble solids	QTL SSC_1, SSC_2,$SSC_{1,2,}$ LG 4, 5	Sweetness Red skin colouration	QTL Swe_2, LG 5 QTL $Srcolour_2$LG5	Quilot et al., 2004
	Sucrose	QTL Suc_1,Suc_2 LG3, 6, 7	-n/a-	-n/a-	
	Glucose	QTL $\underline{Glu_1}$, $\underline{Glu_2}$, LG 2, 4, 5, 7	-n/a-	-n/a-	
	Fructose	QTL $Fru_{1,2}$, $\underline{Fru_1}$, $\underline{Fru_2}$,$Fru_1^{S,Z}$ LG1, 2, 4, 7	Sweetness	QTL Swe_2, LG1	
	Total sugars	QTL $Tsugar_1$, $Tsugar_2$, LG1, 5, 6	Sweetness	QTL Swe_2, LG5	
	Malic acid	QTL Mal_2, $\underline{Mal_2}$,Mal_1^Z, $Mal_{1,2}$, LG2, 3, 4, 5, 6	Sweetness	QTL Swe_2, LG5	
	Citric acid	QTL $\underline{Cit_1}$, $\underline{Cit_{1,2}}$, Cit_2^Z, $\underline{Cit_2}$, LG1, 3, 4, 7	Red flesh colour	QTL $\underline{FRcolour_2}$, LG3	
	Quinic acid	QTL Qui_1, Qui_2, Qui_2^Z,Qui_1^S, LG1, 4, 5, 6, 7	Skin speckle	QTL $SSpeckle_2^S$, LG6	
	Shikimic acid	QTL Shi_1, Shi_2, $\underline{Shi_1}$,$\underline{Shi_2}$, Shi_2^S, LG3, 4, 6, 8	Sweetness	QTL Swe_2, LG3	
	Total acids	QTL $TAcid_1$, $\underline{TAcid_2}$, $TAcid_2^Z$, LG2, 3, 4, 5	Sweetness	QTL Swe_2, LG5	
	Diameter	QTL $FPolarD_1$, LG7	Juiciness	QTL Jui_2, $\underline{Jui_2}$, LG 4, 7	

Table 1.2 Genetic regulations of traits in fruit quality in important *Rosaceae* fruit crops: QTLs and genes (Cont.).

Fruit/Cross	**Trait**	**Gene/QTL, LG**	**Associated sensory traits**	**Gene/QTL,LG**	**Reference**
PEACH P. *persica* cv. Ferjalou Jalousia x	Soluble solids	QTL SSC95+, SSC96+, LG4, 6	-n/a-	-n/a-	Dirlewanger et al., 1999
P. *persica* cv. Fantasia	Sucrose	QTL Suc95+, Suc96+, LG5, 6	-n/a-	-n/a-	
	Glucose	QTL Glu96-*, LG8	-n/a-	-n/a-	
	Fructose	QTL Fru95+, Fru96+, LG3, 4, 5, 8	-n/a-	-n/a-	
	Sorbitol	QTL Sor95+, Sor96+, LG1, 6	-n/a-	-n/a-	
	Malic acid	QTL Mal95+, Mal95-,Mal96-, LG1, 5, 6	-n/a-	-n/a-	
	Citric acid	QTL Cit95-, CIT96-, Cit96-, Cit95+, LG5, 6, 9	-n/a-	-n/a-	
	Quinic acid	QTL Qui95*, LG1	-n/a-	-n/a-	
P. *persica* cv. Dr. Davis x P. *persica* cv. Georgia Belle	Texture	Gene PL2, PME1, RIN, endoPG, Ara, PMES, PG4, LG1, 4, 5, 7, 8	-n/a-	-n/a-	Ogundiwin et al., 2009
	Pigment	Gene BCH, PpLDOX, ZXE2, LG2, 5, 7	-n/a-	-n/a-	
	Flavour	Gene SPS	-n/a-	-n/a-	

Table 1.2 Genetic regulations of traits in fruit quality in important *Rosaceae* fruit crops: QTLs and genes (Cont.).

Fruit/Cross	Trait	Gene/QTL, LG	Associated sensory traits	Gene/QTL,LG	Reference
APPLE					
Malus cvs. Telamon x Braeburn	°Brix Green fruit	QTL BrixG, LG2, 10	-n/a-	-n/a-	Kenis et al., 2008
	°Brix Red fruit	QTL BrixR, LG2, 10, 16	-n/a-	-n/a-	
	Acidity	QTL Acidity, LG2, 8, 10, 13, 15-17	-n/a-	-n/a-	
Malus pummila cvs. Prima x Fiesta	Wedge fracture	QTL on LG1, 6, 10	Crispness	QTL on LG1, 6, 10	King et al., 2001
		QTL on LG1, 6, 10, 15, 16	Hardness	QTL on LG1, 6, 10, 15, 16	
				QTL on LG1, 16	
	Compression	QTL on LG1, 16	Juiciness	QTL on LG 1, 12	
		QTL on LG1, 12	Crispness	QTL on LG6, 15, 16	
		QTL on LG6, 15, 16	Hardness	QTL on LG1	
	Specific gravity	QTL on LG1	Juiciness	QTL on LG12, 16	
		QTL on LG 12, 16	Crispness	QTL on LG5, 15, 16	
	Weight	QTL on LG6, 15, 16	Hardness	QTL on LG16	
		QTL on LG16	Juiciness	QTL on LG12, 16	
		QTL on LG12, 16	Crispness	QTL on LG6, 16	
	Penetrometer readings	QTL on LG6, 16	Hardness	QTL on LG16	
		QTL on LG16	Juiciness	QTL on LG1, 10	
		QTL on LG1, 10	Crispness	QTL on LG10	

Table 1.2 Genetic regulations of traits in fruit quality in important *Rosaceae* fruit crops: QTLs and genes (Cont.).

Fruit/Cross	Trait	Gene/QTL, LG	Associated sensory traits	Gene/QTL, LG	Reference
RASPBERRIES					
R. *idaeus* subsp. *idaeus* x subsp. *strigosus*	Anthocyanin pathway	bHLH- transcription factor marker LG1	Anthocyanins content	QTLs on LG1	Kassim et al., 2009
	Anthocyanin pathway	bZIP transcription factor (FRUITE4) marker LG4	Anthocyanins content	QTLs on LG4	
	Hairy canes	Gene H on LG 2	Bud break, late bloom, open flowers and green fruit	QTLs on LG2	Graham et al., 2009
			Open flower, fruit colour-green, green/red and plant height		
	Ripening	MYB-transcription factor marker on LG3	Illuminance and reflection,Y	QTLs on LG3	McCallum et al., 2010
			Colour wavelength, y	QTLs on LG3	
			Visible colour	QTLs on LG3	
			Titratable acidity	QTLs on LG3	
			Illuminance and reflectance, Y	QTLs on LG2	
			Visible colour	QTL on LG2	
	Metabolite transport Flavanol	TIP marker on LG2	Titratable acidity	QTL on LG2	
	Anthocyanins	flavanone synthase marker on LG4	Titratable acidity	QTL on LG4	
		bZIP transcription factor (FRUITE4) marker LG4	Titratable acidity	QTL on LG4	

1.2.3 Sugars and organic acids accumulation

Sugars and organic acids contents are important with notably fructose and citric acid contents linked to sweet and sour taste intensities. Limited accumulation of citric acid is desirable to produce less tart fruit, but absence of acids will result in blandness. Low contents of both sugars and organic acids will result in tasteless fruit (Kader, 1991). The citric to malic acid ratio also affects sweetness perception- citric acid masks sweet taste in sucrose solutions if added to tomato puree (Schifferstein and Fritjers, 1990; Baldwin et al., 2008). Hexoses (glucose and fructose) are the primary fruit sugars that determine sweetness. They are derived from cleavage of sucrose - the primary assimilated form of carbon - transported into fruit cells for storage. Two classes of enzyme, sucrose synthases (SUS) and invertases, assist sucrose cleavage (Wang et al., 1993). Invertases, particularly the acidic vacuolar type, are involved in accumulation of fructose and glucose in tomato, peach and pears (Bucheli and Dévaud, 1994; Moriguchi et al., 1991; Yamada et al., 2007). Biosynthesis of organic acids utilises precursors from sucrose breakdown, metabolised via the tricarboxylic acid (TCA) cycle (Figure 1.1). Biosynthesis and accumulation in fruit is complex with a number of rate-limiting enzymes- phosphoenolpyruvate carboxylase (PEPC), pyruvate dehydrogenase, citrate synthase, aconitase and malate dehydrogenase. In ripening peaches, increased PEPC activity without change in NADP-malic enzyme activity is correlated more with malic than citric acid contents (Borsani et al., 2009). Also in peach, mRNA expression studies showed PEPC activity was higher in high-acid cv. Fantasia compared to low-acid cv. Jalousia; the high-acid variety had higher mRNA expression. Other roles of PEPC are apparent in tomato, including participation in fruit growth and in turgor pressure important for cell expansion (Guillet et al., 2002). A cDNA encoding PEPC LYCes;Ppc2 (LG7), is specifically expressed in fruit tissue; whereas an alternate cDNA LyCes;Ppc1, mapped to LG 12, is expressed throughout the plant. Although PEPC may be crucial for organic acids accumulation, variations in enzyme activities and fluxes suggest other metabolism influences final concentrations.

Although QTLs for organic acids contents (Table 1.2) have been reported, it was unexpected that these were not linked to QTLs for sourness but to sweetness and

flavour quality QTLs (Quilot et al., 2004; Obando-Ulloa et al., 2009). Therefore, this suggests acids may have significant effects on overall fruit flavour that can affect consumer acceptance and hedonics.

1.2.4 Aroma in fruit

Fruit volatile contents also changes with ripening, as a signal to insects and other seed dispersers. Aroma also imparts unique characteristics to fruit: raspberry ketone (p-hydroxyphenylbutanone) has been reported to give raspberries their characteristic aroma (Harrison et al., 1998) but is present at very low concentrations in fresh berries (Borejsza-Wysocki et al., 1994). Other volatiles important in raspberry aroma include- ionones (α- and β- ionone), hexanoic acid, ethyl esters, aliphatic aldehydes, alcohols and hydrocarbons (e.g. linalool, citral and linalyl-acetate). Certain volatiles impart unripe or 'green' aroma characters, notably aldehydes (Robertson et al., 1995; Ibáñez et al., 1998). Receptors in the nasal cavity detecting aroma volatiles are more abundant than those on palate to detect taste (Pérez, 2008): contribution of aroma to overall flavour quality is thought substantial.

1.2.5 Pigmentation in fruit

Colour is also used as a quality index in purchasing fruit - mature fruit is often intensely coloured and immature fruit often green. In raspberries, change from 'green' to 'green-red', 'pink' and finally the 'red' of maturity is through breakdown of chlorophyll and production of carotenoids and anthocyanins. The red raspberry colour is from anthocyanin pigments, dominated by cyanidin-3-glucoside, -3-sophoroside and -3-rutinoside; reddest berries exhibiting highest anthocyanin contents (de Ancos et al., 1999; Kassim et al., 2009; McCallum et al., 2010). Some volatiles utilise carotenoid degradations as a basis for synthesis, suggesting close associations of volatiles and pigment productions and subsequent aroma and colour development (Lewinsohn et al., 2005). Apart from visual effects, anthocyanins impart antioxidant characteristics (Deighton et al., 2000; Wang and Lin, 2000; Kähkönen et al., 2001; Pantelidis et al., 2007; Çekiç and Özgen, 2009).

1.2.6 Protected cultivation and fruit ripening

Ripening is affected by environmental conditions, particularly in the onset and length of ripening times. Such effects can be minimised or eradicated by protected cultivation. Thus with polytunnels, growth under plastic films, problems in seasonal variation, pest infestation, light and water availability can be overcome and production of crop out of season achieved (Cohen et al., 2005; Kittas et al., 2006; Hanafi et al., 1999). Growth out of season had minimal effects on flavour in loquats (Polat et al., 2005), strawberries (Atkinson et al., 2006; Voća et al., 2009) and red raspberries (Sønsteby et al., 2009). Fruit yield increases, an effect observed both in this present study and other studies.

1.3 Fruit flavour quality

1.3.1 Flavour attributes

Flavour is important in consumer evaluations - taste, aroma and mouthfeel. Each group of attributes (e.g. *sweet, sour, juicy, mealy*) is evaluated in different sensory systems responding to complementary stimuli and information differentially brought together in the brain to yield virtual images.

1.3.2 Integration of oral, retronasal and orthonasal information to form flavour

Taste and aroma perception are linked in flavour character. Sweetness perception is a complex combination of gustatory, olfactory and oral somatosensory cues (Zampini et al., 2007). Taste perception is strictly from neural information in the brain provided by receptors reacting to compounds on the tongue and palate. This can be isolated and examined by use of clips to block air flow into the nose. Aroma is information gathered through orthonasal (front) - as opposed to retronasal (rear) passage - of volatile compounds: experimental evidence suggests differences in interpretation (Landis et al., 2005). Combinations of aroma and taste information take place in flavour perception, interacting with brain pleasure centres.

1.3.3 Taste detection

Taste detection, effected on tongue and palate, includes mouthfeel and is influenced by temperature and interactions with saliva. Flavour-active compounds react with

receptors on epithelial cells of the tongue and palate, encased in taste buds that lie atop protrusions (*i.e*. papillae), of three types: circumvallate, foliate and fungiform (Fig 1.2) (Chandrashekar et al., 2006). Small pores on surfaces of taste buds permit flavour active compounds to come into contact with receptor cells, opening voltage-gate ion channels with direct relationships between taste sensation and increasing ion concentrations.

The outcome is transmission of electrical potentials to the brain via three nerves- facial, glossopharyngeal and vagus - interpreted as taste or flavour characters (Fig. 1.3). Contrary to previous models of 'tongue map' where tastes - sweet, sour, bitter, salty and umami - were detected on specific regions, all tastes are now thought to be detected across the tongue (Chandrashekar et al., 2006). Taste responses depend on stimuli provided by specific compounds - salt, saccharides, alkaloids and amino acids. Continued presence of such compounds reduces taste receptor sensitivity.

Saliva is another important factor in taste perception, providing an ionic environment required for flavour-active compounds to initiate opening of receptor cell ion channels. Saliva contains many electrolytes - immunoglobins, proteins, mucins, glucose, urea and ammonia that facilitate transport of molecules to receptors and enhance perception of specific tastes (Spielman, 1990; Humphrey and Williamson, 2001). Glucose is present at 40-50 μmol/L, lower than the detection threshold for sweetness of 10 mmol/L, sodium chloride (at 1-80 mmol/L) is above the threshold for saltiness, although prolonged exposure makes this taste undetectable and thus saliva is tasteless (Spielman, 1990). Salivary flow rate also affects taste perception and various conditions with impaired flow rate diminish taste ability: age (Grzegorczyk et al., 1979; Mojet et al., 2001), renal complications (Fernström et al., 1996; Middleton and Allman-Farinelli 1999) and oral related disorders- burning mouth syndrome (BMS), xerostamia and taste aberrations (Hershkovich and Nagler, 2004). These roles of saliva in taste perception and on overall flavour character development seem complex.

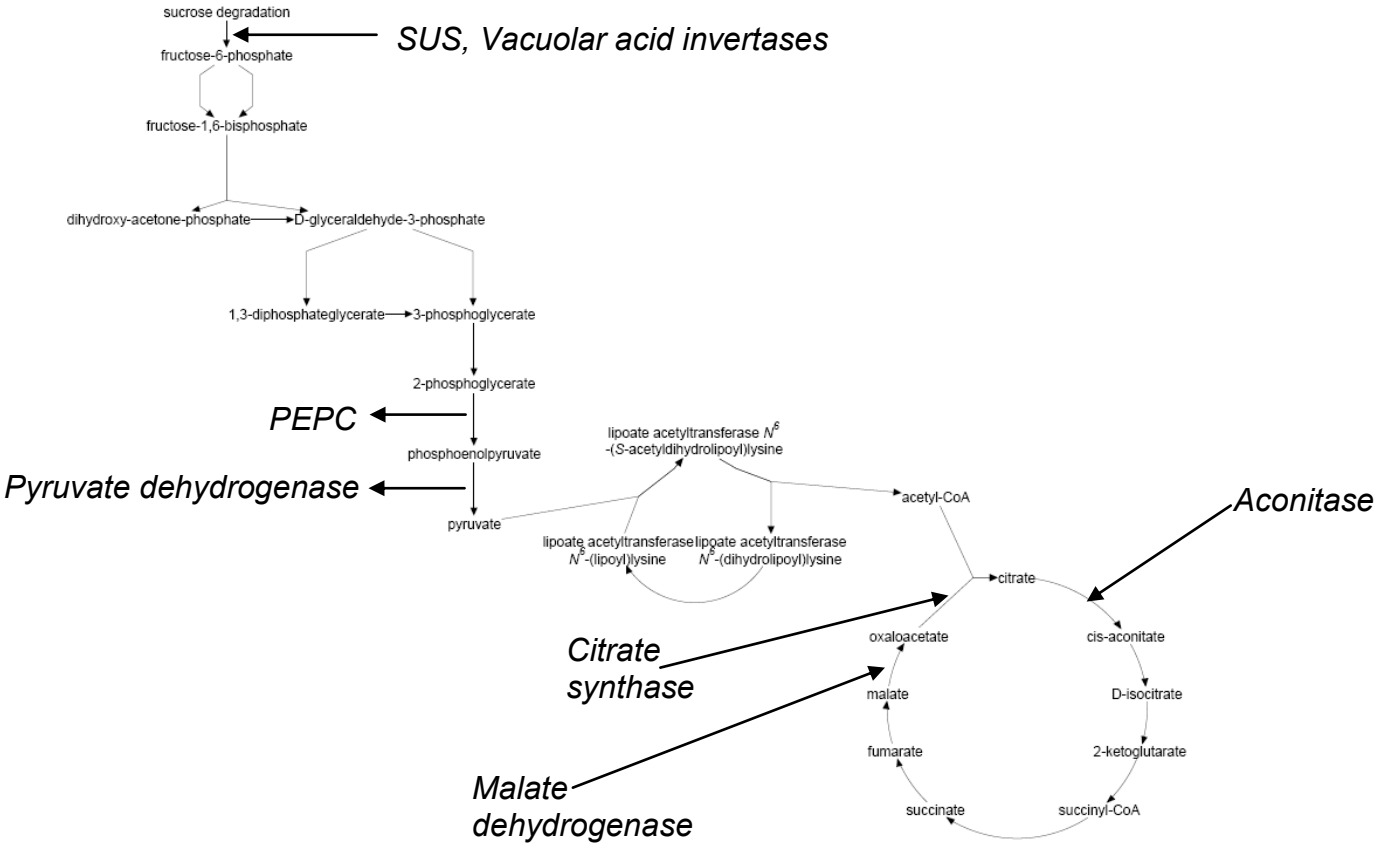


Figure 1.1 Sugars and organic acids biosynthesis are closely associated with precursors for tricarboxylic acids (TCA) cycle derived from sucrose degradation. Certain enzymes (in italics) are rate limiting.

1.3.4 Fruit flavour: role of aroma in taste perception

Aroma contributes to flavour character and intensity, through integration of information from the olfactory system with that from the tongue and palate. Once in the nasal cavity volatiles are absorbed or mixed with mucus on the surface of epithelial cells, and as in taste receptors, chemical stimuli evoke electrical impulses transferred to the olfactory bulb then to the brain for interpretation (Figure 1.4) (Firestein, 2001). Receptors for taste and aroma are both G-coupled protein receptors, dependent on ligand activation- binding to a bigger molecule to perform complex tasks - for activation of taste and olfactory genes (Kim et al., 2004; Firestein, 2001). This mechanism also influences taste sensitivity. The olfactory system is sensitive to more compounds than the gustatory (tongue and palate).

Flavour complexity is contributed to greatly by volatiles. Aroma information can also modify perceived taste: shown in sucrose/odourant and wine matrix experiments (Schifferstein and Varlegh, 1996; Martin, 2002). Sweetness and sourness have been shown to have additive and subtractive vectors to flavour intensity perception (Schifferstein and Frijters, 1990).

One theory relates intensity perception to chemical stimuli, as in Steven's power law (Stevens, 1957). Therefore, variation arises from assessor receptor sensitivities to chemical stimuli, i.e. difference in taste threshold. The relationship is described by the equation:

$$R = k\,C^{n},$$

where, R = perceived intensity (sensory response)

C = physical intensity (chemical stimuli)

k = constant

n = rate of growth of perceived intensity as function of stimulus intensity

From this equation, data variation is assumed exclusively from differences in sensory response directly and linearly correlated with intensity of physical stimuli, e.g. from concentration of chemical compounds. In real situations, sensory responses are not always linearly correlated with stimuli and differ between individuals which, in

sensory panels, is a source of variation (Baek et al., 1999). The sensory score for aroma intensity in panellists differed for a set of sucrose-gelatine gels (different concentrations) flavoured with furfuryl acetate. Sensory score was correlated with rate and technique of mastication rather than volatile concentrations, as well as individual differences in threshold and response.

An alternative theory to Steven's Law is proposed, which includes assessor adaptation to stimuli before perception. This (Reed et al., 2006) more accurately represents the reality of sensory perception of soft fruit characters, as it takes into account a range of compounding factors: relative metabolite quantities and ratios; oral dilution by saliva and mucus in nasal cavity; juice contents and receptor sensitivities of taste and olfactory systems of assessors.

1.3.5 Sensory panel limitation: Variation in taste and aroma perception

Assessors score stimuli differently in part through differences in taste and aroma sensitivities but also through variations in use of scales. Sensory sensitivity is influenced by genetic determinants of receptor sensitivities. In humans, bitterness perceptions of 6-n-propylthiouracil (PROP) have been shown to influence food preferences in children (Anliker et al., 1991) and also in diet, namely vegetable consumption (Dinehart et al., 2006). Receptors for bitterness are genetically determined in both children and adults, influencing preference, as well as linked to sweetness perceptions (Mennella et al., 2005).

Increased sensitivity to specific tastes may influence food choice e.g. variations in human bitter detection in dietary vegetable intake. Thus it is important to determine if sensory data variance arises from differences in samples or assessors.

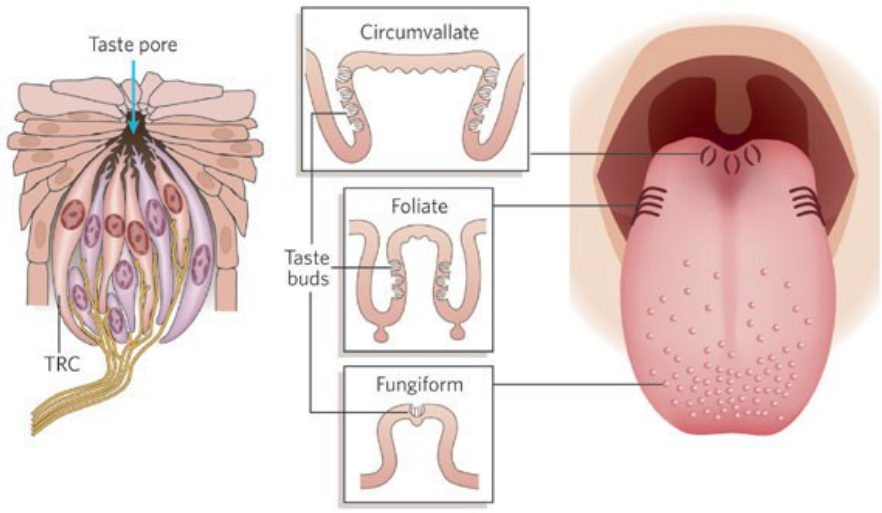


Figure 1.2 Lingual sensors of taste as summarised by Chandrashekar et al., 2006. TRC=taste receptor cells.

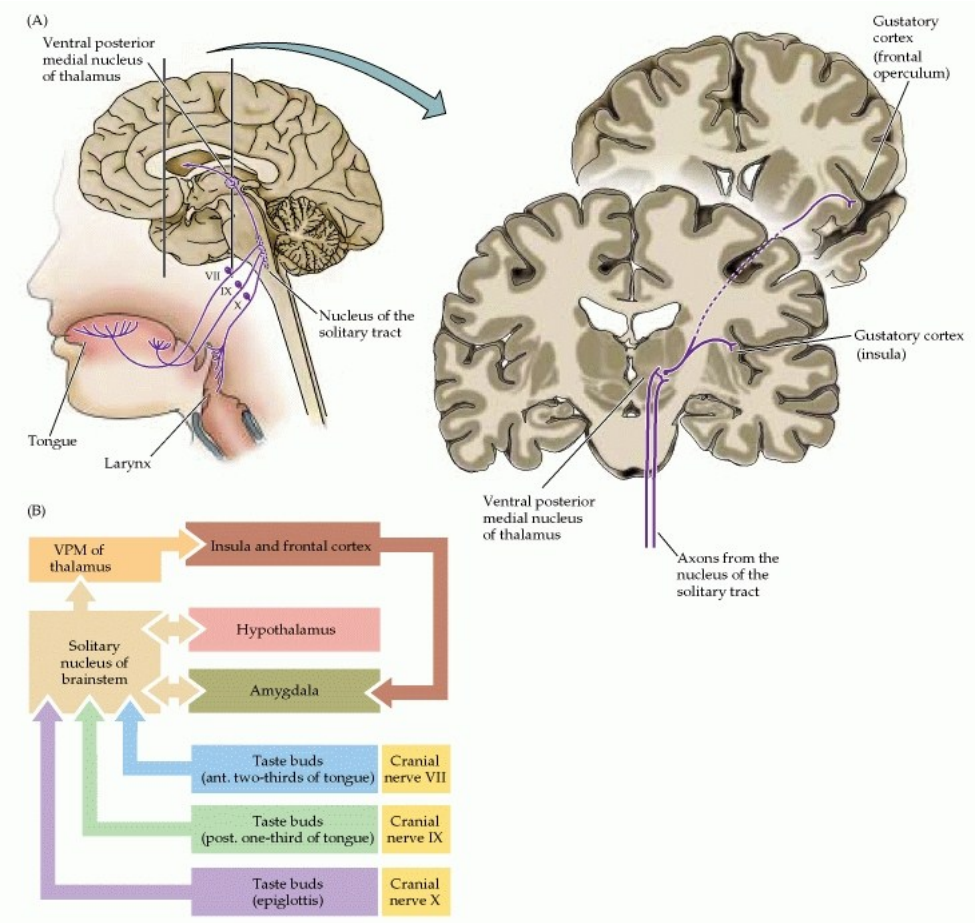


Figure 1.3 Relay of electrical potential from taste receptors via nerve cells to the brain for interpretation of taste (*www.ncbi.nlm.nih.gov*)

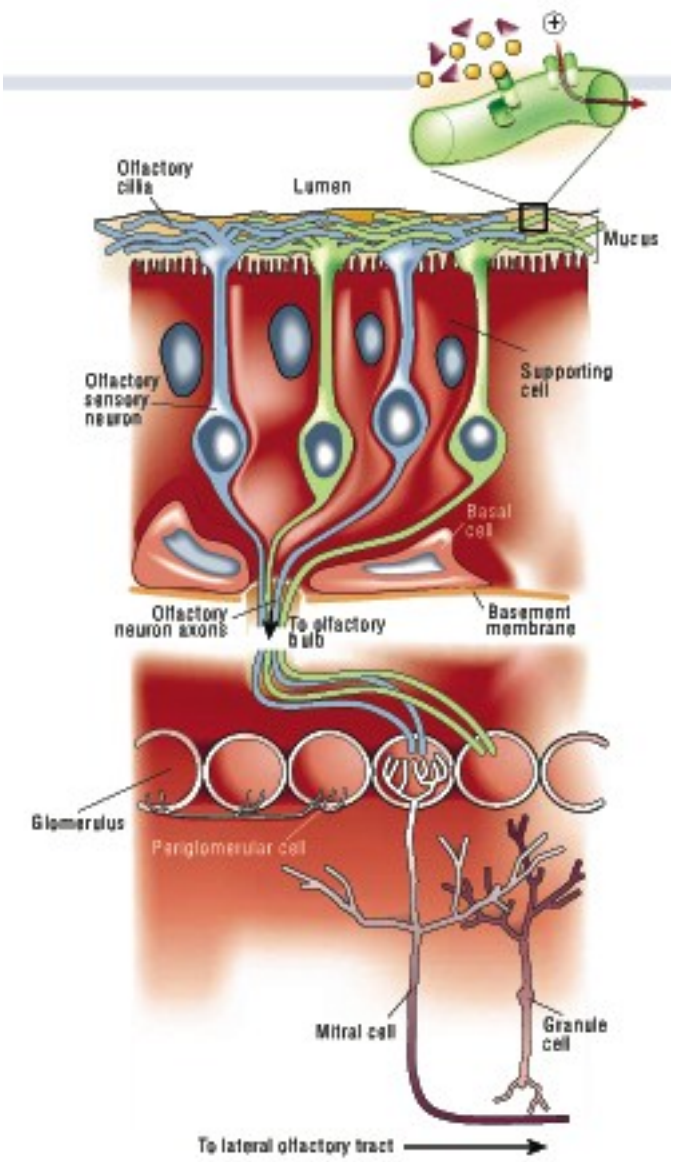


Figure 1.4 Aroma detection: olfactory system (Firestein, 2000).

1.4 Sensory analysis

1.4.1 Role in fruit

Instrumental analyses can quantify fruit metabolite contents that are often used to represent quality. But only sensory analysis can provide interpretation of fruit metabolite stimuli by the brain: flavour interpretation as well as sensation. Quantities of flavour-active metabolites in ripe fruit vary, through environmental and genetic factors, and are reflected in differences of final flavour characters. Correlating flavour metabolites and sensory data could reveal quality information (quantitative and qualitative) which, linked to genetic data, facilitate marker-assisted breeding for fruit flavour quality.

1.4.2 Panel selection: Expert vs. semi-trained vs. consumer

Strategies in sensory analysis can seek to meet different objectives, e.g. product characterisation, product preference or quality assessment, requiring different types of assessor: expert, trained and semi-trained. Differences in training intensity influence product information and descriptions. For example, untrained consumer assessors give information on product acceptance, preference and segmentation within target groups; trained assessors provide more detailed definitions of product attributes. It has been concluded that different training levels provide different information, but are equally important in product success (Moskowitz et al., 2006) (Table 1.3).

Table 1.3 Differences in objective of sensory analysis (Moskowitz et al., 2006).

Type	Consumer acceptance test	Market research
Scale	**Medium panel**	**Large panel**
Assessors	50-100	≥100
Purpose	Determine overall preference, or sensory properties such as appearance including colour, flavour and texture	Usually focuses on consumer populations and identifying the consumers to whom the product would appeal, and developing the understanding of such segmentation

Due to objectives, time and cost constraints, assessors that are trained, semi-trained, consumer or expert can be selected, but for each panel there are concerns over data robustness and validity of product representations. Parallel panel studies (i.e. trained

vs. untrained, expert vs. trained, trained vs. consumer) have suggested advantages to each alternate strategy. In sensory comparisons of toasted almonds, expert assessors exhibited objective analysis, produced more non-redundant descriptors of products and were more successful in detecting small differences between samples (Guerrero et al., 1997). Furthermore, both expert and trained assessors were successful in discriminating flavour and texture. A goal-oriented approach of expert assessors was also demonstrated in sensory texture studies of Mahon cheese but with continued training of non-expert assessors, inter-session variability decreased and a similar representation of product characters emerged (Gonzáles et al., 2002). In food formulation, changes influencing production costs in food companies, data from both trained and consumer assessors were more advantageous than data from in-house trained panel alone to decide if changes altered product acceptance (Ishii et al., 2007). Although training helps improve panel performance, some panels show a similar degree of accomplishment, regardless of training level. Profiling data of 12 perfumes by expert and consumer assessors were comparable, reproducible and showed similar abilities to discriminate products (Worch et al., 2010). The ability to detect differences in products is not always improved with training; trained and untrained panellists performed equally well in groupings of beer when a descriptors list was provided (Lelièvre et al., 2008). A priority in any sensory analysis is relevance of information to both industrial and commercial sectors, as these are trend drivers and ultimately determine product success. Information such as of fruit character must be a representative collection of data covering most factors important through supply chains. Nevertheless, it is generally reported that training improved panel performance in assessment of product character, with the smallest variation found in experts and the largest in consumers (Guerrero et al., 1997; Husson and Pagés, 2003; Gonzáles et al., 2002; O'Sullivan et al., 2003; Barcenas et al., 2004; Ishii et al., 2007; Lelièvre et al., 2008).

1.4.3 Importance of correlations in sensory and instrumental flavour analyses

It is thus evident that successful fruit flavour research requires a combination of instrumental measurements of flavour-active metabolite contents, sensory analyses and consequent correlation by statistical modelling. Such strategies should identify

factors, both objective and subjective, that have most impact in overall fruit flavour character.

Association of instrumental measurements and sensory data of flavour in key fruit crops (i.e. tomato, peach, melon) are listed in Tables 1.2 - 1.4 as are also QTLs and candidate genes. In tomato, there were consensuses of sensory and electronic nose (EN) data in off-odour volatile production in two situations- damage by growth conditions and transport and organic vs. commercial cultivation practices. In comparative studies, an electronic nose performed better than sensory assessment at grouping similarly damaged fruit (Johansson et al., 1999; Sinesio et al., 2000). From such results, instrumental monitoring seems suitable for routine flavour quality assessment, whereas sensory analysis is needed to determine if consumer expectations of quality are met.

1.4.4 Genotypic, environmental and seasonal effect on flavour quality

Chromosomal regions encoding sensory QTLs indicate genetic determinants of fruit quality. Sensory data co-localised with continuous trait QTLs for flavour-active metabolites imply common genetic expression and control mechanisms.

There is genetic evidence in other studies of flavour quality control through metabolite content in *Rosaceae* fruits. Different genotypes can segment fruit populations based on flavour - peach and nectarine cultivars grouped into 4 or 5 groups based on sensory sweetness, sourness, peach/nectarine flavour and aroma intensity scoring (Crisosto et al., 2006). In apricot genotypes (43), high soluble solids content (SSC) and ^{o}Brix values were identified in fruit of premium character with significant effects ($p < 0.05$) of genotype on flavour attractiveness and SSC levels (Ruiz and Egea, 2008). Genotypic factors also helped define flavour character objectives in kiwifruit breeding, notably a flavour wheel linking sensory descriptors to flavour-active compounds from comparison of 10 kiwifruit genotypes (Wismer et al., 2005). Acquiring information on genotypic control and its effects on flavour development through metabolite biosynthesis is a desirable breeding objective: advanced development would be advantageous to both species and overall quality.

Cultivation conditions have varied effects on crops for example they can be a source of off-flavours in tomato (Sinesio et al., 2000). Information on cultivation conditions (i.e. organic vs. commercial) can also influence flavour perception by assessors with a significant effect also reported on preference scoring in tomatoes (Johansson et al., 1999). Sources of bias should be kept to a minimum to ensure sensory data is both robust and representative of the product.

Effects of cultivation on quality have been established in tomato. Clearly established are variations in yield, weight and diameter of fruits as well as sensory scoring for overall taste (Causse et al., 2002). A growing system that both protects and allows root development, used commercially, produced fruits scored highly for flavour (Thybo et al., 2006). In contrast, another study showed no significant influence on juiciness and firmness, important for quality (Žnidarčič et al., 2003).

Fewer studies have been reported for soft fruits. Protected cultivation increased both sugars and acids contents in blueberries (Molina et al., 2008) and yield, SSC and ^{o}Brix in strawberries (Voća et al., 2009). Quantities of flavour-active metabolites and sensory character have a close relationship, with predicted effects on quality.

Seasonal variation can also influence flavour quality and sensory scoring, with effects minimised but not eradicated by protected cultivation although genotype is thought more important (Bunning et al., 2010). Pigmentation is also affected by seasonal variation: pomegranate arils of three cultivars of differing ripening times (i.e. early, mid and late- ripening) varied in colour intensity development through the season, but not in antioxidant capacity (Borochov-Neori et al., 2009).

1.5 Relating flavour to fruit composition

1.5.1 Statistics

Understanding flavour characters in fruit requires modelling relationships between different datasets, which requires care. One preliminary stage is to examine how much variance is from experimental error. Analysis of data is necessary before complex regression analysis to model relationships through multivariate strategies.

To increase interpretive capabilities of relationship models, variables should be selected to represent most variance in data, indicating strongest influence on response. Models can be both representative and comprehensive for variables important to flavour development. Listed below are some examples of data deconstruction strategies.

1.5.2 Initial strategy: Reduction in variables through univariate analysis

Analysis of means: analysis of variance (ANOVA) identify variables most associated with specific traits. Types of analyses are: one- (single) and two-way ANOVA (independent variables); MANOVA (multiple dependent variables); and balanced ANOVA (balanced experimental designs). Significance of relationships between variables and trait, indicated can be calculated (p-value).

Pearson correlation: this analysis estimates strength of linear correlations with significance calculated as p-value and correlation coefficient as r. Values of r range from +1 (positive association) to -1 (negative association); values near zero are variables of little influence. High values of r do not imply a causative link, only additive or subtractive effects of contribution of variables.

1.5.3 Data processing prior to multivariate analyses

Multivariate analyses of instrumental data are often regarded (by reductionists) as objective. Sensory data and instrumental measurements, can yield valuable regression models, predicting effects of metabolites on character. Each variable should be considered initially to ensure an equal chance of contributing to trait (Abbott, 1999). Typically, data matrices are normalised: centred by subtracting with group mean value - (i.e. $X - \mu_{group}$) and weighted by division with group standard deviation value (i.e. $[X / \sigma_{group}]$) (Meullenet et al., 2007). Outlier data points should be examined prior to modelling.

1.5.4 Multivariate modeling: Principal Component Analysis (PCA) and Regression (PCR), Partial Least Squares Regression (PLSR)

Principal component analysis (PCA) identifies factors (principal components, PC) with most effect (variance) on data. The first PC, from scree plots, explains

maximum variance, repeated cycles of correlation yield successive factors (i.e. PC2, PC3….PCn) explaining decreasing amounts of variance on the original data set. Generally, instrumental data yield more significant factors, and better overall explanation (higher % variance) than sensory: more variables explain greater variation. Although each factor explains variance, the likelihood of correlation with any individual variable is small: there are likely to be additive effects. Correlations between independent x-variables can affect overall total variance in dependent y-variables.

To overcome multicolinearity in independent x-variables, principal component regression (PCR), which combines PCA and regression analysis, produces factors most representative of variability. Dependent x-variables are assigned numerical factor loading values close to +1 or -1. Therefore, x-variables with most impact on dependent y-traits are easily interpreted. Independent x-variables in each factor are also not correlated. A shortcoming of this statistical approach is although all x-variables are assured to have no correlation to each other (to minimise the multicolinearity effect), there is a possibility that they will also bear no correlation to dependent y-traits. Therefore, a further analysis of factor inclusion and exclusion most related to a dependent y-trait is needed before a PCR regression model can be thought to be reasonably representative of a relationship (Meilgaard et al., 1999).

Partial least square regression (PLSR) yields multivariate factors from unrelated x-variables that explain the most variance in dependent y (e.g. traits) (Meilgaard et al., 1999). There are two common approaches: explanation of a single y-variable - PLS1; or of multiple y-variables - PLS2. Robust PLS models can predict responses from independent data. There are 2 sets of values for each independent variable, e.g. $x_{original}$, $x_{predicted}$- variable). The difference of $x_{original}$- $x_{predicted}$ is residual, ε. When all ε-values are summed and squared (i.e. SS_{res}), small values indicate the desirable similarity of $x_{original}$ and $x_{predicted}$, hence model- 'method of least squares'. Quality can be examined as effects of x-variable on y-variability: from the fitted regression line; from $\varepsilon_{original}$ and $\varepsilon_{predicted}$ values; and from the R^2 value, with >0.75 considered good (Table 1.6). In PLS1, success in modelling original to predicted data is ascertained from R^2-values, raw and adjusted, whereas in PLSR models performance can be

evaluated from the low value of root mean square errors of prediction (RMSEP) and of calibration (RMSEC). In a study of Manchengo cheese, RMSEC <0.8 was an indicator of a good model and predictive capacity for sensory scoring in profiling (Cabezas et al., 2006). As an alternative,a calculated ratio value can assess the predictive capacity of a model. The ratio of prediction to deviation (RPD) is used or the ratio of standard deviation to RMSEP value (i.e. σ_{group}: RMSEP) with values ≥ 2 indicating good prediction (Meullenet et al., 2007; François et al., 2008).

Table 1.4 Important PLSR numerical outputs to assess model predictive capacity (Meilgaard et al., 1999).

Description	Equation	Term
Residual	$x_{original}$- $x_{predicted}$ or $y_{original}$- $y_{predicted}$	ε_x1, $\varepsilon_x2...\varepsilon_xi$ ε_y1,$\varepsilon_y2....\varepsilon_yi$
Residual sum of squares	$\Sigma\varepsilon_i^2$	SS_{Res}, SS_{Tot}
Residual mean square	$\Sigma[(\varepsilon_x1+\varepsilon_x2,\ldots +\varepsilon_xi)/ n]^2$ $\Sigma[(\varepsilon_y1+\varepsilon_y2,\ldots +\varepsilon_yi)/ n]^2$	MS_{Res}
Coefficient of determination	1- (SS_{Res} / SS_{Tot})	R^2
Coefficient of determination adjusted	1- [((n-1)- MS_{Res}) / SS_{Tot}	R^2_{adj}
Regression coefficient	From equation $y= \beta_0+ \beta_1x+ \beta_2x^2+ \beta_3x^3$ $= \beta_0+ \beta_1x_1+ \beta_2x_2+ \beta_3x_3$ estimation; $b_1= [\Sigma(x_i-x_{mean})* (y_i-y_{mean})] / [\Sigma(x_i-x_{mean})^2$ $b_0= y_{mean}- b_1x_{mean}$	β-coefficient
Root mean square errors of calibration	$[(\Sigma(x_{predicted}- x_{original})^2) / n]^{1/2}$ $[(\Sigma(y_{predicted}- y_{original})^2) / n]^{1/2}$	RMSEC
Root mean square errors of prediction	$[(\Sigma(x_{predicted}- x_{original})^2) / n]^{1/2}$ $[(\Sigma(y_{predicted}- y_{original})^2) / n]^{1/2}$ predicted data when regression model is constructed without samples no.1	RMSEP
Ratio of prediction to deviation	σ_{group}: RMSEP >2, good predictive model	RPD

Important outputs of PLSR analyses are:

1. **Scores plot**: Relative positions of samples in a multivariate space (e.g. factor 1 vs. factor 2) show groupings of similar and discrimination of the dissimilar.
2. **Loadings plot**: Relative positions of independent variables (e.g. sugars and/or acids contents) in multivariate space, in relation to dependent traits (e.g. sweetness and/or sourness scoring).
3. **Bi-plots**: Superimposed scores and loadings plots, relating samples and most closely associated variables (independent and dependent).

1.5.5 Examples of modelling sensory and physicochemical properties in other crops

Firmness is a fruit quality trait often measured instrumentally in crops subjected to lengthy transport. Visible/near infra-red (VIS/NIR) spectroscopy successfully correlated SSC content and firmness from acoustic (i.e. resonance frequency) and spectral (i.e. UV/VIS spectrometers) data of apples before and after harvest (Zude et al., 2006). With apples, 'firmness', as a trait, correlated with sensory characters (roughness, crunchiness, mealiness, sweet and sour taste) and NIR data with varying strengths: *r*-values in the range 0.49 – 0.84 (Mehinagic et al., 2003). PCR identified texture as an important factor in freshness, scoring in 10 apple varieties, for both trained and consumer assessors. Freshness was correlated with sensory crispness, mealiness and juiciness – related to measured firmness data (Péneau et al., 2007). Key quality parameters in satsuma oranges were also predicted (values of $R^2 > 0.80$) from VIS/NIR data, through PCR and PLS, namely- firmness, SSC and acidity (Gómez et al., 2006). Modelling of kiwifruit parameters from NIR data has also been reported (McGlone et al., 1998).

1.6 Other statistical issues

1.6.1 Cluster analysis: groupings in sensory assessors

The usage of scale in panels may differ between assessors despite training, either through physiological differences (i.e. genetic) and/or variation in scale usage. Cluster analysis examines patterns of scoring with, for example, derived dendograms showing similarity or differences between assessors using numerical Euclidean

distances. Hierarchal cluster analysis calculates similarity in assessors and groups that are similar within one branch of a dendogram. Non-hierarchical cluster analysis, based on similarity datasets rather than individual datapoints, is used for multiple large datasets. The association of assessor to cluster is through numerical membership weight value, (between 0 and 1). Cluster analysis can determine whether data variation is from genuine sample differences or assessor sensitivities to sensory stimuli (rogue assessor). Specific assessors can be omitted from further statistical analyses. Collating demographic information on assessors (e.g. consumer preference, age, gender, sensory sensitivities) can ensure assessor clusters are informative and not from data overfitting (Meullenet et al., 2007).

1.6.2 QTL statistics

Loci influencing intensity of specific traits (QTLs) are identified on a linkage map using statistical strategies introduced and outlined by Lander and Botstein (1989). The simplest is to examine associations of traits with polymorphic molecular markers using a *t*-test and ANOVA. Issues surrounding genotypic means of QTLs and the recombination fraction between a single marker and a QTL are resolved with interval mapping (IM) (Zeng et al., 2008). The maximum likelihood association between one QTL and a marker is the statistical basis for interval mapping of Lander and Botstein (1989), whilst Haley and Knott (1992) used regression analysis. However, such analyses identify single QTL in an analysis and may produce bias estimates. Composite interval mapping (CIM) combines both maximum likelihood and regression statistics to identify linked-QTLs more likely than a single QTL (Jansen, 1993; Zeng, 1994; Dupuis and Siegmund, 1999; Zou and Zheng, 2008). Typically preliminary Kruskal-Wallis (KW) analysis identifies chromosomal regions most associated to traits, then interval mapping is used to identify and locate QTLs, as in previous red raspberry linkage maps (Graham et al., 2004; 2006; 2009).

1.7 This study

1.7.1 Aim

The primary aim was to understand influences and determinants of three key flavour characters of fresh red raspberries: sweetness, sourness and flavour intensity. Such knowledge could contribute to progress of marker assisted breeding for premium flavour character in this important Scottish horticultural crop. Information on genetic regulation of flavour can further contribute to understanding of fruit quality development in the *Rosaceae* family.

1.7.2 Objectives

To study factors of genotype, environment (cultivation practices and season) on flavour development in progeny fruits from a cross of two dissimilar red raspberries of difference geographic origin (North America x Europe) through: (i) sensory assessment of sweetness, sourness and flavour intensity (**Chapter 3**) and (ii) fruit metabolite composition (sugars, organic acids and raspberry ketone (**Chapter 2**).

Genotypic:

Fruits were collected from 127 progeny of a cross of two phenotypically differentiated red raspberry (*Rubus idaeus*) varieties: Scottish Glen Moy (subsp. *idaeus*) and North American Latham (subsp. *strigosus*).

Environment:

Replicate progeny plants were established at 3 locations: (i) field and (ii) (covered) polytunnel sites at SCRI, Invergowrie and the (iii) commercial grower polytunnel site in Blairgowrie.

Seasonal:

Field fruit of 2 years, 2006 and 2007, were assessed – 2006 being the warmer summer.

1.7.3 Statistical Analyses Strategies

Strategies central to this study were:

QTL Mapping: of sensory and metabolite data using KW and IM strategies to a red raspberry genetic linkage map of Glen Moy x Latham (Graham et al., 2004, 2006, 2009) based on largely on DNA polymorphisms including: AFLPs, RAPDs, SSRs, SNPs, ESTs etc.

Univariate analyses of seasonal, environmental, and cultivation influences on fruit flavour and metabolite contents: ANOVA and Pearson correlation analyses.

Modelling to relate intensity of key flavour characters – sweetness, sourness and flavour intensity - to fruit contents of metabolites: PLS-1 models with fruit metabolites contents as independent x-variables, individual flavour characters (sweetness, sourness and flavour intensity) as dependent y-trait. Multivariate modelling was also effected including other data: aroma volatiles contents and antioxidant anthocyanins pigment contents; 10-berry weight; ^{o}Brix values; and reflection colorimeter readings on berries. Such additional data were obtained from other members of the research team.

CHAPTER 2

Genetics and Environmental Determinants of Fruit Metabolites

Chapter 2 Genetics and Environmental Determinants of Fruit Metabolites

2.1 Introduction

2.1.1 Raspberry non-volatiles content

Flavour is a crucial factor in quality evaluation and enjoyment of soft fruits, particularly sweetness and sourness, and their balance. These sensory characters are often correlated to sugars and organic acids contents by horticulturalists and food scientists (Malundo et al., 1995; Liem et al., 2004 a, b). Major sugars in red raspberries are fructose and glucose, with citric and malic the most abundant organic acids (Wang, 2003). Cultivated fruit has been reported to have higher sugar contents than wild, which have more acids (Shamaila et al., 1993). However fruit traits of sweetness and sourness traits are correlated not only with contents of certain non-volatile metabolites, specifically sugars and acids, but also with certain volatiles that contribute aroma notes that interact with the basic tastes in human perception of flavour. Wild raspberries are a source of germplasm for breeding of cultivated varieties species to overcome problems relating to narrow genetic diversity. Wild plants can also contribute desirable traits to commercial varieties (Çekiç and Özgen, 2010). Many factors affect accumulation of sugars and acids in fruits, notably seasons, cultivation practices and genotypic influences.

2.1.2 Raspberry ketone and its role in flavour quality

Aroma is an essential component of fruit quality and contributes to signature traits. For example, furaneol (2,5-dimethyl-4-hydroxy-3(2H)-furanone) is a volatile that imparts to strawberry and pineapple characteristic aromas (Buechi et al, 1973; Douillard and Guichard, 1990; Lavid et al., 2002). In raspberries, this characteristic aroma compound is *p*-hydroxyphenyl-butan-2-one, known as raspberry ketone.

Raspberry ketone contents in fresh raspberry fruit are often low and vary between cultivars. Wide content ranges have been reported; 0.9 – 17.4 μg to 109 - 420 μg per 100g fresh fruit (fw) (Larsen and Poll, 1990; Wysocki et al., 1992). Raspberry ketone also has low odour threshold values (0.1 – 1.0 μg / 100g fw), which suggest considerable contribution to overall raspberry aroma in spite of small quantitties

(Larsen and Poll, 1990). However there are also compounded contributions by other volatiles. Low reported quantities of raspberry ketone content in fresh raspberries could be due glycosidically bound forms as glucosides (Pabst et al., 1990) that can be liberated by heat application (Roberts and Acree, 1996). Wysocki and Hradzina (1994) found contents increased with ripening and correlated to fruit anthocyanins, which suggested links between raspberry ketone content and plant pigment development. This link is likely because these metabolites share common precursors in its biosyntheses, 4-coumaryl-CoA and malonyl-CoA (Figure 2.1).

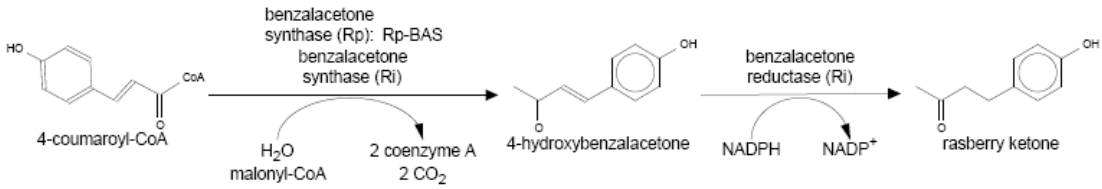


Figure 2.1 Biosynthetic pathway of raspberry ketone (Source: Plant Metabolic Network, *www.plantcyc.org*)

Other than imparting characteristic aromas, raspberry ketone also has nutraceutical benefits, such as anti-cancer (Coates et al., 2007; Çekiç and Ozgen, 2010; Bowen-Forbes et al., 2010) and anti-obesity (Morimoto et al., 2005) properties. This is possibly why both the public health community and the public in general view such berries as 'super-food', due to its promotion of health-enhancing characteristics.

2.1.3 Metabolite quantification methodology

In horticulture, it is common practice to estimate fruit sugar contents and ripeness from ^{o}Brix measurements. Technically ^{o}Brix quantifies total soluble solids content rather than reducing or total sugars and acids contents alone. To quantify sugars and acids, chromatographic and spectroscopic methods are used (Nielsen, 1994a). Enzymatic assay was shown as an effective high throughput measurement method (Vermeir et al., 2007). For analyses in fresh fruit, high performance liquid chromatography (HPLC) is a standard strategy used to quantify sugars and acids. This technique separate components through their differing affinities to stationary phases in matrices of chromatographic columns and the liquid mobile phase flow (Nielson, 1994b). Eluted components can be detected using light absorption,

fluorescence, amperometry or mass spectrometry. Alternatively, a combination of detectors can be used, depending on the components to be resolved and quantified. Accurate quantification is achievable through appropriate selection of extraction method, stationary phase and column type.

2.1.4 Aims of research

This study aimed to quantify sugars, organic acids and raspberry ketone contents in progeny fruit of a cross between Glen Moy (Europe) and Latham (North American) varieties. It also aimed to investigate effects of two environmental factors, cultivation method (open field vs. covered polytunnel) and site (different planting sites in 2007), and different harvest years and genotype, on accumulation of these key metabolites. Through statistical analyses, predicted outcomes were the initial assessment of environmental and genotypic influences on accumulation of sugars, acids and raspberry ketone that contributed to fruit flavour quality, as well as preliminary metabolite QTLs (quantitative trait loci) through genetic mapping of metabolite data to an existing genetic linkage map (Graham et al., 2009).

2.2 Materials and Methods

2.2.1 Fruit collection

Fruits were collected from progeny plants of Glen Moy x Latham cross population and those of commercial varieties. When this study started, the plants in SCRI fields were in their fifth year and 188 progeny had been planted in 3 replicates (564), with 2 identical seedlings for each of the progeny (1128 seedlings). In 2006, only fruit from field plants, 2 plants of 2 replicate plots (R1 and R2) were collected, i.e. total fruits from 4 plants. Collection from 2 replicate plots was to enable assessment of significant differences in fruits in plots. Based on knowledge and experience of SCRI staff in previous years, not all 188 progeny plants bore fruits. Therefore a subset of 149 plants was selected for harvest in the first year of study. In 2007, 2 additional sites of polytunnel plants yielded fruit from progeny of smaller subsets 107, 145 and 86. Thus, fruit of 2 plants and only from one replicate plot, was collected. Samples for analysis were based on availability and viability of plants to produce fruit. The

three sites of fruit sampling in 2007 were SCRI field, SCRI polytunnel and a commercial polytunnel in Blairgowrie. In 2007, poor weather conditions contributed to low fruit yield at SCRI field plants. Fruits of parents (Glen Moy and Latham) were picked from plants of the SCRI field site in 2006 and polytunnel site in 2007. Fruits from commercial varieties (9) were picked only from the SCRI field site in 2006. All fruits were harvested between June and August in 2006 and 2007. Fruits matured at different rates so picking was done daily, on the same side of plant and only mature red fruits (scored for colour) were harvested. After manual picking, berries were placed in polyethylene bags, sealed and labelled with unique 3 digit codes, transported at 5^{o}C to the University of Strathclyde (distance: 80.4 miles) and stored at -20^{o}C until analyses. Fertigation regimes in both polytunnel sites (SCRI and Commercial) were similar with exception of potassium-enriched fertiliser being used at the commercial site. For raspberry ketone contents, quantification was done only for 2007 SCRI polytunnel fruits. These fruits were selected as more aromatic (pers. comm. SCRI staff) and higher in sugars and acids contents compared to that from the field. Fruit was harvested to support 3 parallel projects and each plant varied in fruit yield. Therefore, each project received a finite number of fruit samples and for this project, samples were split into two: for metabolite quantification (sugars, organic acids and raspberry ketone) and sensory analyses. Sensory analyses were performed on fresh fruit and therefore given priority. Metabolite quantification was performed later and on fruits remaining after sensory analyses. Therefore, the number of datapoints for sugars and acids contents varied, depending on fruit availability. Acids contents were analysed first: hence there were more datapoints. However, for each metabolite, the number of datapoints was adequate for statistical treatment.

2.2.2 Extraction of sugars and acids from fruits for quantification

The modified protocol of Sturm et al. (2003) was used to quantify sugars and acids. Berries were thawed for 2 - 4 h at 4^{o}C and approximately 0.3g was weighed into 1.5ml micro-centrifuge tubes. Fruits were pureed using sterile toothpicks and after addition of sterile distilled water to a mark, the mixture was vortexed for 20 seconds and centrifuged at 16 x 1000*g* for 30 minutes. Supernatant (800 µl) was pipetted onto regenerated cellulose filter units (pore size: 0.45um; Alltech, Illinois, USA) placed in

1.5 ml micro-centrifuge tubes then centrifuged at 16 x 1000*g* for a further 10 min. Sugars and acids were quantified from 20 µl aliquots of supernatant

2.2.3 Extraction of raspberry ketone from fruits for quantification

A modified extraction and quantification method from Wyosocki and others (1992) and Hamid (1996) was used for raspberry ketone analysis. Frozen fruit was thawed overnight at 4^{o}C before processing. Approximately 50g fruit was weighed, placed in jellybag (Jelly Bag (Ref no. 3810), Lakeland Ltd., UK) and pressed for juice with a winepress. Collected juice was filtered through a coffee filter placed within a glass funnel over a 20ml glass beaker. Sterile distilled water (1x volume) and 15g / 100g fresh fruit of filter aid (Hyflo SuperCel® Medium; Sigma-Aldrich, MO, USA) were added to collected juice. Mixtures were decanted into 30ml centrifuge tubes (Nalgene Oak Ridge Centrifuge Tubes; Thermo Fisher Scientific Inc., NY, USA) and centrifuged at 10, 000 x *g* for 20 min at ambient to remove filter aid. Supernatant was decanted into 20ml glass beaker, sealed with parafilm and raspberry ketone extracted immediately.

A non-polar solid phase extraction (SPE) (Varian Bond Elut™ C18 SPE column, Varian Inc., CA, USA) cartridge was used to condition the bonded phase, 3 ml methanol and 10 ml sterile distilled water were passed successively through the cartridge, under 17 kPa vacuum, discarding the eluent. Juice (3ml) was then applied to the SPE column, followed by 10 ml sterile distilled water to elute sugars and acids and the eluent was again discarded. To desorb raspberry ketone from the bonded phase, 10 ml of pentane:dichloromethane (1:1; w/v) solvent mix was passed through the column and eluent was collected. A drying agent, 100 mg Na_2SO_4, was added to eluent and the mixture was subjected to rotary evaporation until dryness. To resuspend raspberry ketone into aqueous solution, 1.5 ml HPLC mobile phase (30% acetonitrile, 30mM KH_2PO_4, pH 6.2) was added to the residue and mixed well. The aqueous mix was collected into 1.5 ml centrifuge tubes and centrifuged at 14000 x *g* for 15 min to separate Na_2SO_4. The supernatant was collected, aliquoted into 0.75 ml HPLC vials and kept at -20^{o}C until analysis. All extractions were done in duplicate to assess reproducibility.

2.2.4 Calibration curves

HPLC grade (Sigma-Aldrich, St. Louis, USA) fructose, glucose, citric and malic acid solutions were prepared with distilled water, in increasing concentrations from 0.4 mM to 50.0 mM, and used to derive calibration curves. The volume of solution used was 20 µl. Separate calibration curves were calculated for 2006 and 2007 fruit.

A similar method was used to construct calibration curves for raspberry ketone, with solutions of increasing concentrations from 1 µg – 400 µg / 200 ml mobile phase. Mobile phase was used to dissolve raspberry ketone as it was not soluble in distilled water. Calibration curves were plotted from area under the curve using 20 µl aliquots of known concentrations.

2.2.5 Quantification of sugars

HPLC quantification was performed with mobile phase of degassed deionised distilled water (conductivity 18Ω) at flow rate of 0.6 ml / min with an ion-exclusion column (Varian MetaCarb Pb Plus, 7.8 x 30 mm) held at 85^{o}C. Sugars were quantified using a low-temperature evaporative light scattering detector (Sedex Model 55, Sedere, France) operating at 90^{o}C (Dreux and Lafosse, 1995). For quantification of sugars in 2006 fruit, replicate injections were made on half of the samples (160) from each replicate plant (i.e. 80 from each of rep. 1 and rep. 2) to assess reproducibility and variations between replicate plants. Based on the results in 2006, analysis was performed on only one replicate from each site in 2007, with replicate injections on half of samples from each site, to assess the reproducibility of the method. Data was recorded through an integrator (Varian 4950), which produced chromatograms and calculated areas under peaks. Contents of fructose, glucose and total sugars (fructose + glucose) in fruits were derived from standard equations developed from standard curves of sugars.

2.2.6 Quantification of organic acids

To quantify the acids content of fruit, HPLC was performed with degassed sulphuric acid (4.0 mM H_2SO_4) as mobile phase at 0.4 ml / min resolving acids also by an ion-exclusion column (Varian MetaCarb H Plus Column, 7.8 x 30 mm) at 65^{o}C. Detection was by a variable wavelength UV-Visible spectometer set at 215 nm. Data

was recorded using data management software, ChromPerfect LSi, (Justice Scientific, New Jersey, USA) installed on a PC which generated chromatograms. Replicate analyses were as for determination of sugars. The concentrations of citric, malic and total acids (i.e. citric acid + malic acid) were calculated from standard equations developed from standard curves.

2.2.7 Quantification of raspberry ketone

HPLC analytical parameters for quantification of raspberry ketone contents in fruits were as follows; mobile phase, 30% acetonitrile adjusted to pH 6.2 with 30mM KH_2PO_4 at a flow rate of 1.0 ml / min. Separation was though a reversed-phase C18 bonded phase (Waters µBondapack C18 column, 10µm, 46 x 150 mm, Waters Corp., MA, USA), operated at 25^{o}C. Detection was through a variable wavelength UV-Visible detector set at 280 nm.

2.2.8 Statistical analyses

Minitab v. 14.1 statistical software (Minitab Inc., PA, USA) was used for univariate data analyses, to plot calibration curves and to produce graphic output. To determine reproducibility, analysis of variance (ANOVA) was employed with $p < 0.05$ as significant. To analyse linear correlations of factors (e.g. harvest years and planting sites) on sugars and acids contents, Pearson analyses were done, with significant linear correlations indicated by correlation coefficient values, r, and p- values. For these, r-value = 1 was taken to indicate complete linear correlation and $p < 0.05$ indicated that correlation was significant at the 95% confidence interval. Scatterplots were used to illustrate distributions of sugar and acid contents, with further display of distributions through histograms and boxplots.

2.2.9 QTL analysis

Initial variance analyses across harvest years and sites were performed and quantitative data were mapped onto an existing red raspberry genetic linkage map (Glen Moy x Latham; Graham et al., 2009). This had 7 linkage groups, over 843 cM and 243 molecular markers. A Kruskal-Wallis test was used as a preliminary to identify regions of the genome that were linked to each of the first five principal coordinates, and whether a phenotype was affected by alleles from one parent or

both. A small permutation test was carried out using Genstat 10 for Windows (Genstat Ltd, 2007) to establish appropriate thresholds for the Kruskal-Wallis test. Interval QTL mapping was performed using MapQTL 5 software (Van Ooijen, 2001). If the Kruskal-Wallis analysis indicated that the phenotype was affected by alleles from both parents, the trait was analysed using a four-mean QTL model. If alleles from only one parent were affecting the trait, the marker data was recoded so that MapQTL fitted a two-mean model. QTLs were identified through interval mapping with permutations of 10 rounds or less and with LOD scores > 2.0. Genetic distances of identified preliminary QTLs were calculated using the Kosambi function (Graham et al., 2004, 2006, 2009). Preliminary QTLs were represented as vertical bars spanning the chromosomal regions in each LG with blue representing preliminary QTLs for 2006, black or 2007 and red QTLs of raspberry ketone contents.

2.3 Results

2.3.1 Standard calibration graphs and variation of replicate plants and measurements

Retention times were established for glucose (13 min), fructose (19 min), malic acid (18 min) and citric acid (14 min). Below are standard graphs for sugars (Fig. 2.2), acids (Fig. 2.3) and raspberry ketone (Fig. 2.4). Linear regression values, *R*, for all except malic acid was above 0.8, which indicate good robustness of quantification method.

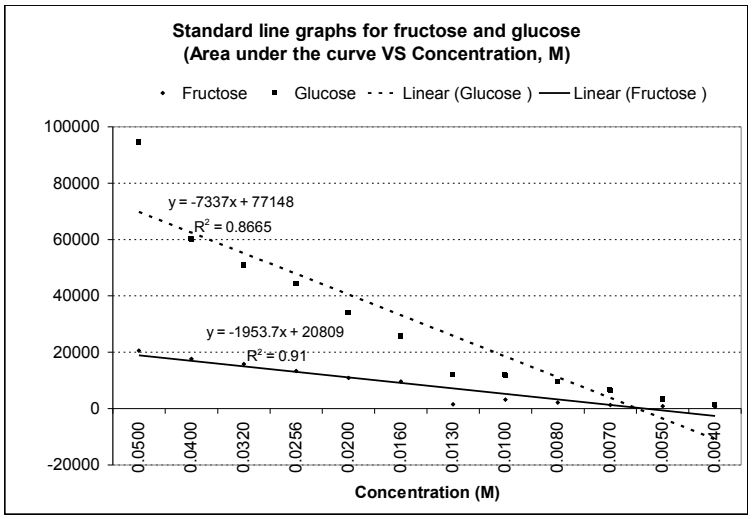


Figure 2.2 Standard graphs and equations for fruit sugars, fructose and glucose, in aqueous solution

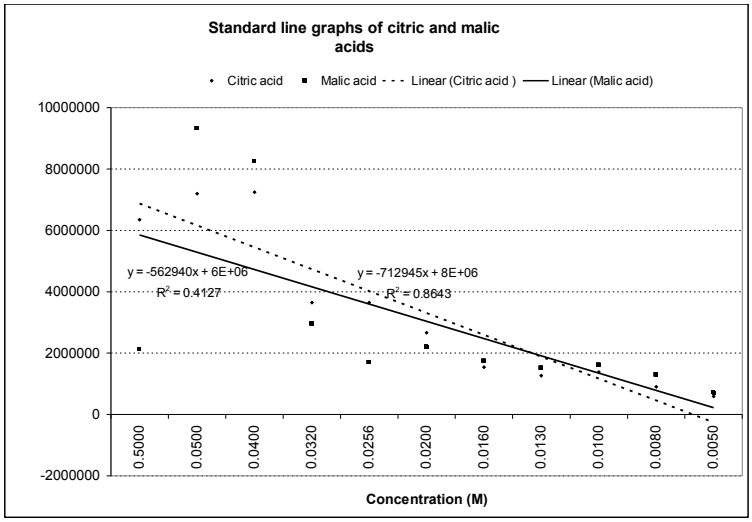


Figure 2.3. Standard graphs and equations plotted for fruit organic, malic and citric, acids, in aqueous solution

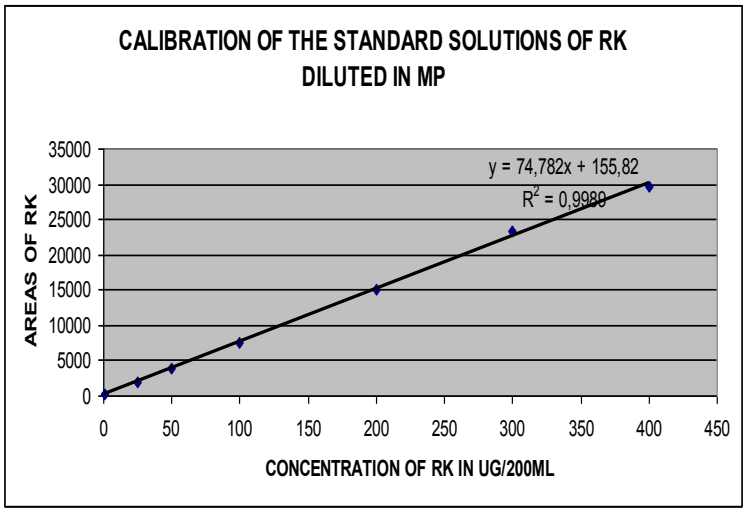


Figure 2.4 Standard graphs and equations for raspberry ketone (RK), diluted in mobile phase (MP).

From ANOVA analyses on replicate extraction and injection data of sugars and acids contents, $p > 0.05$ indicated variations caused by these factors were non-significant. ANOVA analyses also yielded non-significant variations between replicate plants in 2006 ($p > 0.05$). Thus it was decided that data on fruits from a single replicate in each planting site in 2007 would suffice.

In quantification of raspberry ketone contents, extraction and quantification methods were reproducible, as p was > 0.05 from ANOVA analyses on data of replicate injections and extractions. However, variations between progeny plants were significant ($p < 0.05$ in ANOVA).

Key points:

- Differences in fruit sugars and acids contents from replicate plants in 2006 were non-significant.
- Thus in 2007, fruits from plant of only 1 replicate from each planting site were analysed.
- Extraction and quantification methods of fruit sugars, acids and raspberry ketone contents were reproducible.

2.3.2 Sugars and acids contents, and ratios, in commercial and parent fruits

Fruit was analysed from 11 varieties (2 parent and 9 commercial varieties). Latham fruit had higher sugar contents than in Glen Moy, but the quantitative differences between them were not significant (Table 2.1). Highest and lowest quantities were in Autumn Bliss and Octavia varieties respectively, of which both had significantly different contents compared to Latham. In acids content, Glen Moy had significantly lower quantities compared to 7 commercial varieties with highest and lowest quantities in Glen Rosa and Malling Leo respectively. Despite significant quantitative differences between all commercial varieties, fructose : glucose ratios were similar with exception for Malling Leo, which was significantly different to Glen Moy. Citric : malic acid ratios were similar in all varieties with highest value in Glen Magna. Total sugars : total acids ratio is an important quality indicator in the fresh fruit industry. All Gold, a yellow raspberry variety, had highest value, higher by more than 300% higher compared to Glen Moy. Total sugars : total acids ratios were similar amongst all varieties with lowest value in Octavia. Ratio values could

not be calculated for Latham because acid quantification was not performed due to inadequate fruit availability from the 2006 field site. Latham variety also bore small berries, each weighing less than 3 g. In total sugar contents, most commercial varieties had significant differences compared to the 2 parent varieties. In total acids, none had significantly different quantities to Glen Moy.

Key points:

- Sugars contents were higher in Latham than in Glen Moy.
- Latham had 2nd highest contents in fructose amongst all commercial varieties quantified here.
- Glen Moy had one of lowest acids content in all commercial varieties.
- Total sugars : total acids ratio in Glen Moy was one of lowest values in all commercial varieties.

2.3.3 Sugars and acids contents, and its ratios in progeny fruits

2006 field crop had higher total sugars and acids contents compared 2007 field crop, by 19.1% - 235.4% respectively (Table 2.3). Under polytunnel cultivation in 2007, Glen Moy had increased total sugars, by more than 500%, compared to Latham. However, Latham had higher total acids content, by more than 600% compared to Glen Moy.

In 2007, highest overall contents for acids and sugars were in the Commercial polytunnel crop (Table 2.3). In SCRI crops, polytunnel fruit had higher sugar contents than field, by 33 – 35% higher for fructose and glucose respectively. But acids contents in both field and polytunnel crops were similar. In polytunnel crops, Commercial crop had higher total sugars and acids contents than in SCRI, by 41.7% and 27.4% respectively. These quantitative differences observed in polytunnel crops could be related to increased berry size and fruit yield (Graham, pers. comm.).

Table 2.1. Individual and total contents of sugars and organic acids in field fruit of parent (in bold and italics) and commercial varieties in 2006. SED = standard error of mean difference, LSD = least significant difference. Significant difference between parent and commercial varieties is denoted by * and +, using 5% LSD calculated with $t = 2$ and $p = 0.05$. (*different to Latham, + different to Glen Moy and *,+significantly different to both parents).

Site	Values	Samples	Fructose (g/ml± 0.130)	Glucose (g/ml ± 0.511)	Malic Acid (g/ml ± 0.053)	Citric Acid (g/ml ±0.050)	Total Sugars (g/ml ± 0.567)	Total Acids (g/ml ± 0.083)	Fructose: Glucose (±0.103)	Malic acid : Citric acid (±0.578)	Total sugars : Total acids (±1.873)
Field 2006	Mean± SED	***Glen Moy***	0.051	0.357	0.079	0.137	0.408	0.216	0.143	0.577	1.889
		Latham	0.247	1.045	-n/a-	-n/a-	1.293	-n/a-	0.236	n/a	n/a
		Tulameen	0.073*	0.940+	0.074	0.145	1.012+,*	0.219	0.078	0.510	4.621
		Joan J.	0.137+,*	0.902+,	0.076	0.145	1.039+	0.221	0.152	0.524	4.701
		All Gold	0.263+	1.810+,*	0.084	0.145	2.073+,*	0.229	0.145	0.579	9.052
		Glen Ample	0.123+,*	0.802+,*	0.072	0.164	0.925+,*	0.236	0.153	0.439	3.919
		Glen Rosa	0.070*	0.620+,*	0.165+	0.215+	0.692+,*	0.379	0.113	0.767	1.826
		Malling Leo	0.086*	0.945+	0.064	0.097+	1.031+,*	0.161	0.091*	0.660	6.404
		Autumn Bliss	0.432+,*	2.471+,*	0.141+	0.243+	2.903+,*	0.384	0.175	0.580	7.560
		Glen Magna	0.126+,*	0.941+	0.114+	0.125	1.068+	0.239	0.134	0.912	4.469
		Octavia	0.069*	0.375*	0.073	0.190+	0.444*	0.263	0.184	0.384	1.688
	5% LSD		0.060	0.236	0.027	0.026	0.261	0.042	0.048	0.295	0.959

Table 2.2 Ranking of commercial varieties based on differences in sugar and acid contents between each variety and parent varieties (Glen Moy and Latham). Ranking was based on multiple range tests of differences between means.

Site		Ranking	Fructose	Glucose	Total Sugars	Ranking	Malic Acid	Citric Acid	Total Acids
Field 2006		1	Autumn Bliss	Autumn Bliss	Autumn Bliss	1	Glen Rosa	Autumn Bliss	Autumn Bliss
		2	All Gold	All Gold	All Gold	2	Autumn Bliss	Glen Rosa	Glen Rosa
		3	Latham	Latham	Latham	3	Glen Magna	Octavia	Octavia
		4	Joan J.	Malling Leo	Glen Magna	4	All Gold	Glen Ample	Glen Magna
		5	Glen Magna	Glen Magna	Joan J.	5	Glen Moy	Tulameen	Glen Ample
		6	Glen Ample	Tulameen	Malling Leo	6	Joan J.	Joan J.	All Gold
		7	Malling Leo	Joan J.	Tulameen	7	Tulameen	All Gold	Joan J.
		8	Tulameen	Glen Ample	Glen Ample	8	Octavia	Glen Moy	Tulameen
		9	Glen Rosa	Glen Rosa	Glen Rosa	9	Glen Ample	Glen Magna	Glen Moy
		10	Octavia	Octavia	Octavia	10	Malling Leo	Malling Leo	Malling Leo
		11	Glen Moy	Glen Moy	Glen Moy				
	5% LSD		0.060	0.236	0.261		0.027	0.026	0.042

In 2007, total sugars : total acids ratio was highest in Commercial polytunnel crop, higher by 56.7% and 20.1% compared to SCRI field and polytunnel crops respectively. Commercial polytunnel crop also had highest fructose : glucose ratio but only 2nd highest for citric : malic acids ratio. If total sugars : total acids ratio was used here as a flavour quality indicator, then Commercial polytunnel crop would have highest quality in all 2007 crops. Confirmation from sensory results is needed to validate this statement.

Key points:

- Different planting years produced fruits which varied in sugars and acids contents in plants grown in open field.
- Polytunnel cultivation produced fruits with increased sugar and acid contents, berry size and yield. Parent varieties also had significant increase and decrease in sugars and acids contents when cultivated under polytunnel.
- Commercial polytunnel crop had highest total sugars : total acids ratio of all 2007 crops.

2.3.4 Raspberry ketone contents in commercial and parent fruits

The highest ketone content was in Joan J. variety (0.078 μg / 100g fw) with much lower contents in Glen Moy and Latham, by 6% - 17%, respectively (Table 2.7). Contents in both parent varieties were similar, with only 11% difference between them. The lowest content was in Autumn Bliss (0.002 μg / 100g fw) and 2 other varieties had contents lower than 0.01 μg / 100g fw. However, despite these quantitative differences, none was statistically significant, not even with biggest difference between Joan J. and Autumn Bliss (0.076 μg / 100g fw). Raspberry ketone contents reported here are much lower that those reported in literature: 0.9 – 17.4 μg / 100 g fresh fruit Wyosocki et al. (1992). It was proposed raspberry ketone in fresh fruits exists as bound glucosides (Pabst et al., 1990) and most studies only report on unbound raspberry ketone, including this study. Hence it may be possible higher proportion of bound ketones caused lower values reported here. Another possible cause for variation cause in reported quantities here and other studies may be due to solvent degradation from extraction process. This has also been reported as an issue in other studies, which quantified raspberry ketone (Larsen

Table 2.3 Mean individual and total of sugars and organic acids contents (g/ml) in progeny and parent fruit analysed in 2006 (one location- SCRI field and in 2007 (3 locations- SCRI field and polytunnel and commercial polytunnel. SD = standard deviation. Significant difference between parent and commercial varieties was denoted by * and +,from Multiple Range Test (* different to Latham, + different to Glen Moy and *,+ different to both parental varieties).

Season	Fruit		Fructose	Glucose	Malic Acid	Citric Acid	Total Sugars	Total Acids
Field 2006	Progeny	Mean± SD	*,+0.492±0.244	*0.250±0.100	+0.411±0.117	+0.689±0.244	*,+0.737±0.341	+1.100±0.335
		Min-Max	0.107-1.222	0.080-0.576	0.219-1.022	0.199-1.830	0.187-1.797	0.419-2.356
Field 2007	Progeny	Mean± SD	0.385±0.037	0.233±0.015	0.068±0.041	0.260±0.188	0.619±0.046	0.328±0.218
		Min-Max	0.055-1.090	0.005-0.032	0.001-0.208	0.0004-1.549	0.127-1.191	0.001-1.658
Polytunnel 2007	Progeny	Mean± SD	*,+0.592±0.057	*,+0.346±0.018	*,+0.062±0.037	+0.255±0.107	*,+0.938±0.072	+0.317±0.135
		Min-Max	0.067-3.480	0.062-1.062	0.001-0.210	0.019-0.505	0.128-4.542	0.020-0.636
Commercial 2007	Progeny	Mean± SD	0.921±0.092	0.408±0.026	0.081±0.052	0.323±0.086	1.329±0.108	0.404±0.112
		Min-Max	0.088-4.633	0.071-0.957	0.010-0.282	0.052-0.503	0.169-5.018	0.061-0.709
Field 2006	Glen Moy	Mean± 5%LSD	0.051±0.060	0.357±0.236	0.078±0.027	0.085±0.026	0.408±0.260	0.163±0.42
	Latham	Mean± 5% LSD	0.247±0.060	1.045±0.236	n/a	n/a	1.293±0.260	n/a
Polytunnel 2007	Glen Moy	Mean±SD	1.574±0.307	0.824±0.056	0.008±0.002	0.028±0.013	2.398±0.363	0.038±0.005
	Latham	Mean±SD	0.219±0.038	0.151±0.023	0.039±0.002	0.243±0.008	0.370±0.060	0.282±0.005

Table 2.4 Fructose : Glucose, Citric acid : Malic acid and total sugars : total acids ratio values in progeny and parent crops of 2006 and 2007. Significant difference between parent and commercial varieties was denoted by * and +,from Multiple Range Test (* different to Latham, + different to Glen Moy and *,+ different to both parental varieties).

Season	Samples		Fructose: Glucose	Citric acid: Malic acid	Total sugars: Total acids
Field 2006	Progeny	Mean±SD	*,+1.94±0.37	+1.68±0.46	+0.74±0.44
		Min-Max	1.17-3.21	0.83-3.70	0.18-2.79
Field 2007	Progeny	Mean±SD	1.74±1.63	4.37±3.34	2.10±1.19
		Min-Max	0.65-10.85	0.19-22.77	0.46-5.52
Polytunnel 2007	Progeny	Mean±SD	*1.71±1.22	+6.06±6.70	+2.74±1.91
		Min-Max	0.31-7.81	0.77-48.23	0.45- 14.96
Commercial 2007	Progeny	Mean±SD	2.46±2.09	5.18±3.24	3.29±1.92
		Min-Max	0.51-12.24	0.98-24.19	0.37-9.76
Field 2006	Glen Moy	Mean±5% LSD	0.06±0.03	1.09±0.04	3.91±0.56
	Latham	Mean±5% LSD	0.19±0.01	n/a	n/a
Polytunnel 2007	Glen Moy	Mean±SD	1.90±0.24	3.63±1.80	63.11±27.65
	Latham	Mean±SD	1.45±0.03	6.26±0.11	1.31±2.30

and Poll, 1990; Wyosocki et al., 1992; Klesk et al., 2004). Other than solvent extraction issues, degradation from frozen storage (-20^{o}C) is possible, as raspberry ketone is stored in the cell vacuole and thawing may rupture the vacuole.

Key points:

- Raspberry ketone contents in parent varieties were amongst the highest in all commercial varieties, with similar contents in both parent varieties.
- A higher raspberry ketone portion could be glycosidically-bound in fresh berries, causing lower quantified contents reported in this study.

2.3.5 Raspberry ketone contents in progeny and parent fruits

The mean raspberry ketone content in progeny fruit was 0.028 μg / 100g fw, 57 - 62% lower than in parent fruits (Table 2.9). 33% of progeny fruits had contents above parent varieties, which resulted in the distribution being right-skewed (Figure 2.5). Similar issues pertaining to extraction methods and glycosidically-bound raspberry ketone could be reasons for the lower contents observed here.

Key points:

- There is generally lower content in progeny compared to parent varieties.
- Lower quantities reported for progeny population is possibly due to glycosidically bound raspberry ketone in fresh fruit.
- The content distribution was right-skewed, with 33% of progeny having higher contents that parent fruits.

Table 2.5 Raspberry ketone contents (μg/100g fw) in parent and 7 commercial varieties. Quantification was performed on fruits harvested only from the SCRI polytunnel in 2007. There were no significant differences in contents between commercial and parental varieties.

Variety	Raspberry ketone content
Glen Moy	0.073
Latham	0.065
Glen Ample	0.004
Autumn Bliss	0.002
Malling Leo	0.008
Octavia	0.010
All Gold	0.030
Tulameen	0.044
Joan J.	0.078
5% LSD	**0.100**

Table 2.6 Raspberry ketone content (µg / 100g fw) in progeny and parent fruits. Legend: P 2007 = 2007 SCRI polytunnel, SD = standard deviation.

Crop / Site		Raspberry ketone
P 2007	Mean (µg/100g) ± SD	0.028 ± 0.0336
	Min – Max	8.75×10^{-5} – 0.250
Latham P 2007	Mean (µg/100g) (5% LSD = 0.100)	0.065
Glen Moy P 2007	Mean (µg/100g) (5% LSD = 0.100)	0.073

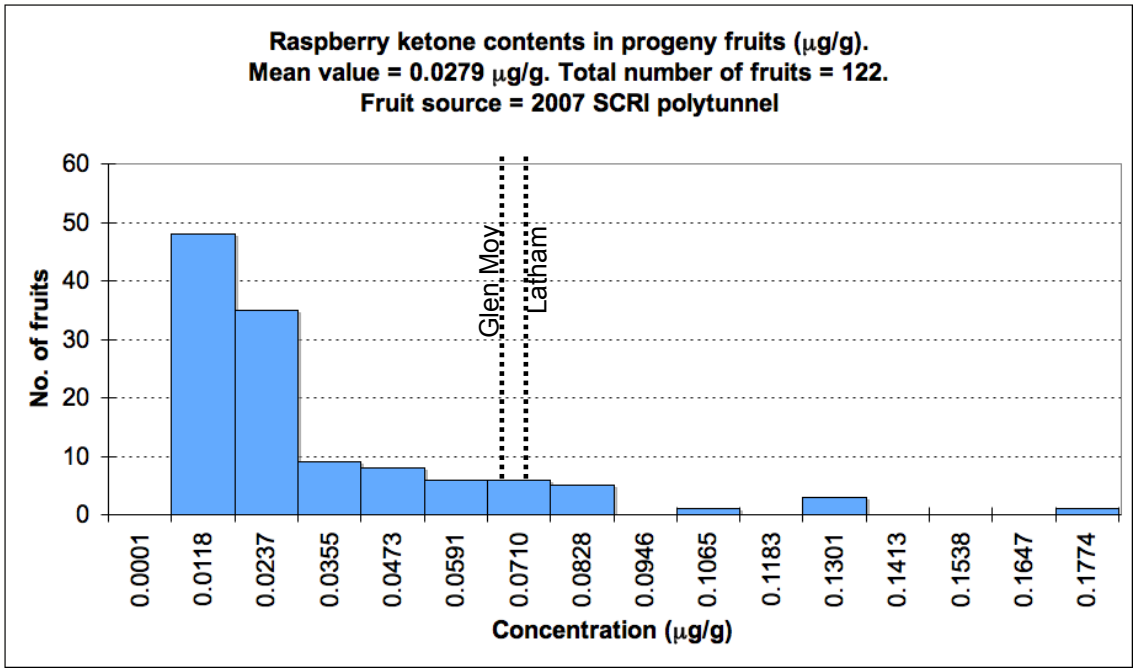


Figure 2.5 Distribution histogram of raspberry ketone contents in progeny fruits. Reference lines (dotted) indicate contents in Glen Moy and Latham.

2.3.6 Distribution of sugars contents in progeny fruit

Glucose contents in field crops were distinctly higher and more widely distributed in 2006 than in 2007 (Fig. 2.6 a). 21.6% progeny in 2006 had contents higher than maximum contents of 2007 (0.032 g/ml). Fructose contents in field crops were similar in both years (Fig. 2.6 b) with only 3.1% of 2006 crop had higher than maximum contents in 2007 (1.090 g/ml). There were similar distributions for total sugars content in field crops as well as for fructose (Fig. 2.6 c). 7.4% of the 2006 field crop had content higher than maximum content in the 2007 field crop (1.191 g/ml).

Generally in 2007, sugar contents in polytunnel crops were higher than in field. Mean glucose contents were higher by more than 1500% and also more widely distributed in the polytunnel crops (Fig. 2.7 a). Fructose contents were also higher in polytunnel crops, by 53.8% and 139.2% in SCRI and Commercial polytunnel crops respectively, but distribution trends were similar for all (Fig. 2.7 b), as was also for total sugars contents (Figure 2.7c). Total sugars contents were however higher in polytunnel crops by 51.5% and 114.7% for SCRI and Commercial sites respectively (Fig. 2.7 c).

Glucose content distribution in field crop was right skewed in 2006, with most of the progeny had contents similar to the mean and median (Fig. 2.8a). In 2006 field crop, 37% progeny had contents within range of parent varieties (0.357±0.511 - 1.045±0.511 g/ml). In 2007, contents were normally distributed, with approximately 14% progeny having median value. For polytunnel crops, distributions were wider and similar between sites (Fig. 2.8b). In SCRI, contents normally distributed around 0.338±0.026 g/ml value, and approximately 17% progeny had mean and median contents; with 94.4% in the range of parent varieties (0.151±0.023 - 0.824±0.056 g/ml). For Commercial polytunnel crop, content was also normally distributed with 20% progeny having mean and median contents.

Fructose content distributions in field crops were right-skewed for both years (Fig. 2.9a), with most of the progeny had contents similar to the mean and median. In

2006, 16% progeny had contents within range of parent varieties (0.051±0.130 - 0.247±0.130 g/ml). In 2007, distributions of fructose contents in all crops were similar (Fig. 2.9b). For polytunnel crops, most of the progeny had contents to the mean and median (0.757±0.092 g/ml), 40.4% and 29.2% in SCRI and Commercial crops respectively. A high proportion (92.1%) of SCRI polytunnel progenies had contents within range of parental varieties (0.219±0.038 - 1.574±0.307 g/ml).

Total sugar contents distributions in 2006 field and 2007 polytunnel crops were right-skewed (Figs 2.10 a, b). In 2006, 23.5% progeny had contents within mean and median values (0.679±0.341 g/ml) compared with 42% of 2007 SCRI polytunnel crop (0.775±0.072 g/ml). In 2006 field crop, 88% of the progeny population were within range of parent varieties (0.408±0.567 - 1.293±0.567 g/ml), lower compared to 93.3% of progeny in 2007 SCRI polytunnel crop.

Sugar content distributions are shown in boxplots (Fig 2.11). Right-skewed distributions were observed in all crops for individual and total sugars contents, and content distributions in polytunnel crops were most skewed. However, distributions were possibly influenced by total number of fruits, which were different between planting sites and years. But, similar distribution trends were observed for polytunnel crops, consistent for individual and total sugars contents. The same was also for field crops.

Despite similarities in distributions of polytunnel crops, indicated by histograms and boxplots, linear correlation analyses showed sugar contents in these crops were significantly different, notably in fructose and total sugar contents ($p < 0.05$) (Table 2.7). Sugar contents in 2007 SCRI field and polytunnel crops were highly significantly different ($p < 0.01$), particularly for glucose and total sugar contents. In field crops, the two harvest years (2006, 2007) produced fruit significantly different in fructose and total sugar content ($p = 0.01, 0.03$).

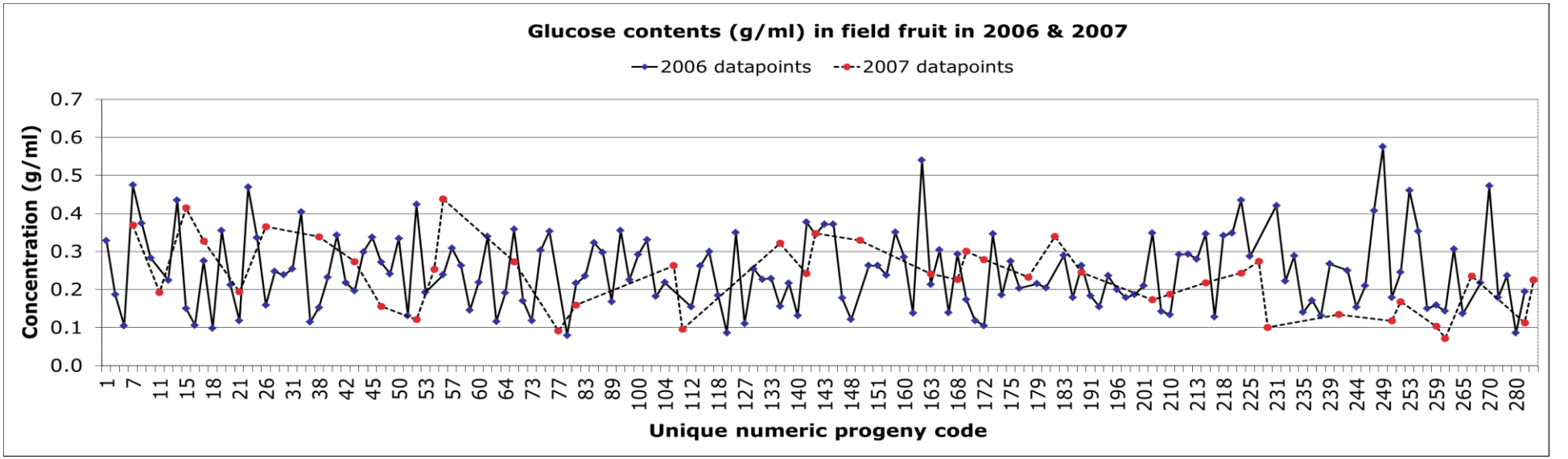


Figure 2.6(a) Mean glucose contents in field fruits of 2006 (—◆—) and 2007 (- ● -). X-axis denotes code of progeny and Y-axis glucose concentration in g/ml. Total number (#) of progeny fruits depended on availability. Mean values (mean±SD g/ml) were: 2006 (149) = 0.250±0.100 g/ml and 2007 (41) = 0.233±0.015 g/ml.

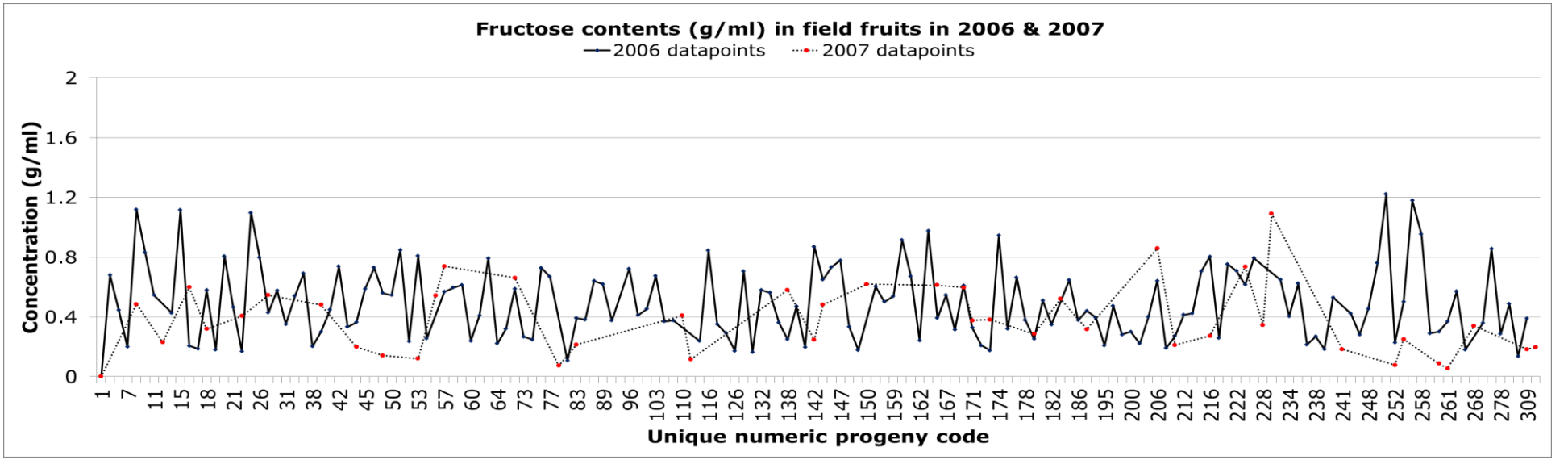


Figure 2.6(b) Mean fructose contents in field fruits of 2006 (—◆—) and 2007 (- ● -). X-axis denotes unique numeric code of each progeny and Y-axis denotes concentration of fructose in g/ml unit. Total number (#) of progeny fruits analysed from each year was different depending on availability. Mean values (mean±SD g/ml) were: 2006 (149) = 0.492±0.244 g/ml and 2007 (41) = 0.385±0.037g/ml.

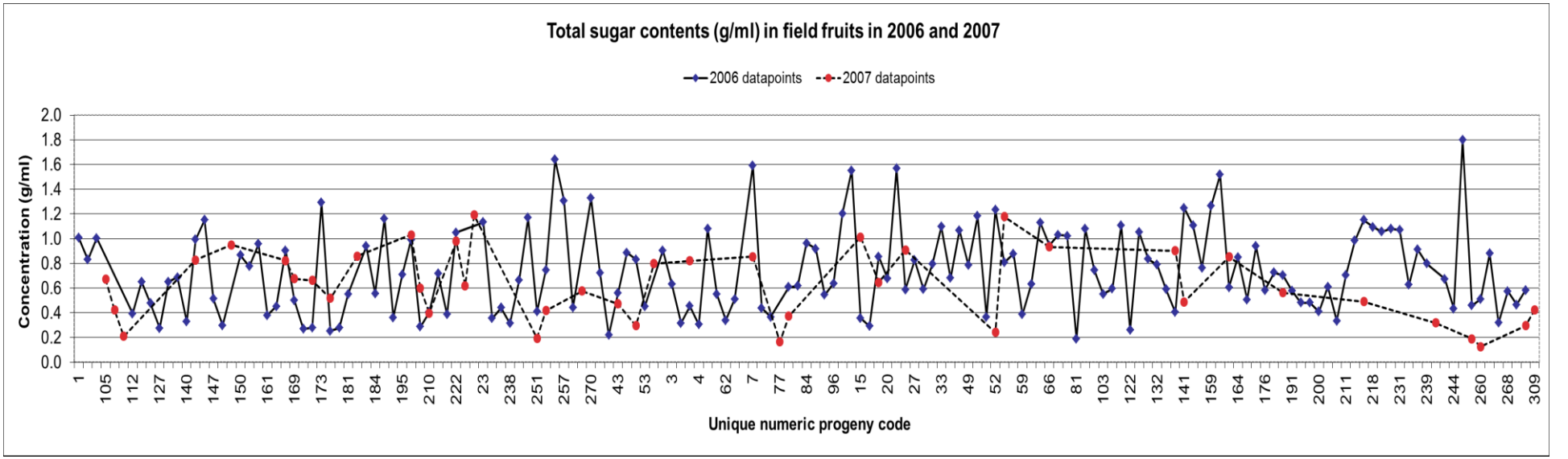


Figure 2.6(c) Mean total sugar contents in field fruits of 2006 (—◆—) and 2007 (- ● -). X-axis denotes unique numeric code of each progeny and Y-axis denotes concentration of total sugars in g/ml. Total number of fruits analysed from each crop (#) Mean values (mean±SD g/ml) for each year were: 2006 (141) = 0.737±0.341 g/ml and 2007 (41) = 0.619±0.046 g/ml.

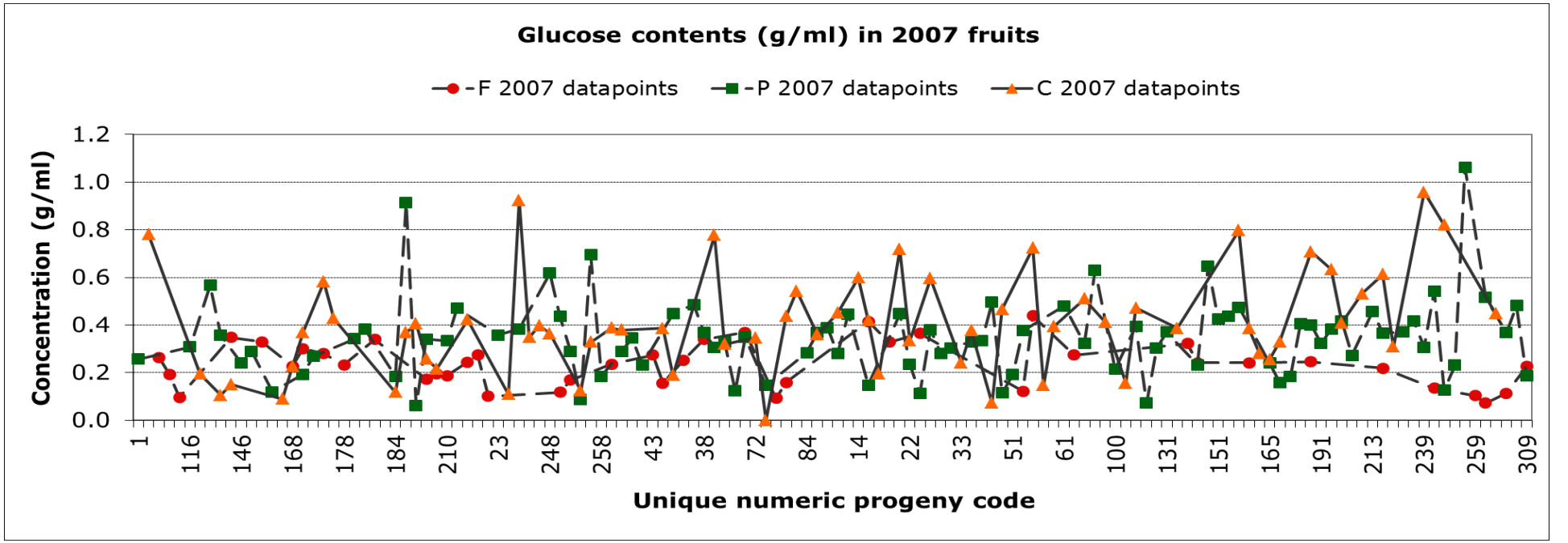


Figure 2.7(a) Mean glucose contents in all 2007 fruits: Field (F 2007 = -●-), SCRI Polytunnel (P 2007 = -■- -) and commercial Polytunnel (C 2007 = —▲—). X-axis denotes unique numeric code of each progeny and Y-axis denotes concentration of glucose in g/ml. Total number (#) of fruits analysed from each site was different depending on availability. Fruits were collected when red-ripe, stored at -20^{o}C (max. 2 months) until analysis. Mean values (mean±SD g/ml) were: F 2007 (42) = 0.233±0.015 g/ml, P2007 (88) = 0.346±0.018 g/ml and C2007 (64) = 0.408±0.026 g/ml.

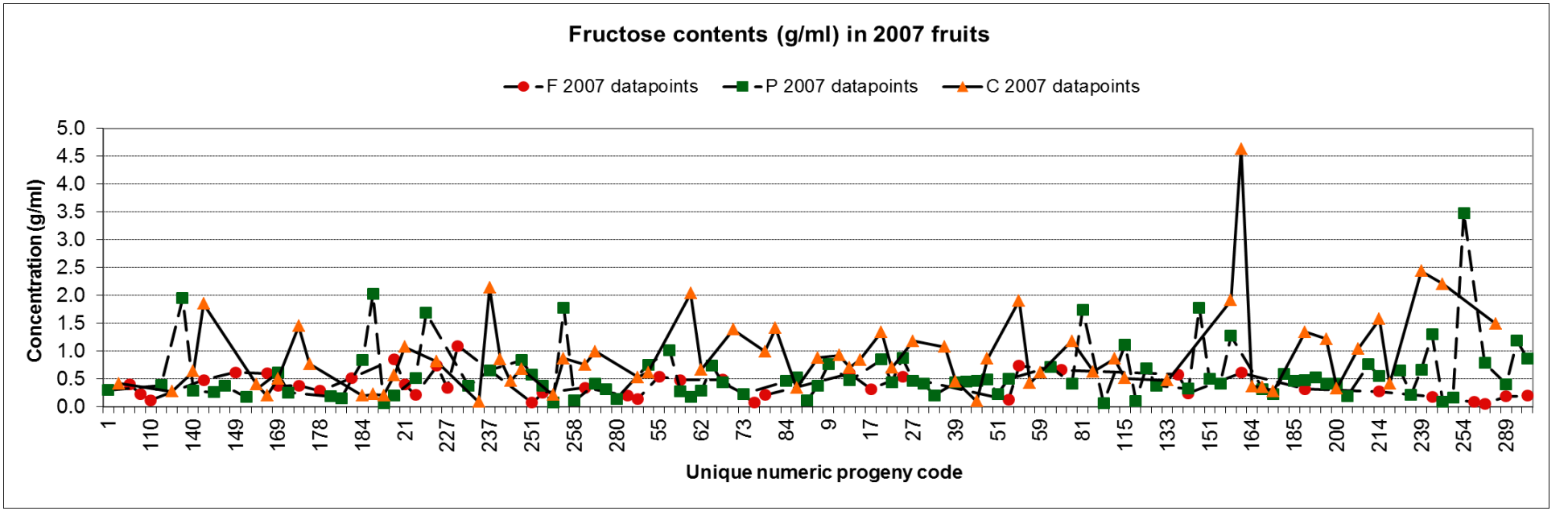


Figure 2.7(b) Mean fructose contents in all 2007 fruits: Field (F 2007 = - ● -), SCRI Polytunnel (P 2007 = - ■ -) and Commercial polytunnel (C 2007 = —▲—). X-axis denotes unique numeric code of each progeny and Y-axis denotes concentration of glucose in g/ml. Total number (#) of fruits analysed from each site was different depending on availability. Fruits were collected when red-ripe, stored at -20°C (max. 2 months) until analysis. Mean values (mean±SD g/ml) were: F 2007 (42) = 0.385±0.037 g/ml, P 2007 (88) = 0.592±0.057 g/ml and C 2007 (64) = 0.921±0.092 g/ml.

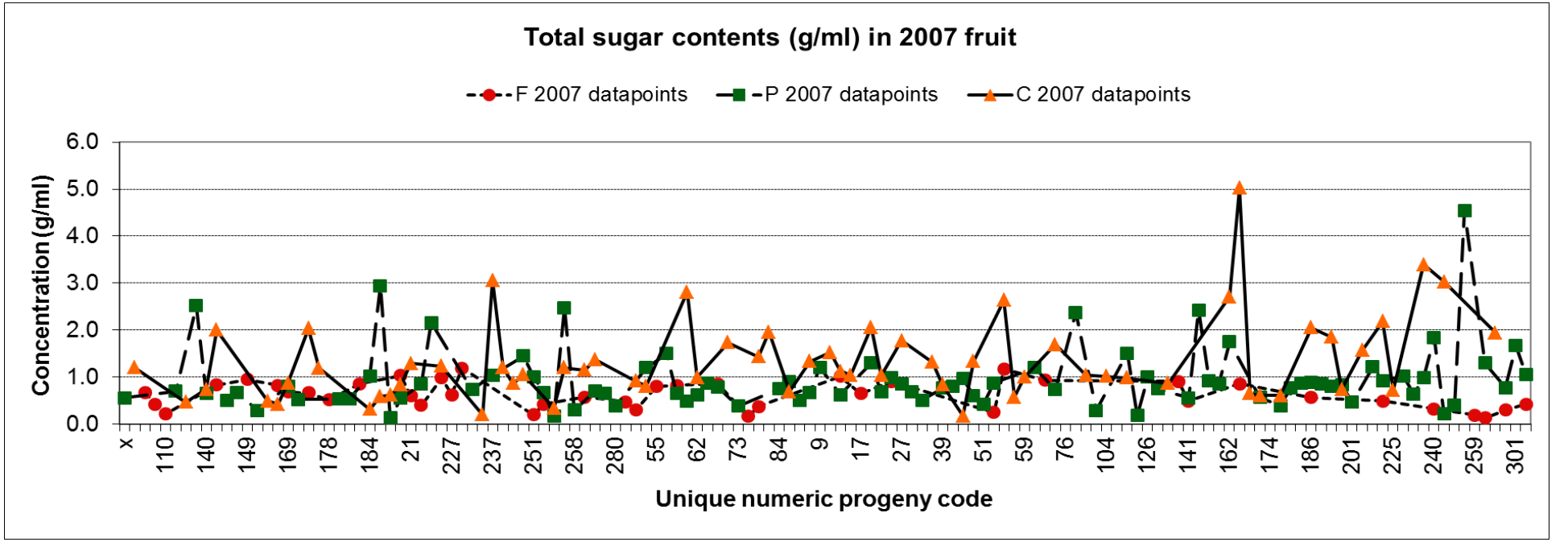
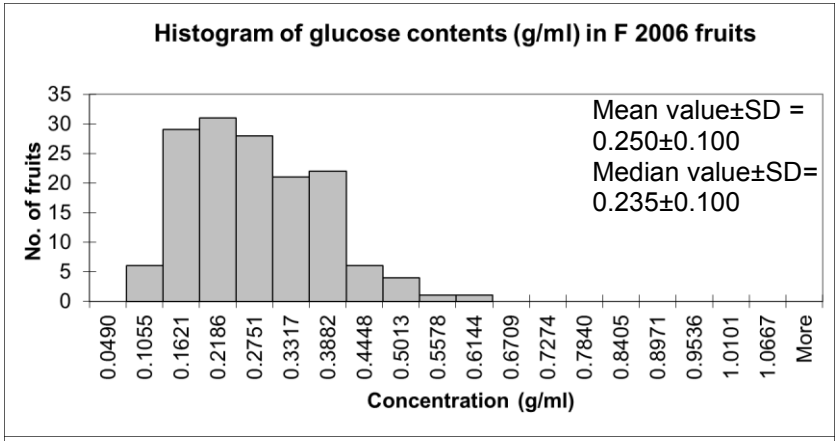
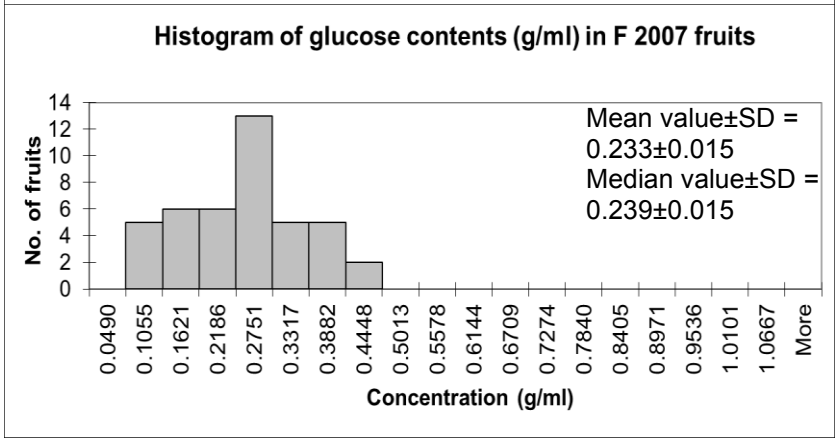


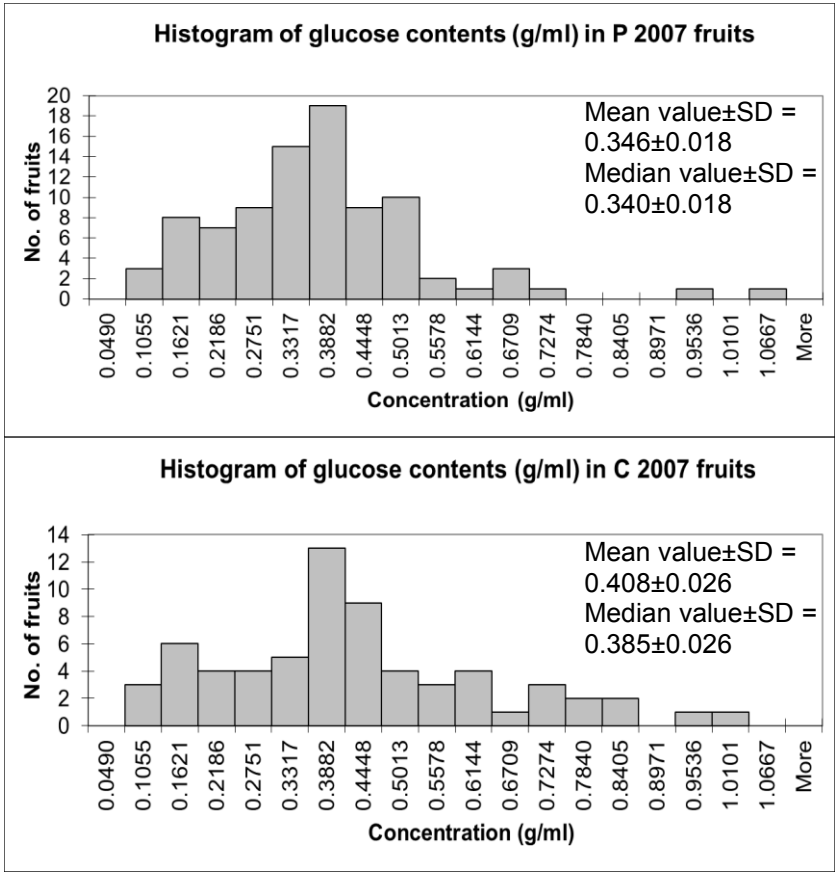
Figure 2.7(c) Total sugars contents in all 2007 fruits: Field (F 2007 = -●-), SCRI Polytunnel (P 2007 = -■- -) and Commercial Polytunnel (C 2007 = —▲—). X-axis denotes unique numeric code of each progeny and Y-axis denotes concentration of glucose in g/ml. Total number (#) of fruits analysed from each site was different depending on availability. Fruits were collected when red-ripe, stored at -20^{o}C (max. 2 months) until analysis. Mean values (mean±SD g/ml) were: F 2007 (42) = 0.619±0.046 g/ml, P 2007 (88) = 0.938±0.072 g/ml and C 2007 (64) = 1.329±0.108 g/ml.





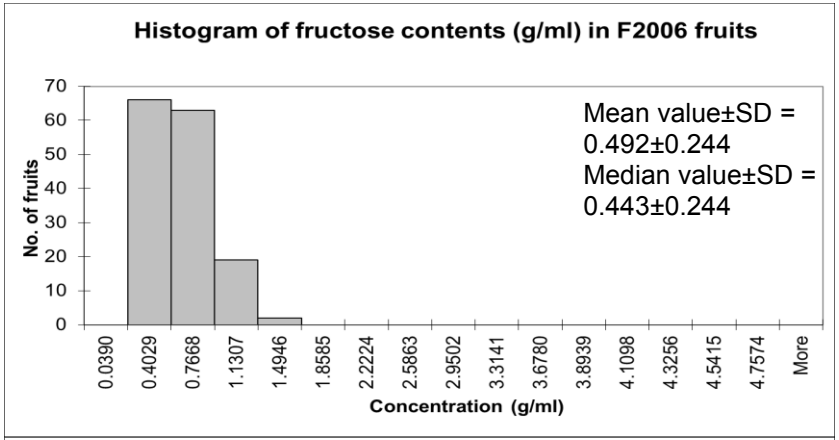
Content in parent fruit (mean value±5%LSD)
Glen Moy = 0.357±0.236
Latham = 1.045±0.236

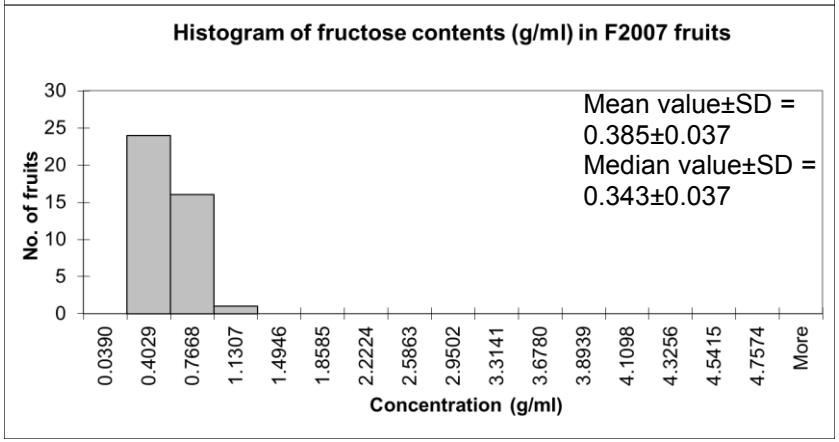
Figure 2.8(a) Glucose contents in field fruits in 2006 (F 2006; #149) and 2007 (F 2007; #42). Parent fruits were unavailable in 2007 open field site due to poor yield and in Commercial polytunnel due to disease. Less fruits were available for harvest in 2007 because of poor growing conditions of low temperature and high moisture.



Content in parent fruit (mean value±SD)
Glen Moy = 0.824±0.056
Latham = 0.151±0.023

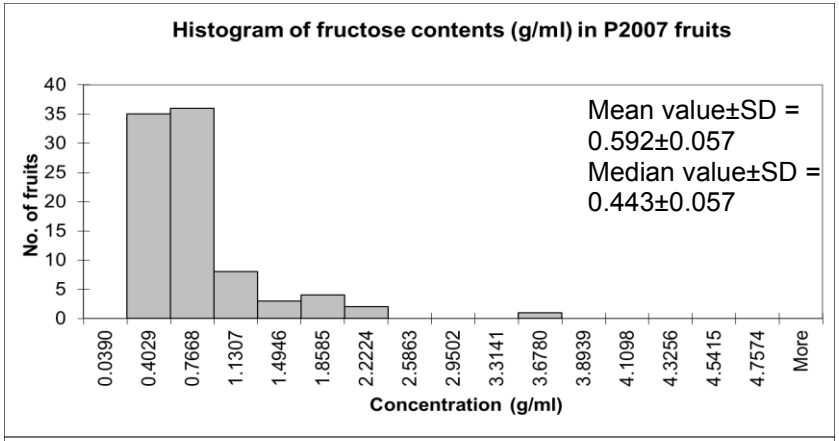
Figure 2.8(b) Glucose contents in polytunnel fruits from SCRI (P 2007; #89) and Commercial (C 2007; #65) sites. Parent fruits were unavailable in 2007 Commercial polytunnel due to disease.

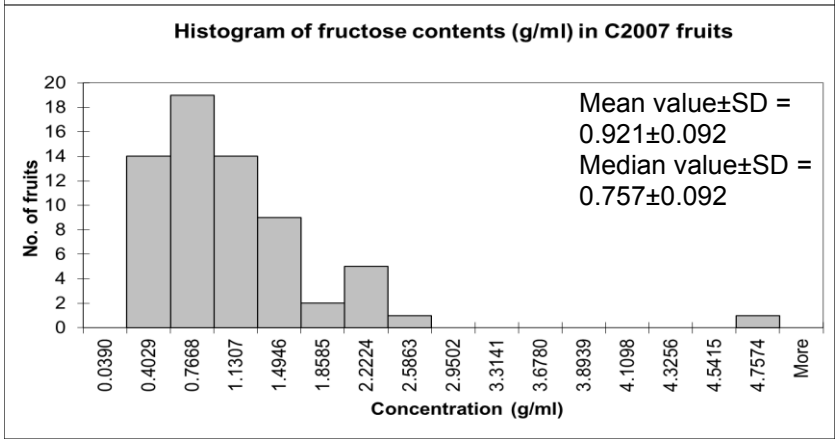




Content in parent fruit (mean value±5%LSD)
Glen Moy = 0.051±0.060
Latham = 0.247±0.060

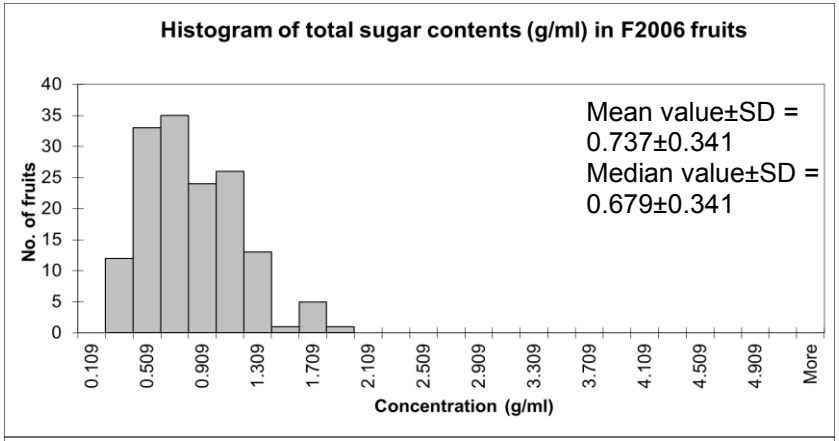
Figure 2.9(a) Fructose contents in field fruits in 2006 (F 2006; #149) and 2007 (F 2007; #42). Parent fruits were unavailable in 2007 open field site due to poor yield and in commercial polytunnel due to disease. Less fruits were available in 2007; due to poor growing conditions

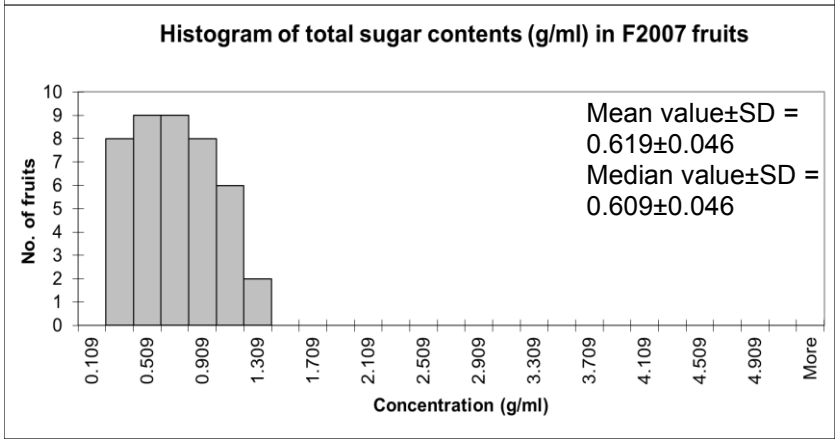




Content in parent fruit (mean value±SD)
Glen Moy = 1.574±0.307
Latham = 0.219±0.038

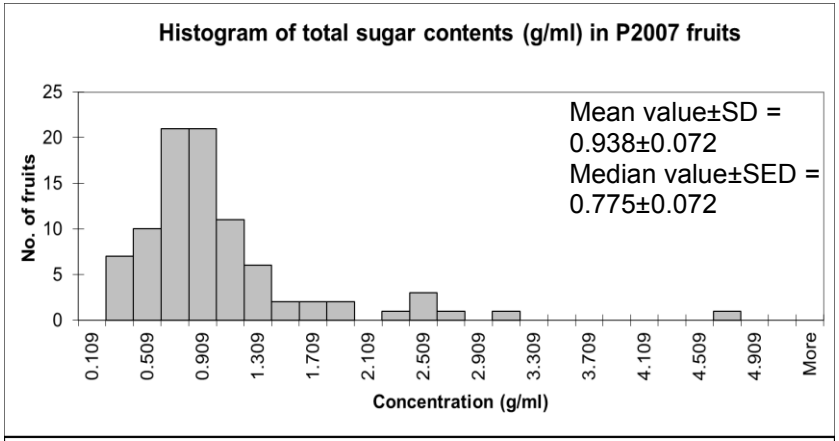
Figure 2.9(b) Fructose contents in polytunnel fruits from SCRI (P 2007; #89) and commercial (C 2007; #65) sites. Parent fruits were unavailable from the 2007 commercial polytunnel, due to disease.





Content in parent fruit (mean value±5%LSD)
Glen Moy = 0.408±0.260
Latham = 1.293±0.260

Figure 2.10(a) Total sugar contents in field fruits in 2006 (F 2006; #149) and 2007 (F 2007; #42). Parent fruits were unavailable from the 2007 open field site, due to poor yield, and from the commercial polytunnel, due to disease. Less fruits were available in 2007 because of poor growing conditions of low temperature and high moisture.



Content in parent fruit (mean value±SD)
Glen Moy = 2.398±0.363
Latham = 0.370±0.060

Figure 2.10(b) Total sugar contents in polytunnel fruits from SCRI (P 2007; #89) and Commercial (C 2007; #65) sites. Parent fruits were unavailable from the 2007 Commercial polytunnel, due to disease.

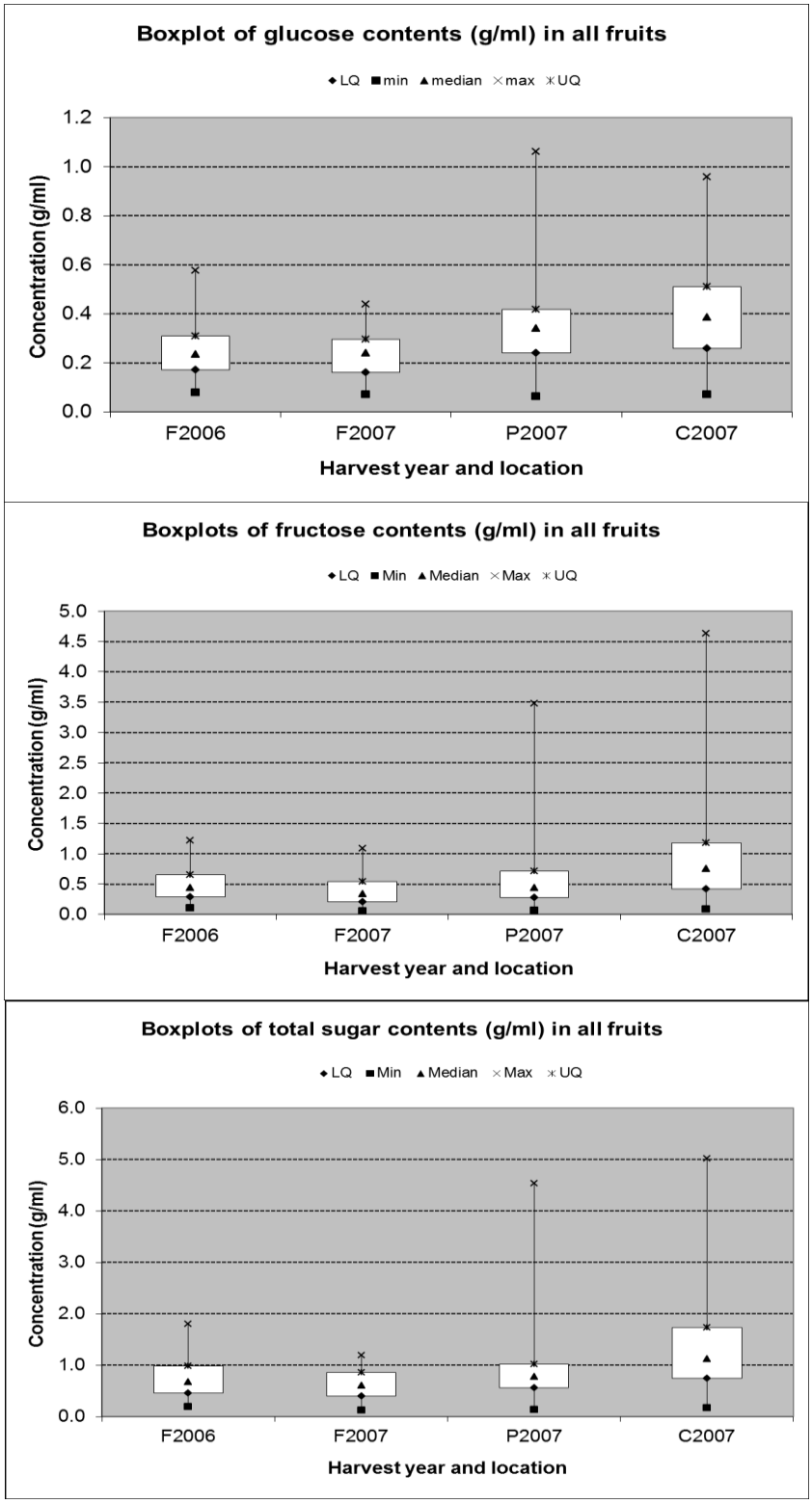


Figure 2.11. Boxplots of progeny fruit glucose, fructose and total sugar contents (g/ml): F2006 - field 2006, F2007 - field 007, P2007 - SCRI polytunnel 2007, C2007 - commercial polytunnel 2007.

Table 2.7 Summary result of 2 sample *t*-test to determine significance of differences in mean sugar contents in progeny fruit from 2 seasons (2006 and 2007) and in 2007 different sites (F – field, P – SCRI polytunnel and C – commercial polytunnel) at 95% confidence level ($\alpha = 0.05$), significant = *p*-value < 0.05.

	p –value			*p* –value			
	F 2006	F2007		F 2007	P 2007	P 2007	C 2007
Fructose	**0.01**			**<0.001**		**<0.001**	
Glucose	0.40			**<0.001**		**0.05**	
Total sugars	**0.03**			**<0.001**		**<0.001**	

It was also observed here a higher progeny proportion was within content range of parent varieties, when plants were cultivated under polytunnel. It is inferred that environmental factors had greater effects than genotype, in determining sugars and acids contents of field crops than in polytunnel crops, which increased number of progeny members with contents within range in parent varieties.

Key points:

- Field crops in both years differed significantly in fructose and total sugars content.
- Polytunnel crops had significantly different sugar contents compared to field crop, but content distributions were similar.
- 2007 SCRI crops, field and polytunnel, had significantly different sugar content.
- Polytunnel cultivation increased number of progenies with contents within range in parent varieties.

2.3.7 Acid contents in progeny fruits

2006 field crop produced fruits distinctly higher in citric and malic acid contents than in 2007. The content distribution of 2006 citric acid contents was wider compared to 2007 (Fig. 2.12a) and 22.4% of 2007 progeny fruits had lower than minimum quantity of 2006 crop (0.199 g/ml). For malic acid contents, distribution in 2006 was also wider compared to 2007 in field crops (Fig. 2.12b); a high proportion (99%) of 2007 crop had contents lower than 2006 minimum quantity (0.219 g/ml). Because 2006 and 2007 contents distributions for citric and malic acids in field crops were both dissimilar, this resulted in distributions in total acids contents to also be different in the 2 years (Fig. 2.12c), with 83.2% 2007 progeny fruits had less than 2006 minimum total acids content (0.419 g/ml).

For acids content in 2007, effects from growing conditions (field vs. polytunnel) were not clear. Citric, malic acid and thus total acid contents were generally distributed over similar ranges over the 3 planting sites (Fig. 2.13a-c). Mean quantities for citric and malic acid in all 2007 crops were also similar (Table 2.2). Three (3) progeny fruits were outliers for citric and total acid content, #16, #53 and #72, in field crop, which were also outliers with 7 other progeny fruit for malic acid contents, from other planting sites.

As mentioned previously, content distribution for acids in 2006 field crop was wider compared to 2007, also supported by histograms (Figs 2.14a – c). For citric acid, distributions in both years were right-skewed, with higher proportions of 2006 progeny fruits (17.4%) having contents higher than its mean and median values (0.701±0.244 g/ml). 2006 crop had all acid contents higher than parent Glen Moy (Fig. 2.14a). In 2007 field crop, highest proportion of progeny fruits (35%) was within mean and median (0.262±0.188 g/ml) contents. For citric acid contents in 2007 polytunnel crops (Fig. 2.15a), distributions were right-skewed for both sites, with 40% and 43% of SCRI and Commercial crops having mean and median (0.265±0.107 and 0.328±0.086 g/ml) contents respectively. Although mean contents were higher than parent varieties, it was more similar to Latham than Glen Moy, with only 4.7% difference with Latham compared to 89.0% difference with Glen Moy.

For malic acid contents in polytunnel crops, distributions were right-skewed with 40.3% SCRI and 36.0% Commercial crops respectivelye having contents within mean and median (0.061±0.037 and 0.072±0.052 g/ml) values (Fig. 2.15b). In SCRI crop, only 18.7% had contents within range of parent varieties, with mean contents 37.1% - 87.0% higher than parent fruits.

Total acid content in 2006 field crop was normally distributed within a wider range than in 2007, where distribution was right-skewed (Fig. 2.16a). A high proportion of progeny fruits, 16.0% and 35.8% respectively for 2006 and 2007 had contents higher than mean and median (1.126±0.335 and 0.339±0.218 g/ml) values. For 2006 field crop, all progeny had contents higher than Glen Moy. For total acids contents in 2007 polytunnel crops, distributions were right-skewed with 36.8% and 40.7% progeny fruit respectively in SCRI and Commercial sites having contents within mean and median (0.325±0.135 and 0.413±0.112 g/ml) values (Fig. 2.16b). For SCRI polytunnel crop, 53.5% of progeny had contents within quantities of parent varieties. Mean contents were higher, but more similar to Latham than Glen Moy, with only 11.0% difference with Latham and a higher 88.0% difference with Glen Moy.

Boxplots (Fig. 2.17) show a widest distribution of acid contents in 2006 field crop compared to all other crops, regardless of harvest year or sites. Content distributions of field crops were right-skewed, and in polytunnel crops, normally distributed. Content ranges were similar in all 2007 crops.

From summary data (Table 2.1), fruit sugar and acid contents in field crops differed between harvest years. All acid contents differed significantly ($p < 0.01$) between 2006 and 2007 field crops (Table 2.8). Despite similarities in distributions, citric and total acid contents of polytunnel crops were significantly different ($p < 0.01$). Surprisingly, despite different cultivation methods in 2007 SCRI crops (field vs. polytunnel), acid contents of crops were not significantly different ($p > 0.05$).

Key points:

- Field crops of both years differed significantly in all acid contents. Polytunnel crops had similar distributions between the two sites, but differed significantly in contents.
- Despite different growing methods of 2007 SCRI crops, this did not produce fruits with significantly different acids contents.

2.3.8 Total sugars : total acids ratio in parent and progeny fruits

Total sugars : total acids ratio values are important indicators of flavour quality used in the fresh fruit industry, and a good balance of sugars and acids contents is considered indicative of balance between sweetness and sourness taste traits in fruits. A high ratio value is desirable and a low value results from low total sugar and high total acid contents, could indicate bland or very tart fruits; both undesirable sensory fruit traits.

There was an outlier for 2007 field crop ratio values; progeny #163 with a ratio value of 113.8 due to its low total acids content. This value made comparative analyses of ratio values in other progeny fruits difficult. Graphical data representation is therefore presented either with or without ratio value of progeny #163. In field crops, 2007 crop had ratio values higher and distributed over a wider range (Fig. 2.18a, b). Mean ratio value in 2006 field crop (0.74), reflected its generally higher total acids contents and was 64.8% lower than mean ratio value for 2007 crop (2.10). In 2007 crops, values were distributed similarly in all 3 planting sites (Fig. 2.19a, b). SCRI crops had only 23.1% difference in values between field and polytunnel sites and highest ratio value was in Commercial polytunnel site, higher by 56.7% and 20.5% compared to SCRI field and polytunnel crops respectively.

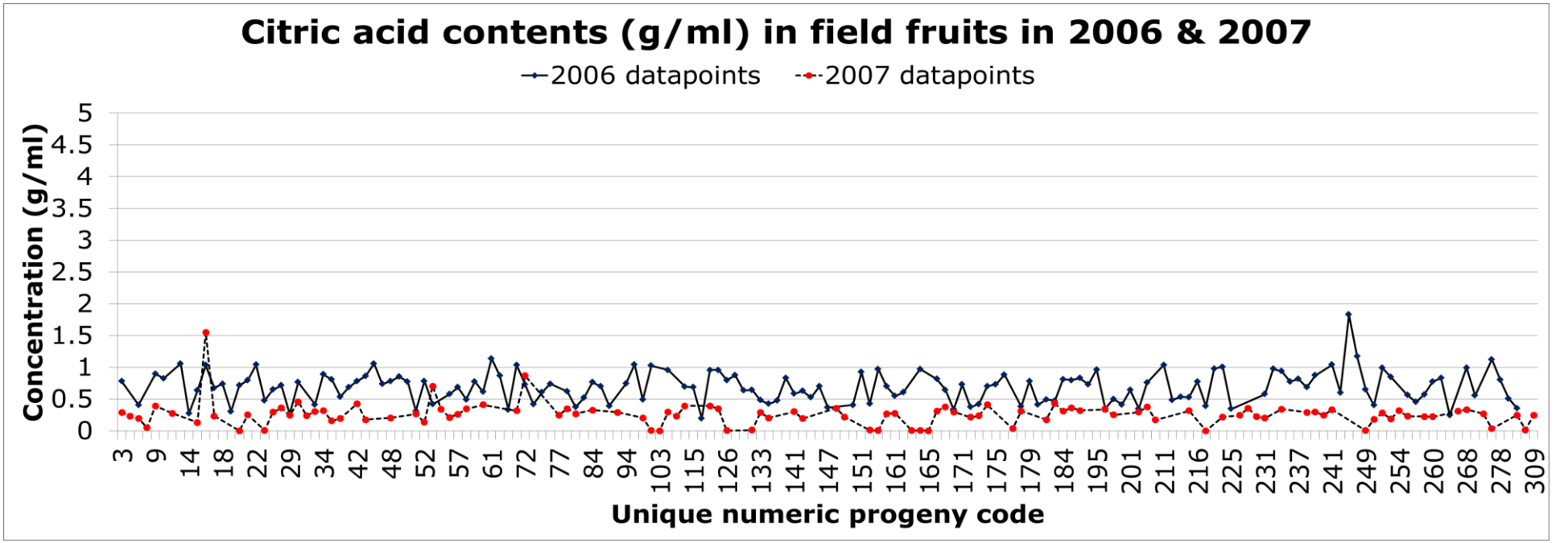


Figure 2.12(a). Mean citric acid contents in field fruits of 2006 (—◆—) and 2007 (- ● -). X-axis denotes unique numeric progeny code and Y-axis glucose concentration in g/ml. Total number (#) of progeny fruits analysed from each year was different depending on availability. Mean values (mean±SD g/ml) were: 2006 (149) = 0.689±0.244 g/ml and 2007 (107) = 0.260±0.188 g/ml.

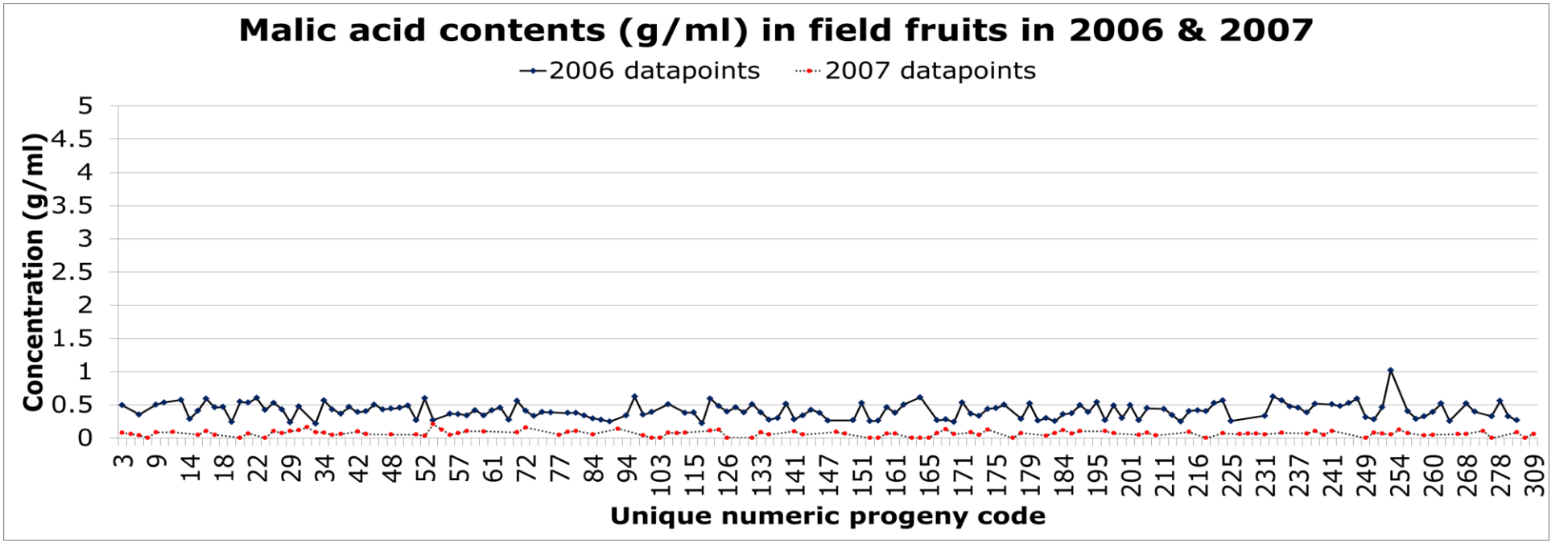


Figure 2.12(b). Mean malic acid contents in field fruits of 2006 (—◆—) and 2007 (-● -). X-axis denotes unique numeric progeny code and Y-axis glucose concentration in g/ml. Total number (#) of progeny fruits analysed from each year was different depending on availability. Mean values (mean±SD g/ml) were: 2006 (149) = 0.411±0.117 g/ml and 2007 (107) = 0.068±0.041 g/ml.

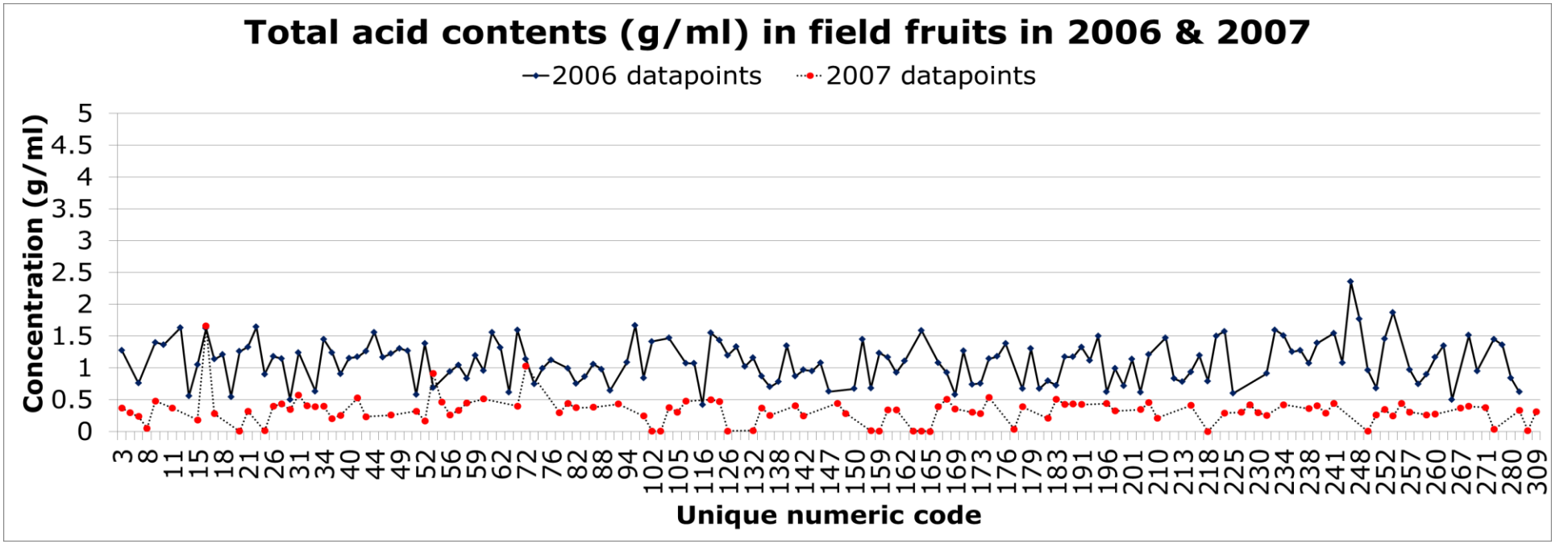


Figure 2.12(c). Mean total acid contents in field fruits of 2006 (—◆—) and 2007 (- ●-). X-axis denotes unique numeric progeny code and Y-axis glucose concentration in g/ml. Total number (#) of progeny fruits analysed from each year was different depending on availability. Mean values (mean±SD g/ml) were: 2006 (149) = 1.100±0.335 g/ml and 2007 (107) = 0.328±0.218 g/ml.

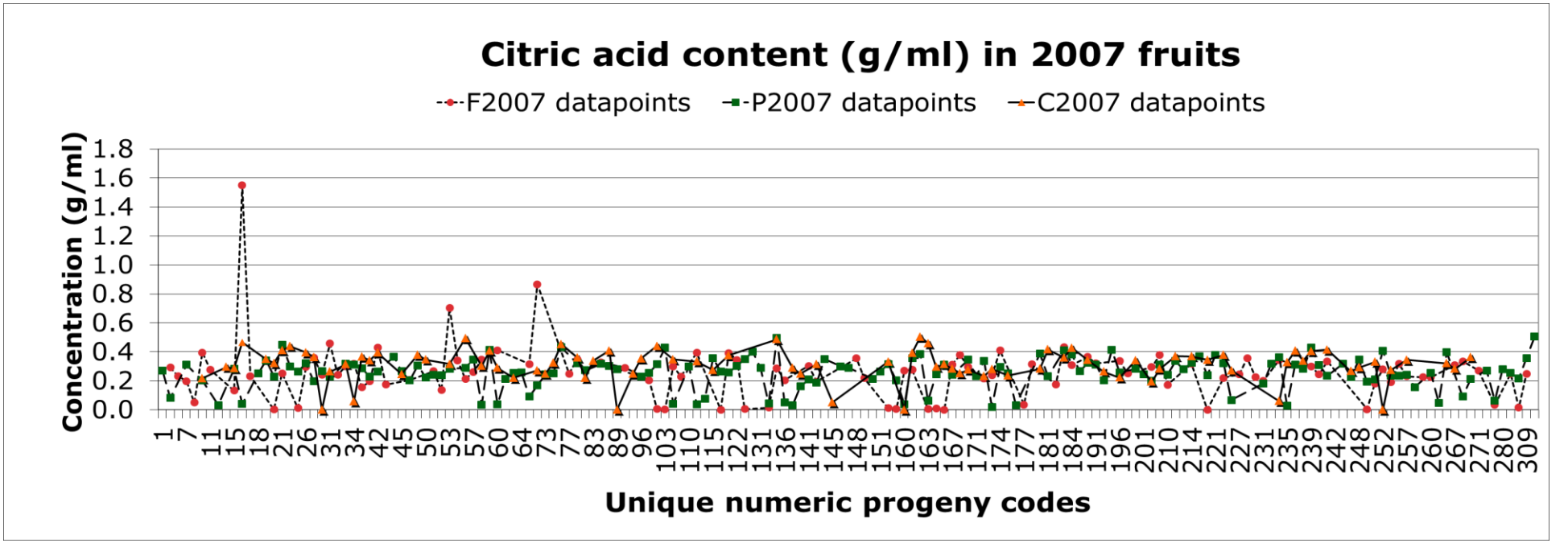


Figure 2.13(a). Mean citric acid contents in all 2007 fruits: Field (F 2007 = -●-), SCRI Polytunnel (P 2007 = -■- -) and commercial Polytunnel (C 2007 = —▲—). X-axis denotes progeny unique numeric code and Y-axis citric acid in g/ml . Total number of fruits (#) analysed from each site was different depending on availability. Fruits were collected when red-ripe, stored at -20^{o}C (max. 2 months) until analysis. Mean values (mean±SD g/ml) for each site were: F2007 (107) = 0.260±0.188 g/ml, P2007 (145) = 0.255±0.107 g/ml and C2007 (86) = 0.323±0.086 g/ml.

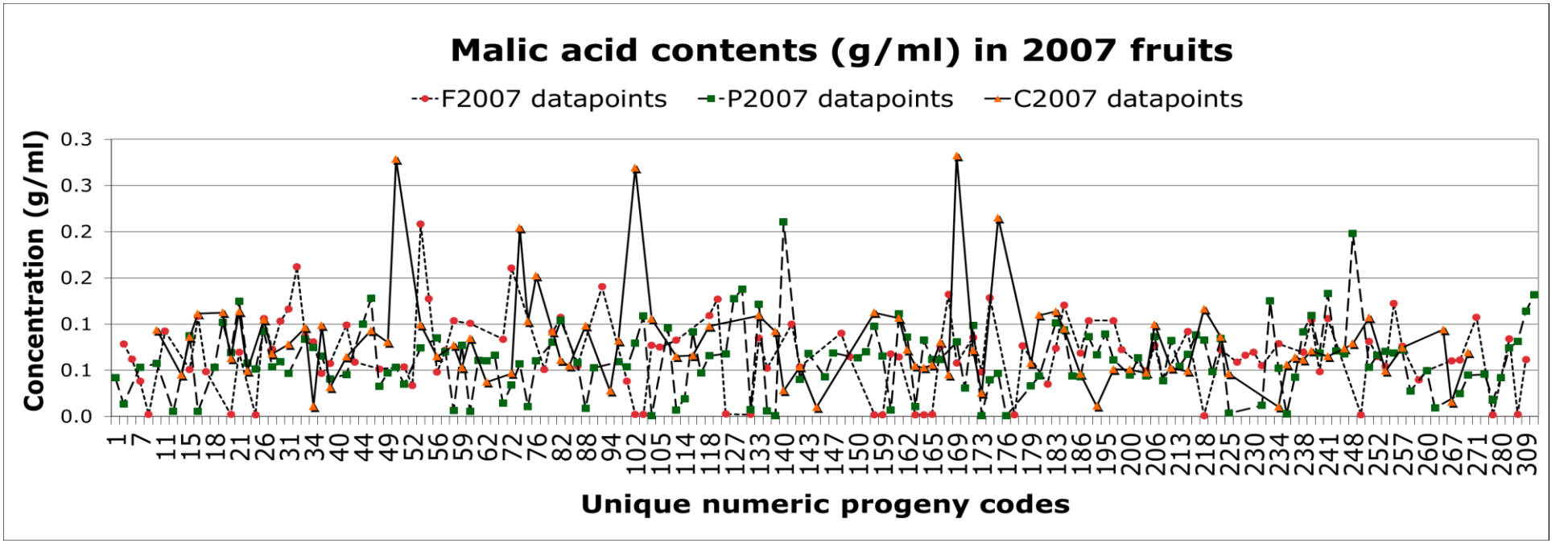


Figure 2.13(b). Mean malic acid contents in all 2007 fruits: Field (F 2007 = ● -), SCRI Polytunnel (P 2007 = ■ - -) and Commercial Polytunnel (C 2007 = –▲––). X-axis denotes progeny unique numeric code and Y-axis citric acid in g/ml . Total number of fruits (#) analysed from each site was different depending on availability. Fruits were collected when red-ripe, stored at -20^{o}C (max. 2 months) until analysis. Mean values (mean±SD g/ml) for each site were: F2007 (107) = 0.068±0.041 g/ml, P2007 (145) = 0.062±0.037 g/ml and C2007 (86) = 0.081±0.052 g/ml.

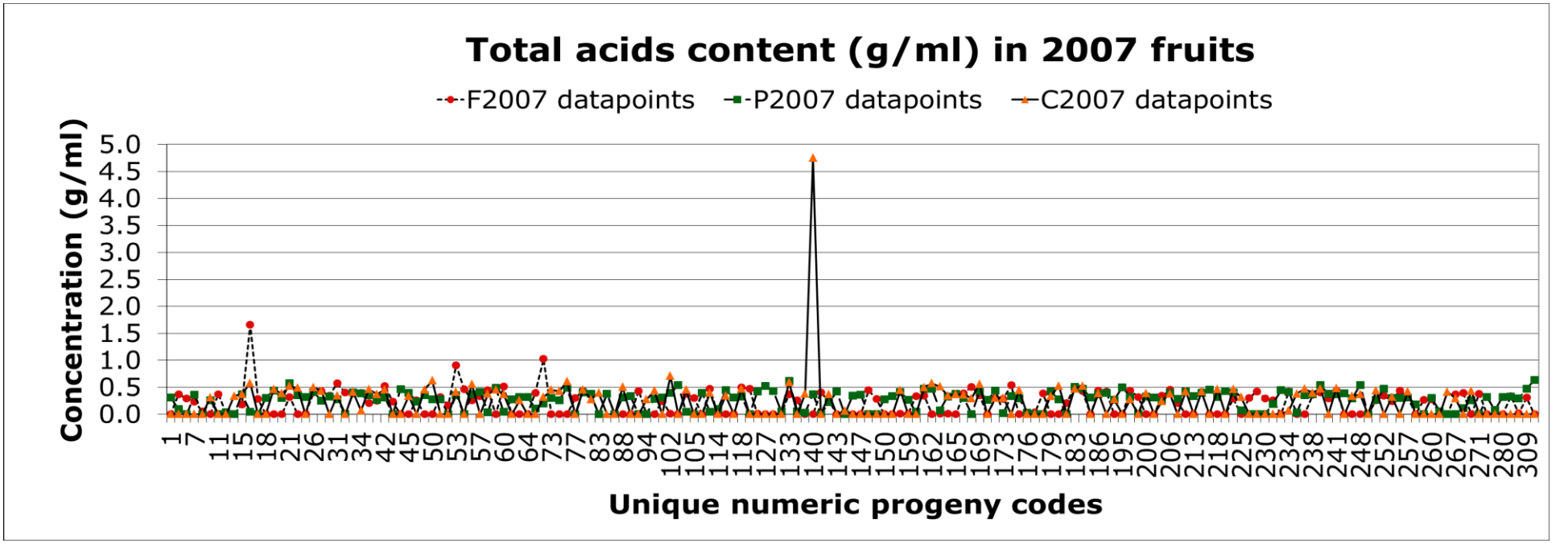
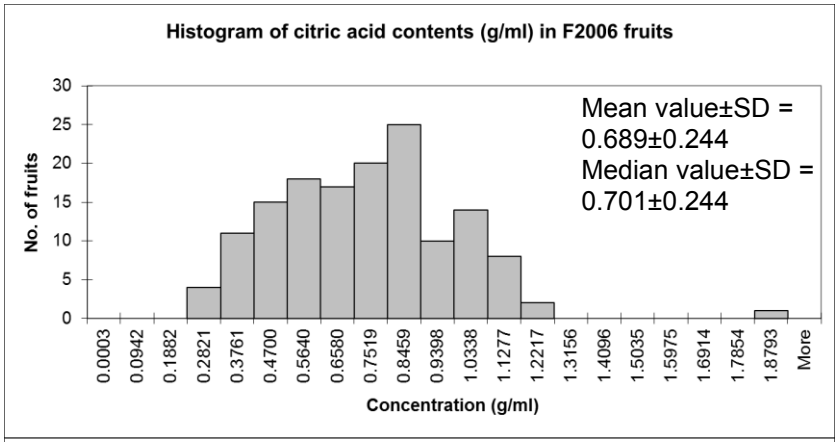
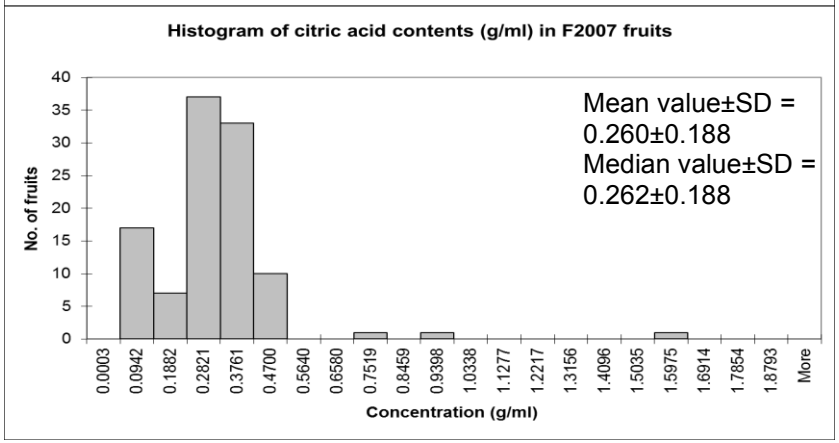


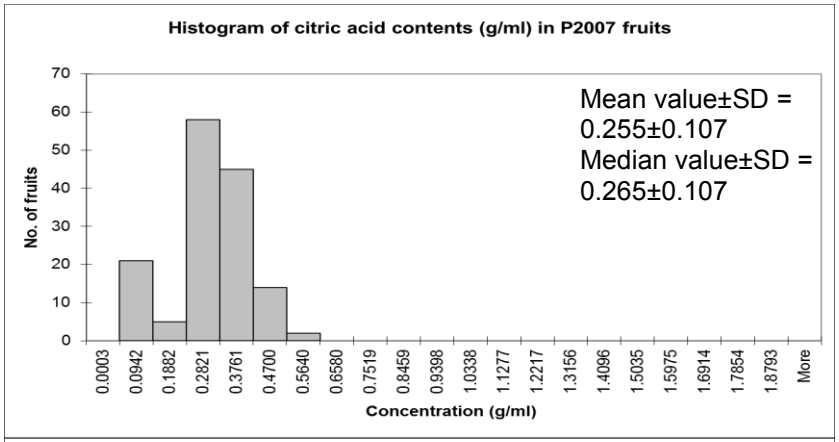
Figure 2.13(c). Mean total acid contents in all 2007 fruits: Field (F 2007 = -●-), SCRI Polytunnel (P 2007 = -■- -) and Commercial Polytunnel (C 2007 = —▲—). X-axis denotes progeny unique numeric code and Y-axis citric acid in g/ml. Total number of fruits (#) analysed from each site was different depending on availability. Fruits were collected when red-ripe, stored at -20^{o}C (max. 2 months) until analysis. Mean values (mean±SD g/ml) for each site were: F2007 (107) = 0.328±0.218 g/ml, P2007 (145) = 0.317±0.135 g/ml and C2007 (86) = 0.404±0.112 g/ml.

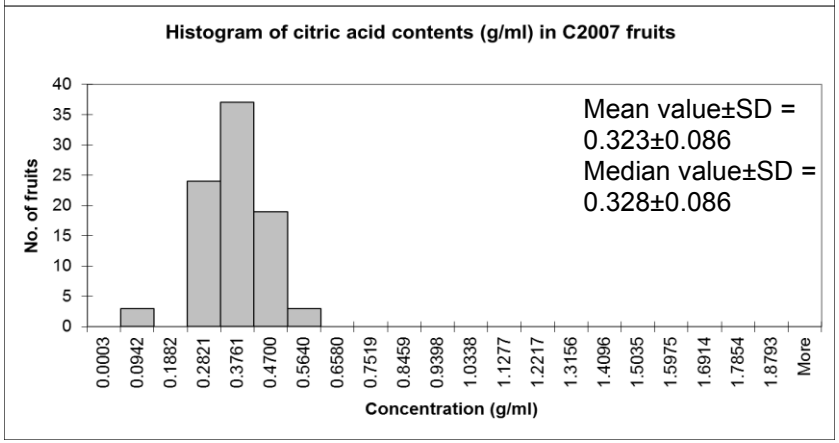




Content in parent fruit (mean value±5%LSD)
Glen Moy = 0.085±0.026
Latham = n/a

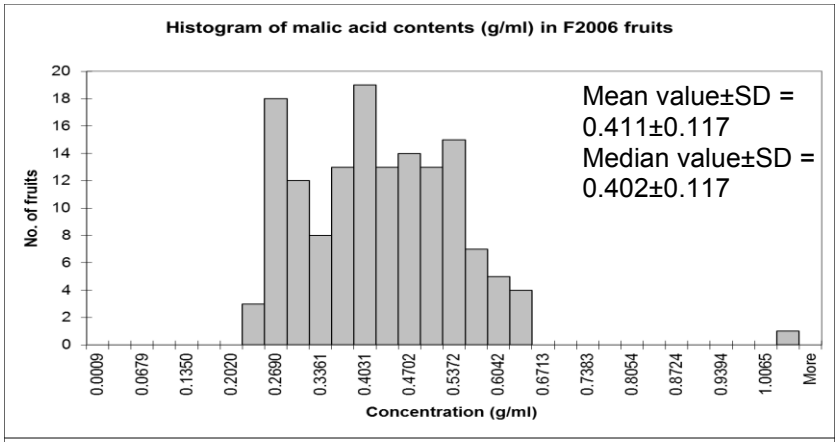
Figure 2.14(a) Citric contents in field crops in 2006 (F 2006; #149) and 2007 (F 2007; #42). Parent fruits were unavailable in 2007 open field site due to poor yield. Less fruits were available for harvest in 2007 because of poor growing conditions of low temperature and high moisture.

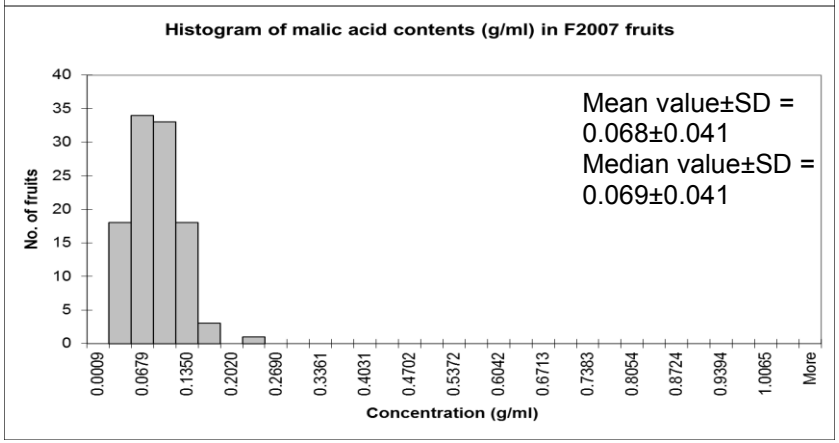




Content in parent fruit (mean value±SD)
Glen Moy = 0.028±0.107
Latham = 0.243±0.107

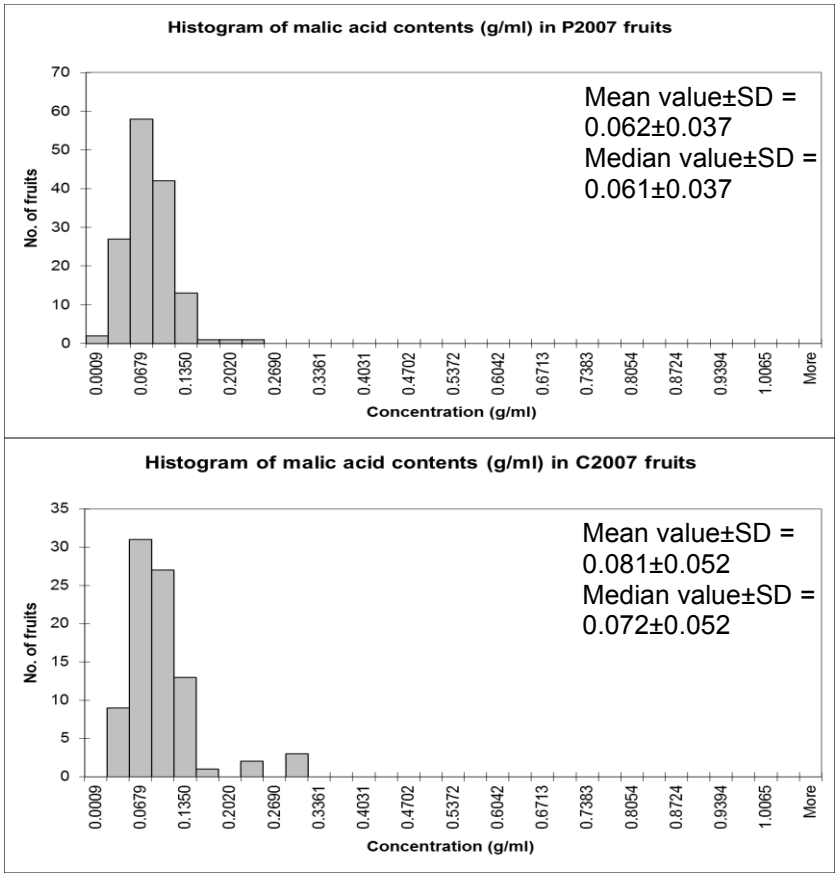
Figure 2.14(b). Citric acid contents in polytunnel crops from SCRI (P 2007; #145) and Commercial (C 2007; #86) sites. Parent fruits were unavailable in 2007 Commercial polytunnel due to disease.





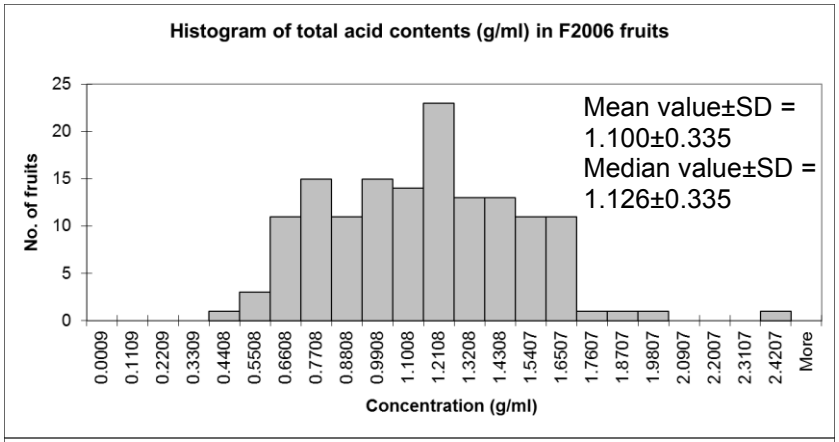
Content in parent fruit (mean value±5%LSD)
Glen Moy = 0.078±0.027
Latham = n/a

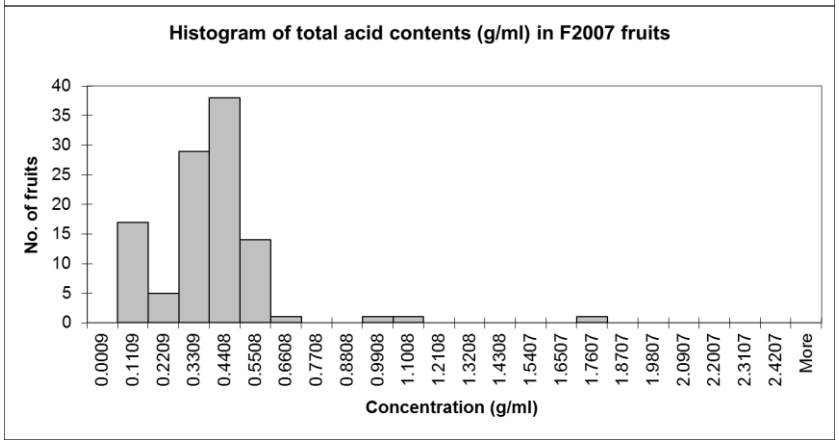
Figure 2.15(a). Malic acid contents in field crops in 2006 (F 2006; #149) and 2007 (F 2007; #42). Parent fruits were unavailable in 2007 open field site due to poor yield. Less fruits were available for harvest in 2007 because of poor growing conditions of low temperature and high moisture.



Content in parent fruit (mean value±SD)
Glen Moy = 0.008±0.037
Latham = 0.039±0.037

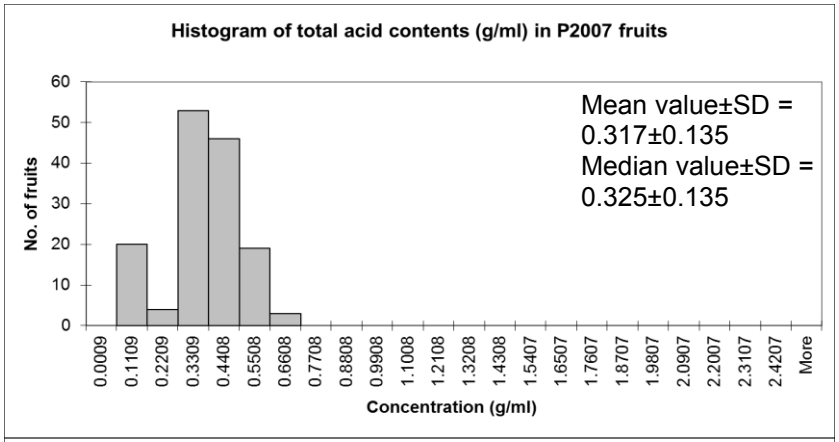
Figure 2.15(b) Malic acid contents in polytunnel crops from SCRI (P 2007; #145) and Commercial (C 2007; #86) sites.

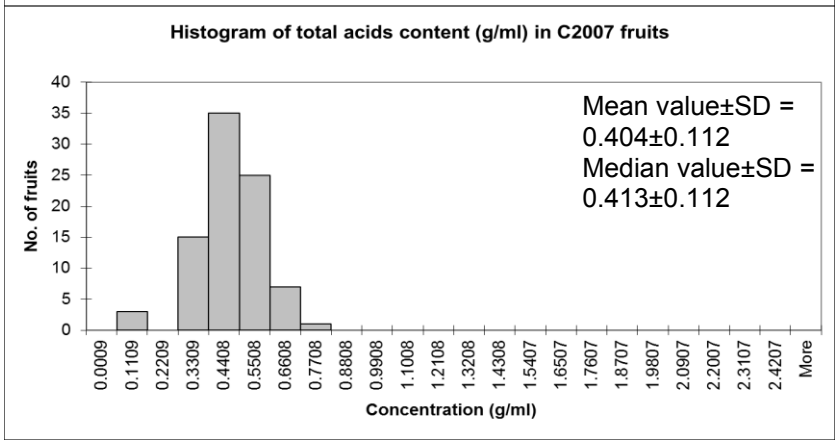




Content in parent fruit (mean value±5%LSD)
Glen Moy = 0.163±0.420
Latham = n/a

Figure 2.16(a) Total acid contents in field crops in 2006 (F 2006; #149) and 2007 (F 2007; #42). Parent fruits were unavailable in 2007 open field site due to poor yield. Less fruits were available for harvest in 2007 because of poor growing conditions of low temperature and high moisture.





Content in parent fruit (mean value±SD)
Glen Moy = 0.038±0.135
Latham = 0.282±0.135

Figure 2.16 (b) Total acid contents in polytunnel crops from SCRI (P 2007; #145) and Commercial (C 2007; #86) sites. Parent fruits were unavailable in 2007 Commercial polytunnel due to disease.

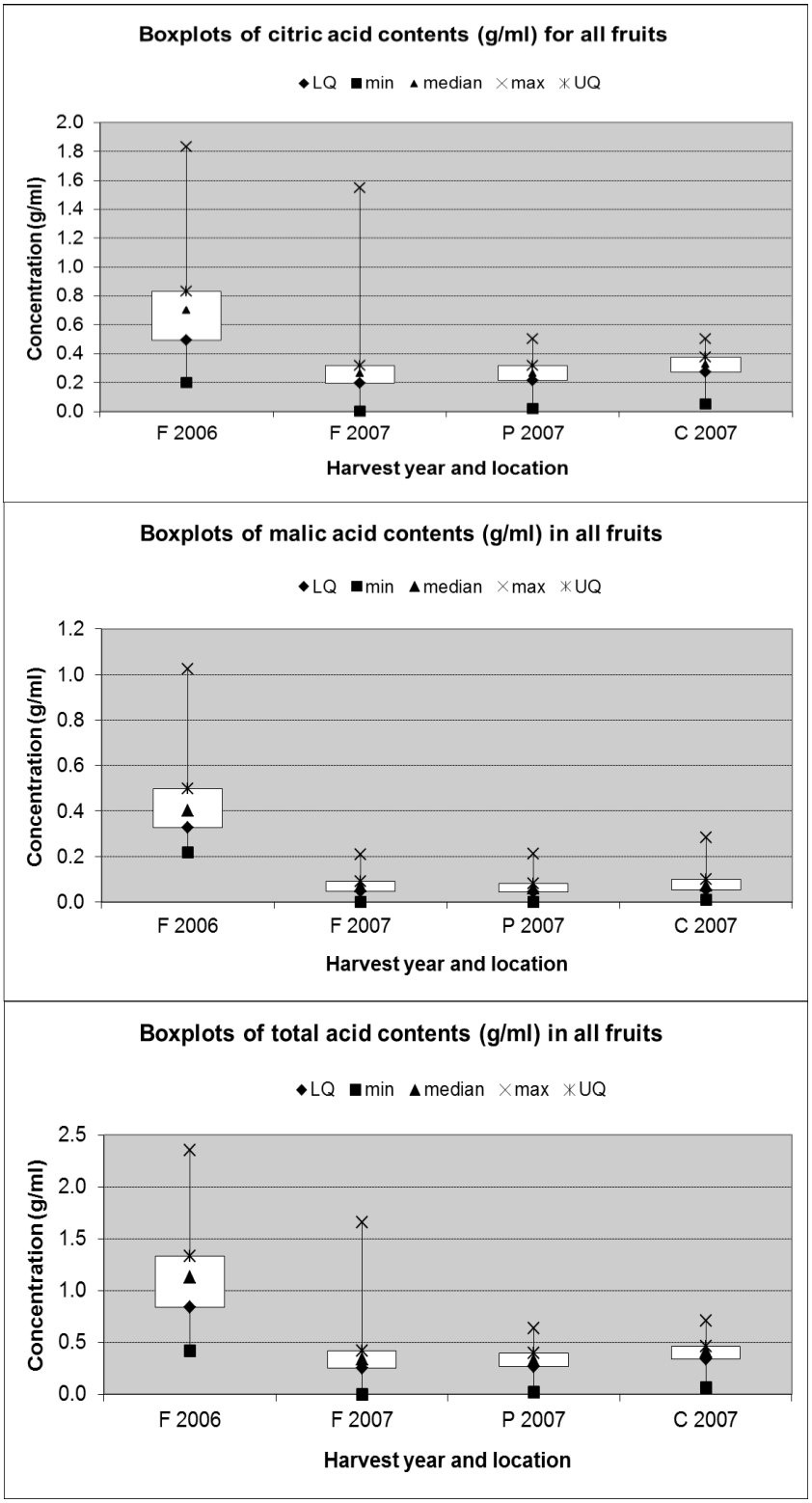


Figure 2.17. Distributions of citric, malic and total acid content (g/ml) in all fruits. Legend: F2006 - field fruit 2006, F2007 - field fruit 2007, P2007 - SCRI polytunnel fruit 2007, C2007 - Commercial polytunnel fruit 2007.

Table 2.8 Summary result of 2 sample *t*-test to determine significance of differences in mean values of acid contents in all fruits; from different years (2006 and 2007) and different sites (F – field, P – SCRI polytunnel and C – commercial polytunnel) at 95% confidence level ($\alpha = 0.05$), sig = p-value < 0.05.

Variable	*p*-value			*p*-value			
	F 2006	F 2007		F 2007	P2007	P 2007	C 2007
Malic Acid	**< 0.001**			0.19		**0.002**	
Citric Acid	**< 0.001**			0.93		**< 0.001**	
Total Acids	**< 0.001**			0.67		**< 0.001**	

Distributions for ratio values in 2007 crops were right skewed and wider compared to 2006 field crop (Figs 2.19 – 2.20). Narrowest distribution range was for values in 2006 field crop, with 85.2% progeny having ratios within range of mean and median values.

Differences in distribution ranges between 2006 and 2007 crops were apparent in boxplots (Fig. 2.21). In 2007 crops, SCRI polytunnel crop had widest distribution range the most right-skewed distribution. There was generally wider distribution ranges in polytunnel crops compared to field in 2007. Despite differences in distribution ranges, differences in ratio values between field crops of both years or between planting sites in 2007 crops were not significant ($p > 0.05$; Table 2.9). However, as environmental factors were previously demonstrated to have influenced total sugars and total acids contents, its impact on ratio values is inferred here.

Key points:

- Progeny #163 from 2007 field crop had a relatively higher ratio value compared to all crops, due to its lower acids content.
- Distribution ranges for ratio values differed between crops, with narrowest distribution range in 2006 field crop.
- However these differences were not significant
- As environmental factors were demonstrated to have influenced total sugars and acids contents, its impact on ratios is inferred.

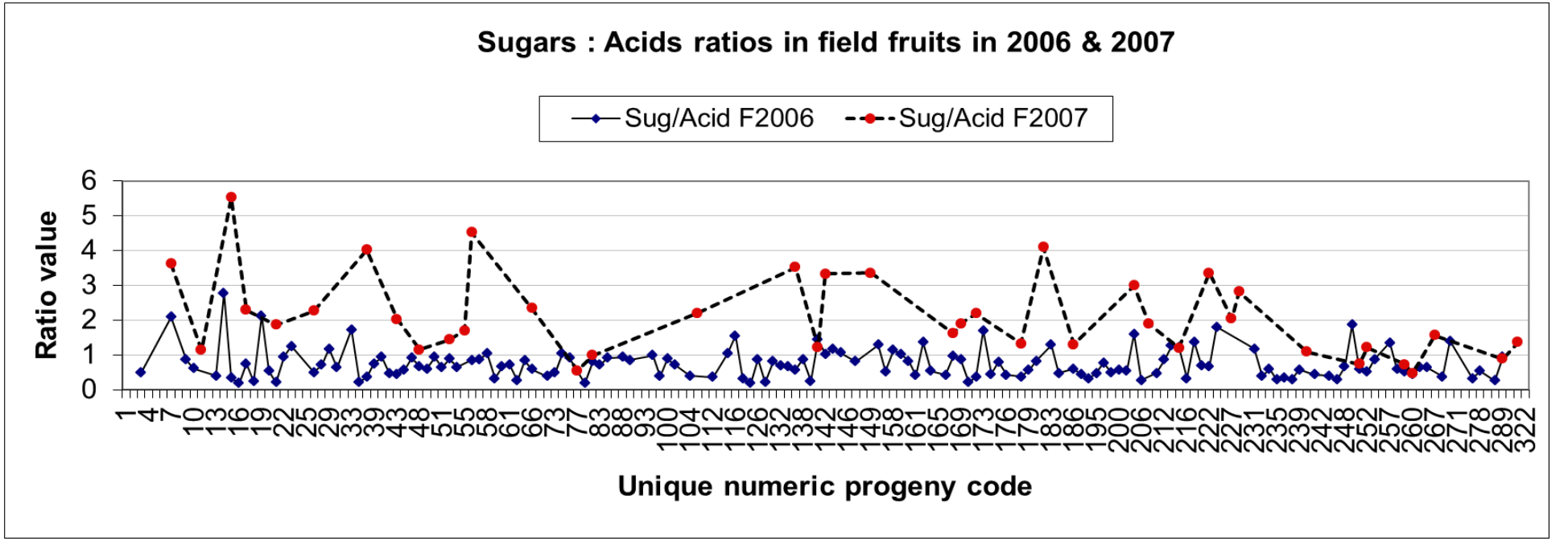


Figure 2.18 (a) Total sugars : acids in field crops of 2006 (—◆—) and 2007 (- ● -) **without** value in progeny #163 included. X-axis denotes unique numeric code of each progeny and Y-axis ratio. Mean values (mean±SD g/ml) were: 2006 (149)= 0.74±0.44 g/ml and 2007 (106) 2.10±1.19 g/ml (**without** #163).

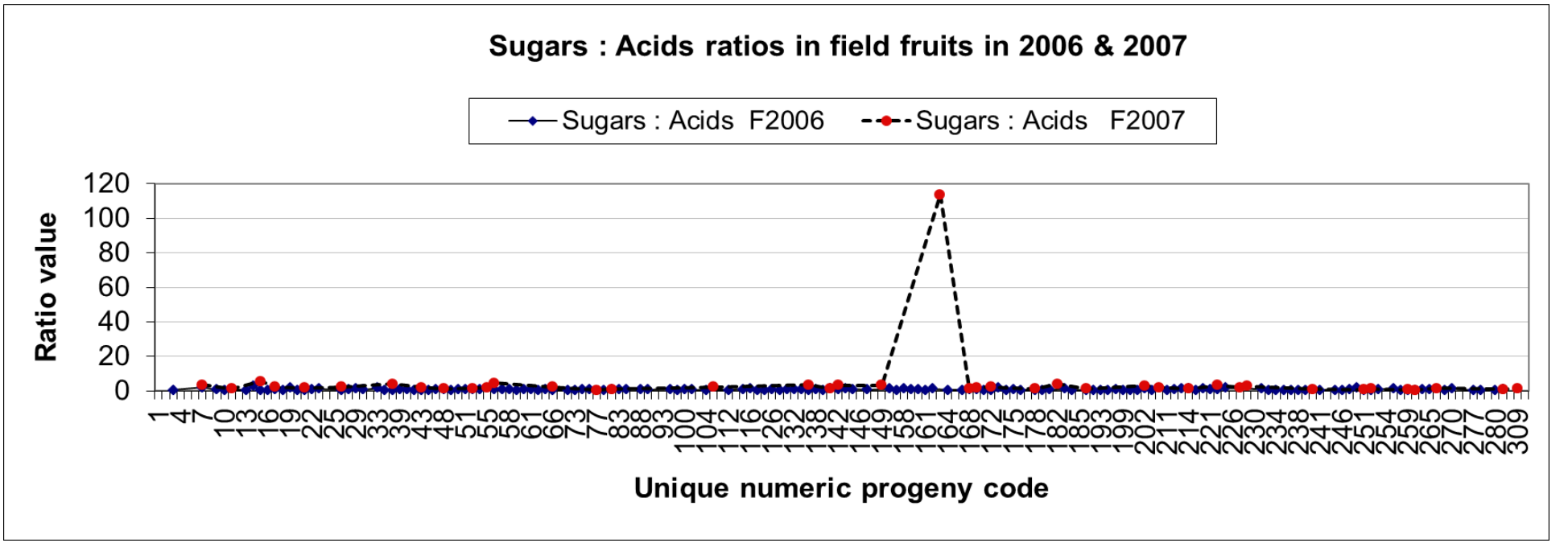


Figure 2.18 (b) Total sugars : acids in field crops of 2006 (—◆—) and 2007 (-●- -) **with** value in progeny #163 included. X-axis denotes unique numeric code of each progeny and Y-axis ratio. Mean values (mean±SD g/ml) were: 2006 (149)= 0.74±0.44 g/ml and 2007 (107) = 4.82±17.48 g/ml (**with** #163).

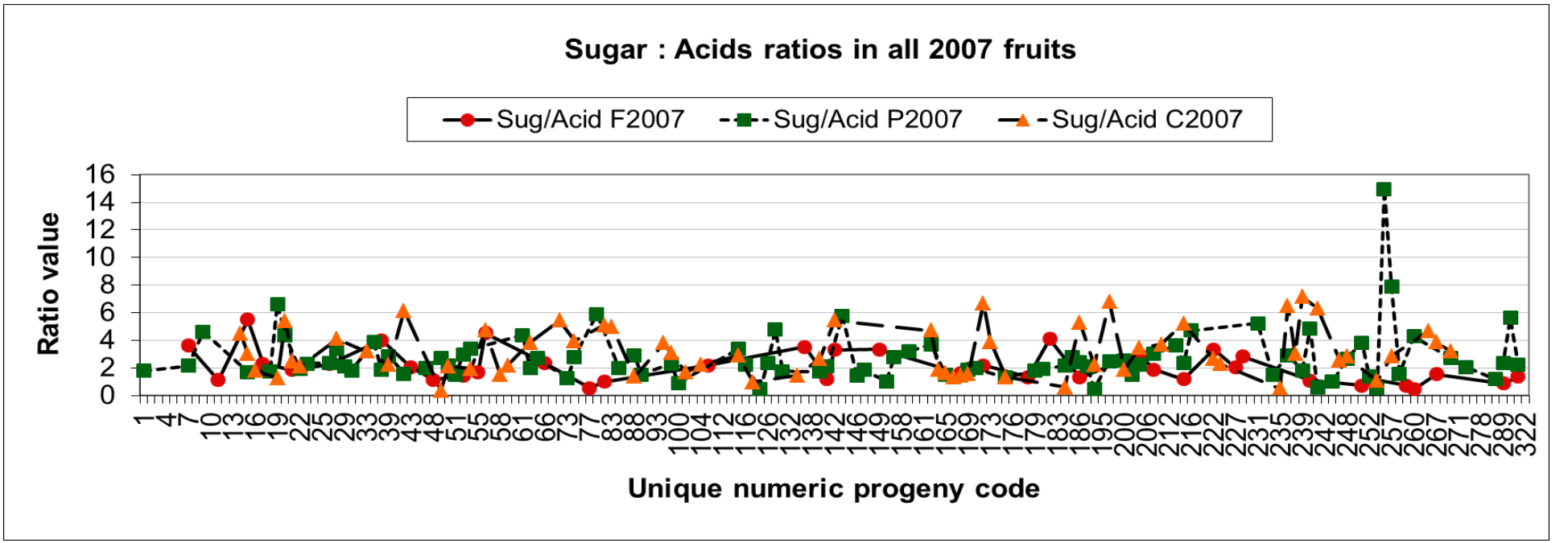
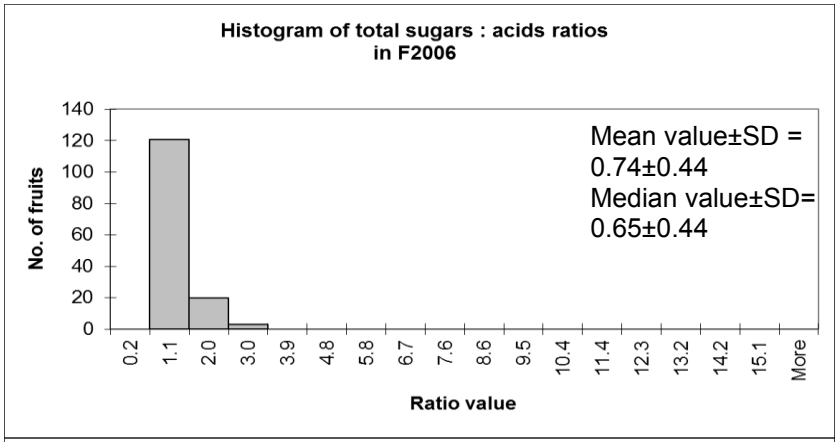
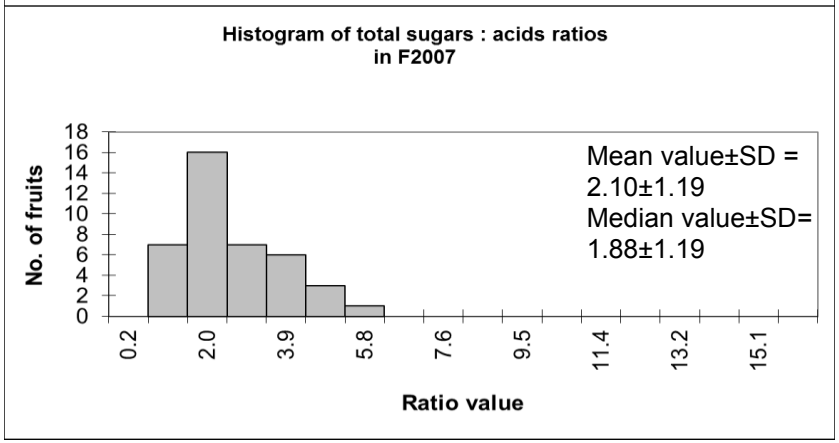


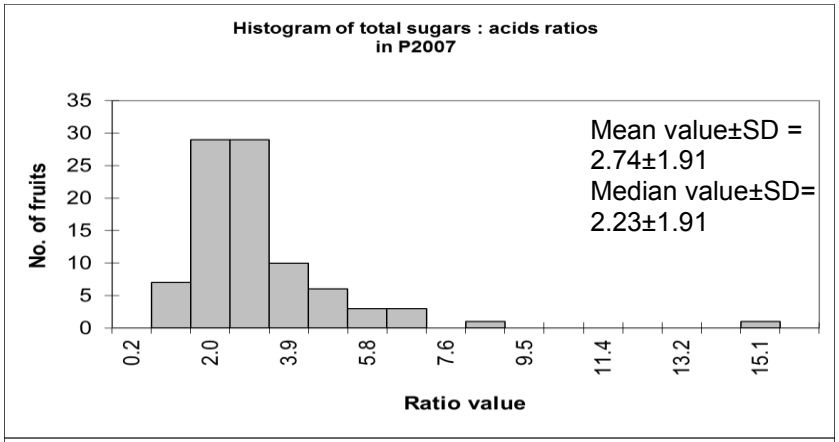
Figure 2.18 (c) Total sugar : acids ratios in all 2007 crops **without** value in progeny #163 from field site. Legend: Field (F 2007 = - ● -), SCRI Polytunnel (P 2007 = - ■ -) and Commercial Polytunnel (C 2007 = —▲—). Mean values (mean±SD g/ml) for each site were: F 2007 (106) = 2.10±1.19 (**without** #163), P 2007 (145) = 2.74±1.91 g/ml and C 2007 (86) = 3.29±1.92 g/ml.

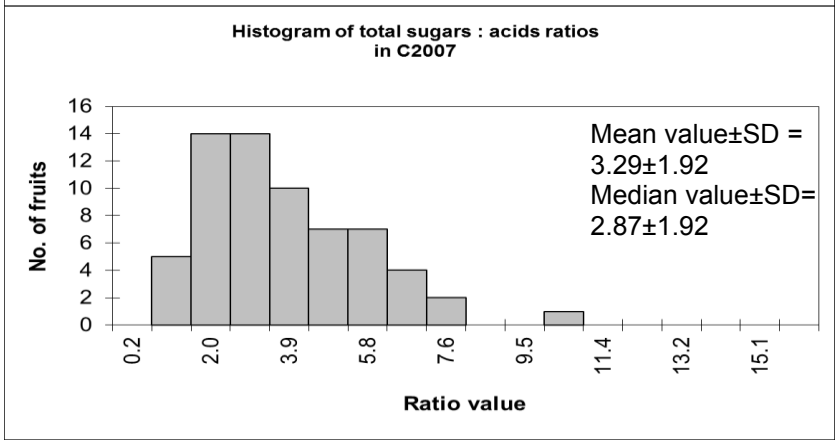




Ratio in parent fruit (mean value±5%LSD)
Glen Moy = 3.91±0.56

Figure 2.19 Distribution of total sugars : total acids ratios in field crops in 2006 (F 2006; 149) and 2007 (F 2007; 107). Parent fruits were unavailable from the 2007 field site due to poor yield. Value for progeny #163 from 2007 field crop was excluded.





Ratio value in parent fruit (mean value±SD)
Glen Moy = 63.10±1.91
Latham = 1.31±1.91

Figure 2.20 Distribution of total sugars : total acids ratios in 2007 polytunnel crops in 2 sites; SCRI (P 2007; 145) and Commercial (C 2007; 86) sites. Parent fruits were unavailable from the 2007 Commercial polytunnel due to disease. Value for progeny #163 was excluded.

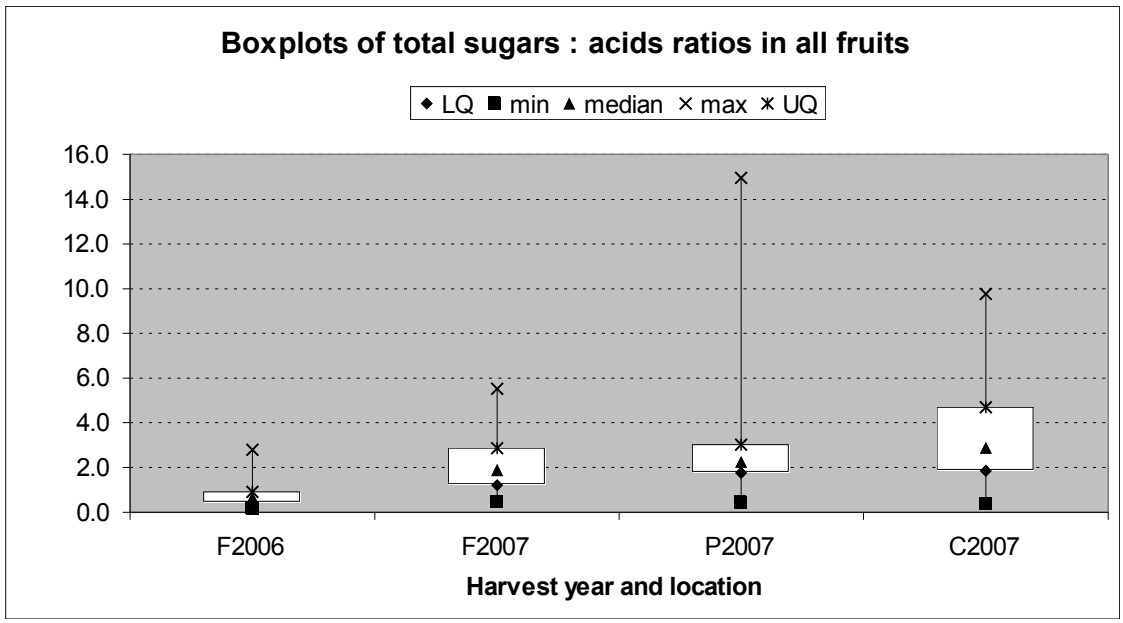


Figure 2.21 Distributions of total sugars : total acids ratio values. Legend: F2006 – 2006 field crop, F2007 - 2007 field crop, P2007 – 2007 SCRI polytunnel crop, C2007 – 2007 Commercial polytunnel crop.

Table 2.9 Summary of results of 2 sample *t*-test to determine significance of differences in fruit total sugars : acids ratio from different years (2006 and 2007) and 3 sites (F – field, P – SCRI polytunnel and C – commercial polytunnel) at 95% confidence level ($\alpha = 0.05$), sig = p-value < 0.05.

	p -value			*p* –value			
	F 2006	F 2007		F 2007	P2007	P 2007	C 2007
Total sugars: Total acids	0.14			0.45		0.08	

2.3.9 Heritability of metabolite contents

Heritability estimates of metabolite contents were 6.5 – 41.2% higher for 2006 field crop compared to estimates for all 2007 crops (Table 2.10). Heritability estimate values were highest for glucose and total acid content in 2006 and similar for all other sugars and acids content. For 2007 crops, heritability was highest overall for Commercial polytunnel crop. However, highest individual estimate value was for citric acid content in field crop. Heritability estimates values for sugar contents in 2007 field crop were 30%-34% lower than for acids content.

Under polytunnel cultivation, estimate values increased with exception of citric and total acids content, which had a 19% - 28% reduction. From heritability estimate values, it was deduced that, with polytunnel cultivation, the genotypic influence on fruit metabolite contents increased, with exception of citric and total acids. However, only up to 40% of variance in metabolite contents was due to genotypic factors. Environmental influence accounted for more than half of this trait variance.

For raspberry ketone contents, heritability estimate value of polytunnel crop was 50.4%. Whether environmental factors influenced genotypic effects on raspberry ketone content could not be determined, because contents were quantified from fruit of only a single site. Generally, for sugars and acids content, managing cultivation conditions is possibly effective in regulating its accumulation in fruits, but from varying heritability estimates values between sites indicated here, it also infers a strong 'genotype linked to environment' interaction that results in the final metabolite content of fruits.

Key points:

- Metabolite formation and accumulation into fruits had an estimated 46.7% percent heritability estimate.
- Polytunnel cultivation and genotypic interaction had the strongest influence in determining metabolite content variance.
- Genotypic influence on metabolite contents of fruits cultivated under polytunnel was accountable for < 40% of trait variance.

Table 2.10 Variance components and heritability estimates for fruit metabolite content traits in 2006 and 2007. Heritability was estimated using different equations (explained in 3.3.5) in 2006 and 2007 because of different replications. Legend: F 2007 = 2007 SCRI field , P 2007 = 2007 SCRI polytunnel and C 2007 = 2007 Commercial polytunnel crop.

	2006	2007								
Metabolite contents	h^2 (%)	σ^2_G			σ^2_{GS}			h^2 (%)		
		F 2007	*P 2007*	*C 2007*	*F 2007*	*P 2007*	*C 2007*	*F 2007*	*P 2007*	*C 2007*
Fructose	**46.0**	0.0555	0.2868	0.5377		0.8801		**5.9**	**24.6**	**37.9**
Glucose	**47.1**	0.0088	0.0274	0.0439		0.0801		**9.9**	**25.5**	**35.4**
Malic acid	**46.6**	0.0016	0.0013	0.0027		0.0056		**22.6**	**19.2**	**32.0**
Citric acid	**46.7**	0.0357	0.0115	0.0074		0.0546		**39.5**	**17.4**	**12.0**
Total sugars	**46.6**	0.0857	0.4522	0.7492		1.2872		**6.2**	**26.0**	**36.8**
Total acids	**47.0**	0.0476	0.0181	0.0125		0.0783		**37.8**	**18.8**	**13.8**
Raspberry ketone	**-**	-	-	-		-		**-**	**50.4**	**-**

2.3.10 QTL for sugars and acids content

A permutation test was carried out using Genstat 10 for Windows (Genstat, 2007) to establish appropriate thresholds for the Kruskal-Wallis test. Interval mapping was then carried out using MapQTL. If the Kruskal-Wallis analysis indicated that the phenotype was affected by alleles from both parents, the trait was analysed using a four-mean QTL model. If alleles from only one parent were affecting the trait, the marker data were recoded so that MapQTL fitted a two-mean model. QTLs were identified through interval mapping with permutations of 10 rounds or less and with LOD scores > 2.0.

A summary of all preliminary QTLs is presented in Table 2.11. For 2006 field fruit, 6 QTLs were identified in 3 linkage groups: 3 QTLs on LG 1 (Fig. 2.22), a QTL on LG4 for sugar content (Fig. 2.25) and 3 QTLs on LG3 for acid content (Fig. 2.24) which explained the 9.9% - 10.7% trait variance (Table 2.10). For LG1 (Fig. 2.22), which had most QTLs for sugar content of 2006 field crop, the QTLs co-located with a marker for bHLH (basic-helix-loop-helix) transcription factor. The QTL for glucose content in 2006 field crop also co-located with the FRUITE4 marker on LG4 (Fig. 2.25). These 2 markers (bHLH transcription factor and FRUITE4) are implicated in metabolism of cyanidin anthocyanin contents in raspberry pigment development (Kassim et al., 2009). For 2007 crops, a total of 13 QTLs were identified for metabolite content. The QTL with the highest explained trait variance (40.9%) was for the glucose content of the commercial polytunnel crop, on LG 3 (Table 2.10). This QTL co-localised with the total sugars QTL for the same crop, although this co-localisation must be verified with further fine mapping due to the large QTL interval. QTLs for malic acid content in polytunnel crops were identified on LG2, accounting for 8.8% - 18.8% of the trait variance. These QTLs co-localised with gene *H* and a marker for raspberry intrinsic tonoplast protein (RaspTIPSNP). Gene *H* affects presence of hairs on canes and is also associated with early ripening (Graham et al., 2009) and RaspTIPSNP, is a member of the aquaporin family, responsible for transport of water and small molecules into plant cells. This was also found to have genetic associations with raspberry colour development and the

ripening process (McCallum et al., 2010). For metabolite QTLs in LG3 (Fig. 2.24), care in interpretation must be applied to malic acid and total sugar QTLs for polytunnel crops. This is due to the large QTL intervals for both QTLs. Further fine mapping should reduce and lend verification to its validity. Therefore, at best, the genetic associations reported here are possibly just simple linkages. Markers of interest on LG3 (Fig. 2.24) are the MYB-transcription factor, also implicated in anthocyanin production of raspberries (Kassim et al., 2009) and closely placed near the acid contents QTLs of the 2006 field crop. In LG4 (Fig. 2.25), the citric acid QTL for the commercial polytunnel crop co-located with a RiD4R2 marker, which was found to also co-localise to colour development QTLs in raspberries (McCallum et al., 2010). Other metabolite QTLs in LG5 for 2007 crops did not co-localise to any other known markers.

2.3.11 QTLs for raspberry ketone contents

All QTLs for raspberry ketone contents were located on LG2 (Fig. 2.23). The trait explained why the variance for these QTLs was between 12.0% - 13.5% and all 3 QTLs clustered from 70.8cM – 99.1cM on the LG2 (Table 2.11). These QTLs co-located to malic acid QTLs of 2007 polytunnel fruits and therefore, to markers of interest for Gene *H* and for metabolite transport, RaspMIPSNP and RaspTIPSNP.

2.3.12 QTL analysis of flavour metabolites

Key points:

- QTLs for sugar contents co-located with markers for bHLH transcription factor and FRUITE4, implicated in pigment development.
- QTLs for acid contents co-located to markers for Gene *H* and metabolite transport proteins, *MIP* and *TIP*.
- Raspberry ketone QTL also co-located to markers for transport proteins.

Table 2.11 Summary of metabolite QTLs: crop year, site, linkage group (LG), location on LG, LOD score and percent (%) trait variance explained. Legend: F = SCRI field, P = SCRI polytunnel and C = commercial polytunnel. Values in italics are highest in trait explained variance.

Metabolites	**Crop**	**Site**	**LG**	**Locus (cM)**	**LOD score**	**% variance**
Fructose	2006	F	1	90.21- 109.34	3.32	11.1
Glucose	2006	F	1	90.21- 106.25	3.46	11.8
		F	4	109.70- 112.90	2.81	12.3
	2007	C	3	*56.39- 58.88*	*5.58*	*40.9*
Citric acid	2006	F	3	89.14- 91.27	2.47	9.9
	2007	F	5	53.20- 55.80	2.24	10.6
		P	3	9.09- 13.60	2.64	8.7
		C	4	*59.70- 62.20*	*2.32*	*14.3*
Malic acid	2007	P	2	99.10- 106.85	2.76	8.8
			3	67.25- 126.80	2.65	9.1
		C	2	*76.12- 82.20*	*2.68*	*16.8*
Total sugars	2006	F	1	90.21- 109.34	3.38	11.5
	2007	C	3	*58.88- 84.68*	*3.55*	*32.1*
Total acids	2006	F	3	89.14- 91.27	2.57	10.7
	2007	F	5	53.20- 55.80	2.12	10.4
		C	5	*10.90- 11.90*	*2.33*	*14.2*
Raspberry ketone	2007	P	2	70.8- 82.2	3.14	12.6
			2	82.2- 95.3	3.49	13.3
			2	*82.2- 99.1*	*3.06*	*13.5*

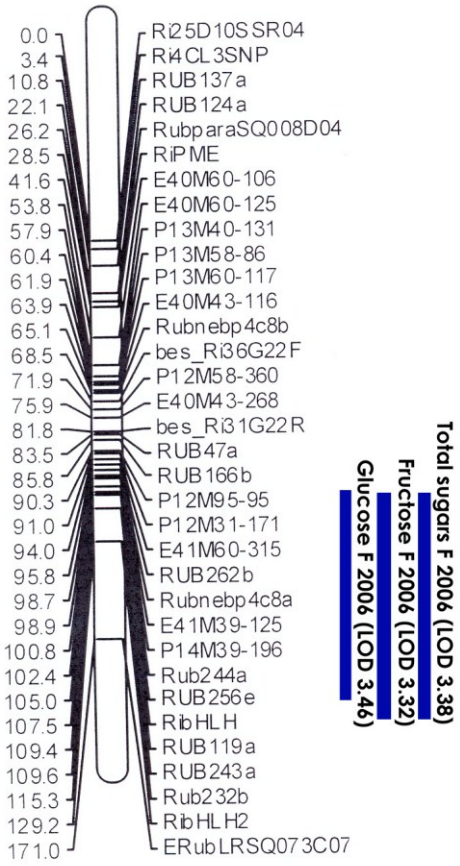


Figure 2.22. QTLs of metabolite contents on linkage Group 1: 3 QTLs covering glucose (1), fructose (1) and total sugar (1) contents, all for 2006 field crop. All QTLs are generally placed on the joint map. Legend: F 2006 = SCRI field crop 2006.

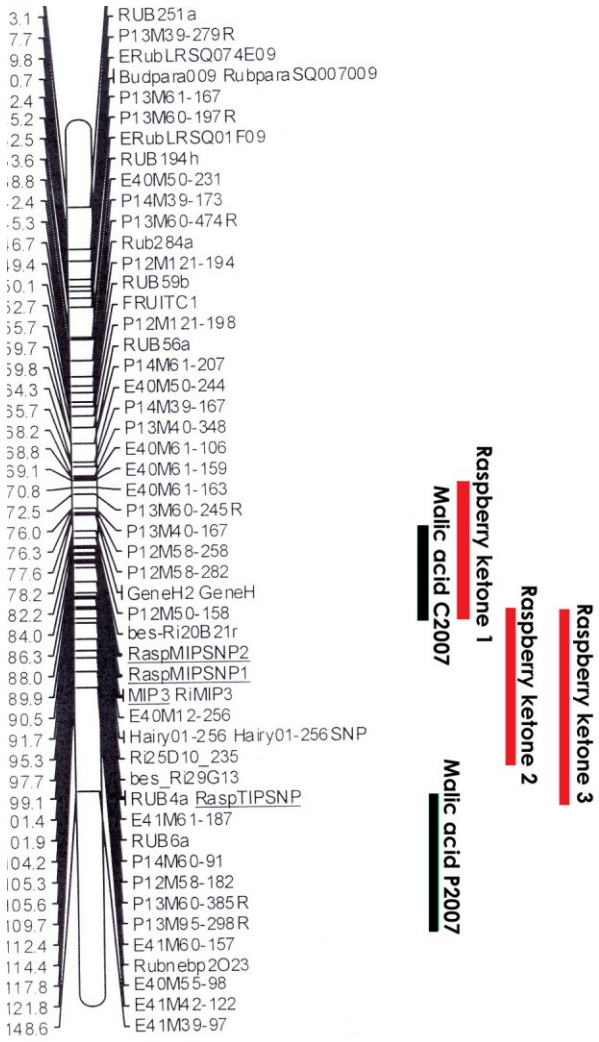


Figure 2.23. QTLs of metabolite contents on linkage Group 2: 5 QTLs covering malic acid (2) and raspberry ketone (3) contents. All QTLs are generally placed on the joint map . Legend: P 2007 = SCRI polytunnel crop 2007, C 2007 = commercial polytunnel crop 2007.

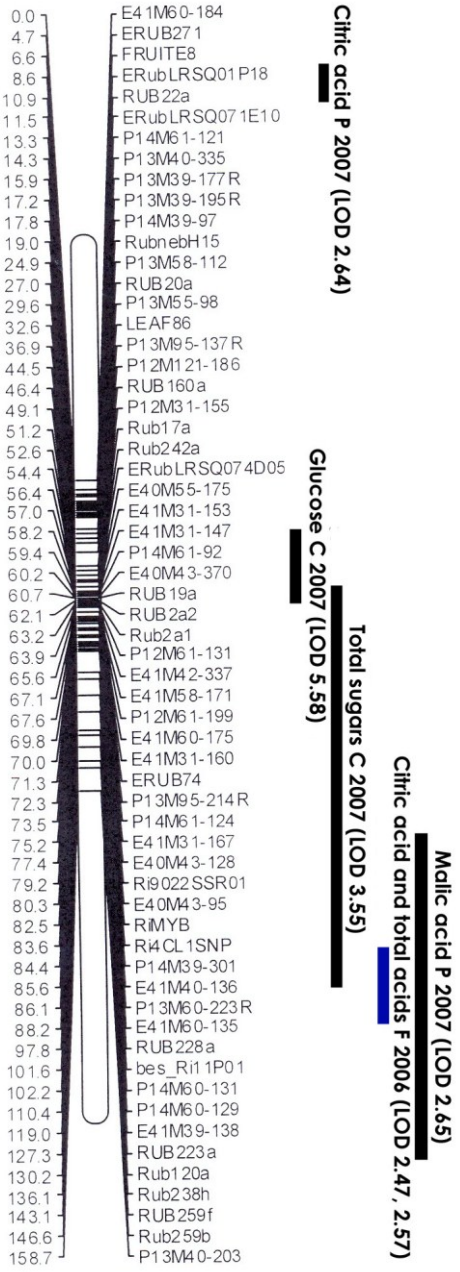


Figure 2.24. QTLs of metabolite contents on linkage Group 3: 4 QTLs covering malic (1), citric and total acids (1), glucose (1) and total sugar contents (1). All QTLs are generally placed on the joint map. Legend: F 2006 = SCRI field crop 2006, P 2007 = SCRI polytunnel crop 2007, C 2007 = commercial polytunnel crop 2007.

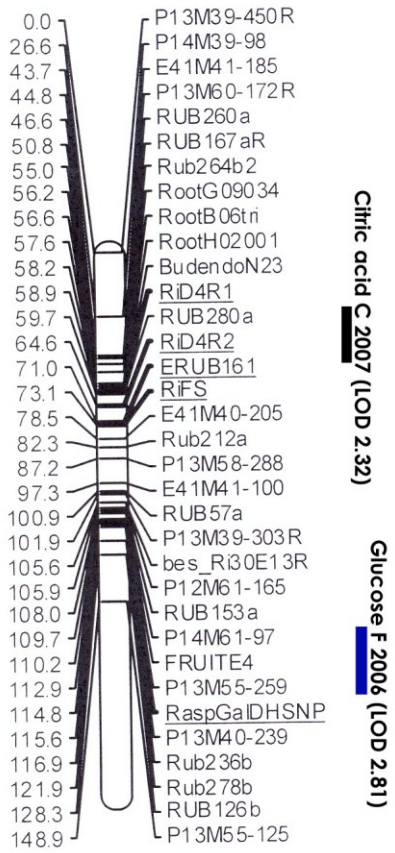


Figure 2.25. QTLs of metabolite contents on Linkage Group 4: 2 QTLs covering citric acid (1) and glucose (1) contents. All QTLs here identified on Latham map. Legend: F 2006 = SCRI field crop 2006 and C 2007 = Commercial polytunnel crop 2007.

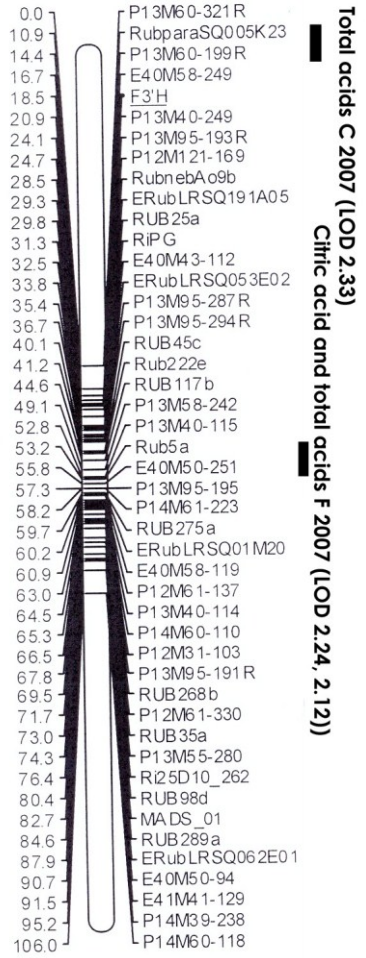


Figure 2.26. QTLs of metabolite contents on Linkage Group 5: 3 QTLs covering citric acid (1) and total acid (2) contents. All QTLs are generally placed on the joint map. Legend: F 2007 = SCRI field crop 2007 and C 2007 = Commercial polytunnel crop 2007.

2.4 Discussion

2.4.1 Seasonal variation

Key findings:

- Increased water availability but lower sun exposure and temperature reduced sugars and acids content of raspberries
- QTLs had genetic links with markers for metabolite transport proteins and other plant development processes

The 2006 field crop was produced during a hotter, drier summer with higher sun exposure: mean temperature, 14.2^{o}C; average rainfall, 49.8 mm; and 537.2 total sunlight hours. Conditions were different in 2007 with mean temperature, 12.7^{o}C; average rainfall, 127.5mm and 368.9 total sunlight hours (Source: *www.metoffice.gov.uk*). Such variation in weather in the 2 seasons resulted in different profiles of fruit sugar and organic acid content (Table 2.5). Such differences in composition suggest accumulation of these key metabolites into fruit cells was influenced by the changing growing conditions.

Temperature affects activity of enzymes involved in plant development and metabolite transport into plant cells. Accumulation of sugars in fruit begins with sucrose cleavage to yield fructose, glucose and uridine diphosphate glucose (UDP-glucose). This process is facilitated by two enzyme classes, sucrose synthases and invertases. Genes encoding expression of enzyme activity are activated at different intervals during plant growth and/or as responses to cellular stress stimuli (Koch, 2004). Sucrose synthases are primarily involved in feeding assimilated carbon (i.e. sucrose) into phloem and invertases convert this to monosaccharides, which with disaccharide are stored in sink cells in fruit (Sturm and Tang, 1999). Sucrose hydrolysis to glucose and fructose also has impact on the activity of the tricarboxylic acid cycle (TCA), the primary source of organic acids. In fruit when hexoses act as the main substrate, factors that influence conversion of sucrose to monosaccharides would impact also on the production of

organic acids production by the TCA cycle. From studies in other crops, it is clear that environmental conditions determine activity (and efficacy) of invertases and sucrose synthases. In tomatoes, elevated temperatures and high solar radiation increased sucrose synthase activity, and through more photosynthesis from longer exposure to sunlight, enhanced sucrose accumulation and cleavage-produced hexoses stored in the fruits. Hexoses were also precursors for antioxidant production and therefore increased fruit hexoses was suggested as an antioxidant defence response to increased exposure to sunlight, protecting plants from cell necrosis (Rosales et al., 2007). However in a recent study on red raspberries, the opposite effect of elevated temperature on fruit metabolites content was observed. Increases in day/night temperatures reduced total soluble solids, sugars and organic acids contents and lower temperatures favoured their accumulation with consequential increase in fruit quality (Wang et al., 2009). Differences in reactions to similar stimuli between plant systems were also observed between sugar apple and cherimoya, both of genus *Annona*, but with different climactic requirements for plant development. Higher temperatures and irradiance increased photosynthetic rate in sugar apple, usually grown in tropical lowlands. Due to large temperature intervals in this area, the sugar apple is better equipped to adapt to these conditions (Higuchi et al., 1999). In this study, higher sugar content of fruits resulted from the hotter, drier summer of 2006, which also increased anthocyanin contents in these progeny (Kassim et al., 2009). This observation was similar to findings for tomato (Rosales et al., 2007) and increase in hexose contents is possibly a response to elevated solar irradiation. Anthocyanin biosynthesis requires malonyl-CoA and phenylalanine as precursors, indirectly produced from sucrose cleavage, (Kassim et al., 2009). In peach, higher temperatures resulted in lower malic acid content of fruits (Lobit et al., 2006). In this present study, a wetter and cooler summer in 2007 produced raspberry fruits with considerably decreased acid and sugar content.

Besides temperature, water availability also influences metabolite accumulation in fruit sink cells. In different grape cultivars, which were subjected to varied ranges of water stress, the outcome was reduced sugar content (Van Leeuwen and Seguin, 1994;

Matthews and Anderson, 1988; Santesteban et al., 2006). The opposite effect was found for mangos, with increased yield, total soluble solids, titratable acids and sugar : acids ratio for plants grown under water stress conditions (Spreer et al., 2009). Similar results have also been reported for pear jujube fruit (Cui et al., 2008), mandarins (Navarro et al., 2010) and 'lane-late' oranges (Pérez-Pérez et al., 2009). Roles for aquaporins as water transporters appear in many aspects of crops. Such proteins were implicated in reduced hydraulic conductivity and sap flow rate, affecting plant growth in a study of drought effect on sugar beets (Shaw et al., 2002). In addition, overexpression of *Arabidopsis* PIP1B gene in transgenic tobacco plants, which controls the genetic expression of a plasma membrane aquaporin, resulted in faster wilting of plants under drought conditions (Aharon et al., 2003).

In this study on red raspberry fruit, increased water availability in 2007 resulted in lower acid and sugar content. A genetic linkage between aquaporins and metabolite content was indicated by the polytunnel malic acid QTL co-localisation with RaspTIPSNP molecular marker, a tonoplast intrinsic protein (TIP), which is a characterised raspberry aquaporin. But the majority of QTLs for 2006 field crop (in LG1 and co-localised with markers for transcription factors (bHLH and FRUITE4) associated with pigment development in berries. Therefore changes in growing conditions from field to polytunnels may favour different genetic regulation systems that impact on metabolite accumulation in fruits. In gene expression studies using yeast models, a tonoplast intrinsic protein is implicated in osmoregulation of plant sink cells (Prudent et al., 2005).

In studies of transgenic tomatoes employing anti-sense membrane intrinsic protein (MIP) constructs, specific aquaporins are exclusively implicated in solute accumulations that influence fruit fructose, glucose, citric and malic acid content (Chen et al., 2001). Antisense plants had lower sugar but higher acid content. A similar study could be conducted on the mapping population to determine if higher sugar and acid contents of Commercial polytunnel crop was due to increased aquaporin genetic expression.

Another indication of possible genetic association in metabolite transport and accumulation in fruits was co-localisation of acids and total sugars QTLs with the marker for transcription factor MYB, implicated in anthocyanin biosynthesis and phenylpropanoid pathway regulation. There is a relationship between fruit organic acids in raspberries and anthocyanin synthesis. This could either be through co-regulation or the contribution of the TCA cycle to pigment and volatile syntheses, both components important contributors to overall final fruit quality (Kassim et al., 2009).

In this study, temperature and water availability influenced accumulation of sugars and acids in fruits. Candidate genes for QTL for these traits include a range of aquaporins and transcription factors. The two environmental factors, from studies in other plants, seem to influence water and metabolite transport and accumulation, and similar roles, together with TCA cycle activity would seem to determine fruit sugar and organic acid contents in red raspberries.

2.4.2 Protected (polytunnel) cultivation

Key findings

- Polytunnel cropping increased sugars and acids contents in raspberries with best cultivation practice found in Commercial site.

Considering the two SCRI crops in 2007, fruits produced under polytunnels had higher sugar and acid content than from the field. Polytunnel cultivation is generally used to overcome problems associated with seasonal effects, crop production out of season, controlled natural light exposure, drought and pest control (Cohen et al., 2005; Kittas et al., 2006; Hanafi et al., 1999). However, for a range of strawberry cultivars, polytunnel cultivation has been reported to reduce yield in comparison of plants grown in the field and under plastic greenhouse (Paraskevopoulou-Paroussi et al., 1991). However, fruit quality was considered similar. The yield may have been reduced due to diminished exposure to specific light wavelengths, which promote plant growth. Fletcher and others for example, reported a 51% increase in marketable yield in ‘Elsanta’ strawberries

grown under a film for highest light transmission (Fletcher et al., 2004). Impacts of protected cultivation on yield in other fruit crops have also been noted. Early ripening and higher mean yield (i.e. yield/tree and yield/ha) was observed with loquat plants grown in glasshouses, bearing fruits 13 - 20 days earlier compared to controls grown in the field (Polat et al., 2005). In this study, increased berry yield was found from plants grown under polytunnels (SCRI and commercial), in terms of increased berry size and number / plant (Graham, pers. comm.). This finding is a replication of a study on raspberry cv. Glen Ample, grown in glasshouses and in the field (Sønsteby et al., 2009). Plants grown under glasshouses bore more fruits, which ripened earlier than controls grown in the field. However, plants grown at temperatures above ambient did not exhibit such differences. Differences in berry yield between protected and field plants were attributed to changes in plant characteristics, i.e. cane height, reduced number of dormant buds and increased berries per lateral.

Different effects of protected cultivation on fruit metabolite contents have also been reported for strawberries: growth of cv. 'Elsanta' under polytunnel films varying in light transmission profiles resulted in reduced contents of glucose and sucrose, with no significant effect on citric acid (Watson et al., 2002). However, in another study, polytunnel cultivation yielded fruit with higher total soluble solids (i.e. higher ^{o}Brix values), with general parallel increase in berry quality (Voća et al., 2009). Such findings indicate protected cultivation could have varying effects in differing and possibly also closely-related Positive effects of protected cultivation consist of minimised environmental effects resulting in earlier ripening, higher berry yield, increased total soluble solids that culminated in enhanced fruit quality.

2.4.3 Genetic inheritance of sugars and organic acids content

Key findings:

- QTLs for sugars and acids contents in raspberry fruit crops were co-located to markers associated to transcription factors and genes that controlled expression of metabolite and water transport proteins.

A total of 16 QTLs for sugar and organic acid content were identified in this study: 5 for 2006 field and 10 for (all three) 2007 crops. For QTLs in 2007 crops, 2 were for field fruit with 3 and 5 respectively in SCRI and Commercial polytunnel crops. LG 3 had highest number of QTLs, which co-located to markers associated with ripening and colour development in raspberries (Graham et al., 2009). Of these markers, MYB-transcription factor and 4-coumarate-CoA ligase were significant, both involved in phenylpropanoid pathways in anthocyanin synthesis (Martin and Paz-Ares, 1997; Ehlting et al., 1999; Feng et al., 2010). These markers were co-located to QTLs for acids content in 2006 field and 2007 SCRI polytunnel crops. Acidity (i.e. pH) was shown to affect anthocyanin stability significantly (Holcroft and Kader, 1999); most but not all are stable at low pH values. Therefore, co-location of acids QTLs in this study to colour-associated QTLs of other studies in red raspberries (Graham et al., 2009; Kassim et al., 2009; McCallum et al., 2010) is of significance. However, more evidence is required to fully characterise these genetic associations, as QTLs identified here are only preliminary data in an ongoing research programme.

2.4.4 Raspberry ketone contents

Key findings:

- Parent varieties had higher contents than progeny fruits.
- Lower contents reported in this study could be from different extraction method.
- Progeny contents not much lower than highest contents in commercial varieties.
- QTLs for raspberry ketone had simple genetic links to markers for metabolite transport pathways, malic acid content, as well as cane hair gene expression.

In this study, contents in parental fruits were higher than in progeny. However contents both parent varieties were lower than previously reported possibly due to extraction methodology. In this study, a high throughput method was applied; raspberry ketone was quantified following a bonded phase extraction while in other studies solvent extraction methods are used (Larsen and Poll, 1990; Wyosocki et al., 1992; Klesk et al., 2004; Malowicki et al., 2008). Raspberry ketone is sensitive to degradation from solvents: in one study severe enough that none was recovered (Malowicki et al., 2008). However, contents in parent fruit from this study were similar to those reported by Wyosocki and others (1992), but average contents in progeny were 64% lower than the maximum in commercial varieties and 57% - 62% lower than in parent fruits. Hence, factors other than heritability are possibly of a stronger impact on raspberry ketone content. Heritability was shown to account for approximately half of this trait variance. Important genetic markers linked to raspberry ketone QTLs are those for metabolite transport proteins and Gene *H*. This suggests common regulatory pathways, where gene expression system of cane hair presence could also possibly impact on raspberry ketone synthesis. However, such genetic links are at best assumptions of correlations, which require further evidence to verify and characterise.

2.5 Conclusion

Sugar and organic acid accumulation in fruit is important in the development of flavour quality at harvest. There are many factors affecting this process and three factors were examined in this study: seasonal variation, cultivation practices and heritability. Seasonal variation had significant effects on accumulation of sugars and organic acids, with hotter, drier summer of 2006 producing fruits higher in sugars and acids compared to lower quantities of sugars in fruits from a wetter and cooler summer in 2007. Longer sun exposure and higher mean temperatures in 2006 could have triggered plant antioxidant defence in progeny plants, which increased sugars content. Polytunnel cultivation resulted in increased fruit sugars and organic acids, compared to field fruit crop.

Analyses indicated co-localisation of fruit sugars and acids content QTLs to markers and candidate genes associated with ripening, colour development, transcription factors and enzymes important in phenylpropanoid pathways. There were also links between sugars and acids QTLs to ripening processes, pigment development and gene expression of other plant processes. Such findings provide preliminary indications that sugars and acids are not only important to raspberry fruit flavour but possibly also in ripening pathways, which impact on overall berry quality. Genetic links of raspberry ketone QTL with malic acid QTLs and genetic markers for plant metabolism indicate a role of raspberry ketone to not only fruit aroma but also in other plant functions. These genetic links are preliminary and would need further verification, but links identified here provide focus for future research.

CHAPTER 3

Genetic and Environmental Determinants of Flavour Quality

Chapter 3 Genetic and Environmental Determinants of Flavour Quality

3.1 Introduction

3.1.1 Raspberry and its flavour characters

Of many factors used as fruit quality indices, flavour is perhaps the most important (Liem et al., 2006; Péneau et al., 2007; Brug et al., 2008). Taste and aroma, integral components of flavour, arise from fruit non-volatile (e.g. sugars and organic acids) and volatile (e.g. raspberry ketone) contents respectively. Flavour however, in practice, cannot be clearly divided into separate sensory cues of taste and aroma, as these share common information assembly in the brain.

There are two primary taste attributes important in raspberries, sweetness and sourness. It is generally the balance of these tastes that make raspberries enjoyable and reason for repeat consumption. Acceptable and favourable raspberry flavours are *fruity, floral sweet* with some *acidity* and no *bitterness* (Harrison et al., 1998). Presently, overall fruit flavour quality is first judged by multiple retailers before becoming available to consumers. Hence retailer viewpoint ultimately determines raspberry cultivar success and its persistence in the market.

3.1.2 QTLs for flavour character

Genomic approaches have become a popular strategy in fruit breeding for flavour quality because it significantly reduces time needed for cultivar development, from genetic seedling screening before planting. Sensory and biochemical data can be linked to specific regions on a genetic linkage map, giving flavour QTLs, often an approach used by researchers to study genetic flavour quality regulation. These QTLs are used as genetic markers to pre-select seedlings for desirable flavour traits before planting.

Tomato flavour has been researched extensively using such genetic strategies, notably using a mapping population derived from crossing two parent varieties of dissimilar phenotypic profile; cv. *Cervil* and *Leovil* (Causse et al., 2001; Saliba-Colombani et al., 2001). The results were 13 QTLs for flavour attributes: sweetness, sourness and aroma profile with 3 QTLs for tomato textural properties. The flavour

QTLs co-located with others for traits which contribute to and / or influence flavour development: soluble solids, sucrose contents, fruit weight, fruit diameter, titratable acidity, elasticity, and dry matter content. There was also correlation with specific tomato aroma volatiles: hex-3-en-1-ol, 3-(methylthio) propanal and 2-isobutylthiozole. In a further study on tomato textural properties, comparison of progenies from *Cervil x Leovil* with *Leovil x VilB* crosses showed QTL interactions over large (20 - 50 cM) chromosomal regions because QTLs were not conserved (Chaïb et al., 2005). In *Rosaceae*, a well-researched crop is apple, where texture is an important flavour quality trait for the fresh fruit market because it influences juice release. Textural properties influence release of flavour compounds during gustation, like sugars, which determine sweetness (Harker et al., 2002). King et al. (2000, 2001) correlated texture and sensory QTLs, which included attributes for texture (*slow breakdown*, *crispness*, *granularity*, *hardness* and *sponginess*) and flavour (*juiciness* and *overall*-liking). Together these traits mapped to nine of sixteen linkage groups (LOD scores ≥ 3.0) (King et al., 2000). *Crispness*, *juiciness*, *sponginess* and *overall-liking* QTLs mapped to a single region on linkage group (LG) 16, which co-localised to instrumental texture QTLs (King et al., 2001). Other than relating textural and sensory properties to genomic regions, these studies also demonstrate that instrumental analyses texture data could also be flavour quality indicators.

From these studies, data from progenies of parental varieties dissimilar in phenotypic profiles to construct genetic linkage maps is well established. Sensory data, associated to biochemical data can be linked to genomic alleles through marker mapping technology, yielding flavour QTLs (i.e. QTL mapping). This approach can also be extended to other variables that affect sensory and consequently, overall flavour quality, for example metabolites content and textural properties.

3.2 Aims of research

The second objective of this study was to characterise three flavour attributes: sweetness and sourness, and flavour intensity in the progeny population of cv. Glen Moy x Latham. These parent varieties differ in geographic origins and sensory profiles. Effects of environmental factors on sensory traits, namely different harvest years and cultivation practices are also studied. A final objective was to identify

genomic loci most associated with sensory trait variance, yielding sensory QTLs. These QTLs, with metabolite QTLs mapped in **Chapter 2**, will give flavour QTLs. The predicted outcomes of this study were flavour QTLs and preliminary understanding of genotype and environmental influences on raspberry flavour.

3.3 Materials and methods

3.3.1 Collection and processing of fruit

Sensory analyses were done on same collected fruits for quantification of sugars and acids (**Chapter 2**). After manual picking, berries were placed in polyethylene bags, sealed and labelled with its unique 3-digit code identity, transported at 5^{o}C to University of Strathclyde (distance: 80.4 miles) and stored at the same temperature for no longer than 48 hours.

For processing, approximately 15 g fruit was pureed using a hand-held blender and aliquots (ca. 2 g) were placed in 15 ml matte plastic cups, labelled with randomly generated numeric codes. For panel selection, supermarket berries were pureed and supplemented with either food-grade aspartame (3.2 mg and 7.2 mg / g fruit puree) (Splenda, Washington, USA) or citric acid (13.4 mg and 26.7 mg / g fruit puree) (E 330 Citric Acid, Young's Home Brew, West Midlands, UK).

3.3.2 Screening of assessors

Sensory panellists were selected through assessment of ability to rank sweetness and sourness of raspberry puree samples, using a category scale of 1 (least) to 3 (most). A reference was provided: raspberry puree without supplementation with either aspartame or citric acid. Individuals who ranked correctly were chosen as panellists for experimental fruit rating. A specimen ranking analysis sheet is given below (Figure 3.1).

Name: Age: Date:

Sensory Evaluation of Raspberries

(1) Rank these samples based on sweetness:

731 571 351

___ ___ ___

(2) Rank these samples based on sourness:

537 931 793

___ ____ ____

Figure 3.1 Ranking form for sweetness and sourness scoring to screen assessors

3.3.3 2006 sensory panel

Assessors were recruited from SCRI students and staff, all healthy non-smokers, aged between 18 – 60 years. The experimental design, used for sample presentation, was an incomplete block design generated using DesignExpress software (QiStatistics Ltd., Reading, UK). The experimental design was to minimise assessor carry-over effects. In 2006, sensory scores for sweetness, sourness and flavour intensity were collected on paper form, using 7-point category scale. A sample of the scoring sheet is provided below (Figure 3.2). Sets of 12 samples were analysed daily, in 2 sessions, each of 6 fruits. Assessors were provided with a plastic spoon, a 50 ml cup of ambient mineral water and a water biscuit to cleanse palates between samples.

Sensory Analysis of Raspberries

Date: Name: Sample No.:

(1) Rate this sample based on sweetness:

☐ ☐ ☐ ☐ ☐ ☐ ☐

A little sweet Sweet Very sweet

(2) Rate this sample based on sourness:

☐ ☐ ☐ ☐ ☐ ☐ ☐

A little sour Sour Very sour

(3) Rate this sample based on Flavour intensity:

☐ ☐ ☐ ☐ ☐ ☐ ☐

Mild Medium Very strong

Figure 3.2 Scoring form for sweetness, sourness, and flavour intensity of fruit

3.3.4 2007 sensory panel

In 2007, assessors were selected from volunteers at the University of Strathclyde, Glasgow. Staff and students, 20 – 60 years, were screened as assessors as in 2006. Fruits were rated in individual booths under red lighting to minimise colour bias, an issue in 2006. Data was collected, stored and analysed using sensory analysis software (FIZZ; Biosystemes, France). Assessors, twelve to fifteen per day (depending on availability) rated fruits using a 7-point category scale. Presentation order was determined by a complete block design with each (3) planting site a block, rating 24 fruits per day, in 2 sessions of 12. There were more assessors in 2007 than in 2006.

3.3.5 Statistical analyses of scoring data

Variations from experimental factors (i.e. replicate plants, panellists, sites, harvest years) were examined by one-way analysis of variance (ANOVA) and least significant differences (LSD) using Minitab v.14 (Minitab Inc., USA) with *p*-values ≤ 0.05 treated as significant (95% confidence level) and also for univariate

correlations through Pearson's analyses. Microsoft Excel produced linegraphs, histograms and boxplots.

3.3.5 Heritability calculations and QTL analyses

Broad sense heritability was estimated differently for each harvest year due to difference in replication. In 2006, heritability of progeny means was estimated as:

$$H^2 = a\sigma^2_G / (a\sigma^2_G + b\sigma^2_p + \sigma^2_e)$$

where, $a\sigma^2_G$, $b\sigma^2_p$ and σ^2_e are the (additive, dominance and epistatic) variance components for genotypes, plots and clones within plots, $a \leq 4$ (number of replicates x number of clones within a plot) and $b \leq 2$ (number of clones within a plot). In 2007, a single replicate of each genotype was available from each site in each year. Therefore the heritability was estimated as:

$$H^2 = c\sigma^2_G / (c\sigma^2_G + d\sigma^2_{GS})$$

where, $c\sigma^2_G$ and $d\sigma^2_{GS}$ are the variance components for genotypes and genotype x site interaction, $c \leq 3$ (number of sites x number of years) and $b \leq 3$ (number of sites).

QTL mapping was carried out using MapQTL 5 software (Van Ooijen, 2004). A Kruskal-Wallis test was used as preliminary analyses to identify genomic regions linked to each of the first five principal coordinates, and if the phenotype was affected by alleles of one or both parents. A small permutation test was carried out with Genstat 10 for Windows (Genstat 2007) to establish appropriate thresholds for the Kruskal-Wallis test. Interval mapping was performed using MapQTL. If the Kruskal-Wallis results indicated the phenotype was affected by alleles from both parents, the trait was re-analysed using a four-mean QTL model. If alleles from only one parent affected the trait, marker data was recoded so that MapQTL fitted a two-mean model. Flavour sensory QTLs were grouped in 4 of 7 raspberry linkage groups on the genetic map (Graham et al., 2009).

3.4 Results

3.4.1 Replication

One-way ANOVA analysis showed that in 2006, sensory score variations between progeny plants were significant but not between replicate progeny plants. Therefore, in 2007 fruit collection, a decision was made to collect fruits from only one replicate plant in each site.

Key points:

- Sensory score variation between individual progeny plants was significant, but not between replicate plants.
- Fruits from only one replicate plant of each planting site were collected for analyses in 2007.

3.4.2 Scoring of sensory attributes in 2006 field fruit

In 2006 field crop, sensory scores of progeny fruits were within score range of parent fruit scores, except for flavour intensity. Mean scores for flavour intensity were 32.7% and 18.1% higher in Glen Moy and Latham respectively, compared to progeny fruits, and these differences were significant (Table 3.1). Mean sweet : sour ratio value in progeny fruits was also significantly lower than in parent fruits, by 11.1% - 237% compared to Latham and Glen Moy respectively. From *p*-value table, field fruits of the two years significantly varied in sourness and flavour intensity, most significant in sourness, but not in sweetness (Table 3.4).

Nine (9) commercial varieties were also scored on sensorial traits in 2006. Mean sensory scores and comparison to parent varieties are listed in Table 3.2. The lowest mean sweetness score in Glen Rosa significantly differentiated it from Glen Moy. Highest and 2nd highest scores in Malling Leo and Joan J. significantly distinguished these fruits from Latham (Table 3.2). In sourness, there were significant differences between Glen Moy and Glen Rosa due to low scores in Glen Moy. With one of the highest scores in sourness in Latham, it was significantly different to Malling Leo, which had lowest mean score. Despite significant differences in mean sweetness and

Table 3.1 Sensory scores and ratio values in 2006 fruits. All analysed fruits were collected from open field site (131 + 2 parent varieties). Significant differences in sensorial trait at 95% confidence level between parent and progeny fruits are indicated by *sig. diff. with Glen Moy, $^{+}$sig. diff. with Latham and $^{*,+}$sig. diff. with both. 5% LSD calculated with, $t = 2$ and $p = 0.05$.

Season	Fruit		Sweetness	Sourness	Flavour Intensity	Sweet: Sour
F 2006	Progeny	Mean±SD	2.59±0.54	3.18±0.63	3.64±0.51*	0.81±0.02*
		Min-Max	1.36-4.44	1.56-5.26	2.24-5.05	0.74-0.96
	Glen Moy	Mean± 5% LSD	3.92±1.46	2.50±1.41	4.83±1.16	1.57±1.47
	Latham	Mean± 5% LSD	2.60±1.46	3.90±1.41	4.30±1.16	0.67±1.47

Table 3.2 Sensory scores (mean value ± 5% LSD) and ratios of parent (2) and commercial varieties (9) in 2006. Significant differences in sensorial trait at 95% confidence level between parent and commercial fruits are indicated by *sig. diff. with Glen Moy, $^{+}$sig. diff. with Latham and $^{*,+}$sig. diff. with both. 5% LSD calculated with $t = 2$ and $p = 0.05$.

Season	Fruit	Sweetness	Sourness	Flavour intensity	Sweet: Sour
F 2006	***Glen Moy***	3.92	2.50	4.83	1.57
	Latham	2.60	3.90	4.30	0.67
	Tulameen	3.54	3.54	4.83	1.00
	Joan J.	4.50^{+}	2.60	4.70	1.73
	All Gold	3.67	2.50	4.25	1.47
	Glen Ample	4.00	2.75	4.75	1.45
	Glen Rosa	1.92*	4.92*	3.92	0.39
	Malling Leo	4.75^{+}	2.25^{+}	4.42	2.11
	Autumn Bliss	3.33	3.83	4.58	0.87
	5% LSD	**1.46**	**1.41**	**1.16**	**1.47**

sourness scores, flavour intensity was scored similarly in parent and commercial varieties, with also no significant differences in mean sweet : sour ratio values.

Key points:

- Sweetness and sourness in 2006 field crop were scored similar to parent fruits.
- Parent fruits were perceived higher in flavour intensity compared to progeny fruits of 2006 field crop.

Compared to other commercial varieties, Latham and Glen Moy were significantly different in sweetness and sourness scores but not in flavour intensity score and sweet : sour ratio value.

3.4.3 Scoring of sensory attributes in 2007 fruits

Different number of progeny fruits was available in 2007: 118 from SCRI open field, 138 from SCRI polytunnel and 65 from Commercial polytunnel. Mean scores for sweetness and flavour intensity were highest in Commercial polytunnel crop with highest sourness score in SCRI field crop (Table 3.3). Percent differences of highest scored crop and mean scores of other crops were: in sweetness, 2.5 – 32.7%, in sourness, 1.5 - 7.4% and in flavour intensity, 5.2 – 12.6%. Therefore, largest difference was in sweetness scoring, with 32.7% difference between open field and Commercial polytunnel crops. From *p*-values, this difference was the only significant difference for sweetness scoring in the 2 cultivation years and over the 3 sites in 2007 (Table 3.4). Sourness and flavour intensity varied significantly in both harvest years and over all 3 sites in 2007. Most significant variation ($p < 0.001$) was in 2007 SCRI crops, between open field and covered polytunnel fruits, for all sensory traits.

Key points:

- Commercial polytunnel site produced fruits perceived most sweet and intense in flavour.
- Field crops were perceived most sour.
- Variations in all sensory traits were significant in 2007 SCRI crops, between open field and polytunnel.

Table 3.3 Sensory scores and ratio values in 2007 fruits over 3 locations- SCRI open field (118) and polytunnel (138) and Commercial polytunnel (65). Data is missing for Latham fruit from open field of both parent fruits from Commercial sites due to insufficient fruit for analysis (small size, very little quantity and disease). Sample priority was given to metabolite quantification analyses. SD = standard deviation, LSD = least significant difference.

Season	**Fruit**		**Sweetness**	**Sourness**	**Flavour intensity**	**Sweet: Sour**
Field 2007	Progeny	Mean±SD	2.51±0.59	3.92±0.51	3.80±0.52	0.64±0.19
		Min-Max	1.25-3.91	2.55-5.00	2.36-4.92	0.32-1.16
Polytunnel 2007		Mean±SD	3.25±0.77	3.65±0.56	4.07±0.59	0.90±0.30
		Min-Max	1.50-4.08	2.42-4.29	1.85-4.64	0.35-1.23
Commercial 2007		Mean±SD	3.33±0.61	3.86±0.59	4.28±0.43	0.86±0.26
		Min-Max	1.77-4.92	2.92-5.23	3.36-5.46	0.35-1.68
Field 2007	Glen Moy	Mean±5% LSD	3.09±0.59	3.82±0.51	3.64±0.52	0.81±0.19
	Latham		n/a	n/a	n/a	n/a
Polytunnel 2007	Glen Moy	Mean±SD	2.69±0.77	3.88±0.56	3.50±0.59	0.69±0.30
	Latham		3.07±0.77	3.87±0.56	3.80±0.59	0.79±0.30
Commercial 2007	Glen Moy		n/a	n/a	n/a	n/a
	Latham		n/a	n/a	n/a	n/a

Table 3.4 One-way ANOVA analysis of sensory scores difference of fruit crops: (i) field crops of 2006 (F 2006, 131 plants) and 2007 (F 2007, 118 plants), (ii) 2007 field crop (F 2007), SCRI polytunnel (P 2007, 138 plants) and Commercial polytunnel crop (C 2007, 65 plants). $\alpha = 0.05$. Values indicated in bold are with $p < 0.05$, therefore significant.

Variable	p-value (i)		p-value (ii)			
	F 2006	F 2007	F 2007	P 2007	P 2007	C 2007
Sweetness	0.10		**<0.001**		0.49	
Sourness	**<0.001**		**<0.001**		**0.02**	
Flavour intensity	**0.01**		**<0.001**		**0.02**	

3.4.4 Sweetness score in progeny and parent fruit

Mean sweetness scores in field fruit from both 2006 (131 plants) and 2007 (118 plants) differed by only 3.1%. Scoring (Figure 3.3 a) range for both years was 1.0 - 4.4 with only 3.0% of 2006 fruit having scores above 2007 maximum score (3.91). Distributions were also similar, slightly right-skewed in both years. In 2006, progeny mean score was 34% lower than Glen Moy score and more similar to Latham score. However, difference in mean score of both parent fruits was not significant (Table 3.2). In 2007, mean field crop score was 18.8% less compared to Glen Moy fruit (3.09). Data is missing for 2007 Latham from open field and of both parent varieties from Commercial sites due to insufficient fruit for analyses affected by small size, which contributed to very little quantity and plants affected by disease.

Sweetness scores did not significantly differ in field crops of the two years (Table 3.4) and score distributions were similar (Figure 3.3a) more right-skewed in 2006 than 2007. This is also indicated by similar standard deviation values between the two crops. From Pearson's correlation coefficient values, sweetness in 2006 field crop was not correlated to scores in any 2007 crop (Table 3.5). From *t*-test, mean scores of 2006 and 2007 field crop were not significantly different but different between 2007 polytunnel and 2006 field crops (Table 3.6).

In 2007, polytunnel crops had 29.5 – 32.7% higher mean scores than field crop, which had scores in lower score range, 1.2 – 5.5 (Figure 3.3b). Differences in score distributions were apparent in the two crops, right-skewed in field and left in polytunnel crops (Figure 3.4a, b). Mean score for SCRI polytunnel crop was 5.9% - 20.8% higher than in Glen Moy and Latham respectively. This implied sweetness is possibly more affected by genotype x environment interaction than genotype alone. Environmental effects possibly contributed to 29.5% lower mean score in 2007 field crop compared to 2006. In 2007, sweetness scores distributions (Figure 3.5) were widest in polytunnel crops and normal and in field crop, distribution was right-skewed. Pearson correlation analyses indicated significant links in scores of all 2007 crops, strongest between field and commercial polytunnel ($p < 0.01$, $r = 0.42$) (Table

3.5). From *t*-test, in 2007, only field and polytunnel crops differed significantly in mean scores, but not between polytunnel crops.

Key points:

- Field fruit of 2 years did not differ significantly in sweetness.
- Genotype x environmental interaction is a possible factor in sweetness variance between field and polytunnel scores.
- Mean scores of parent and progeny fruit under polytunnel cultivation increased.
- Fruits from different polytunnel sites did not differ significantly in sweetness.

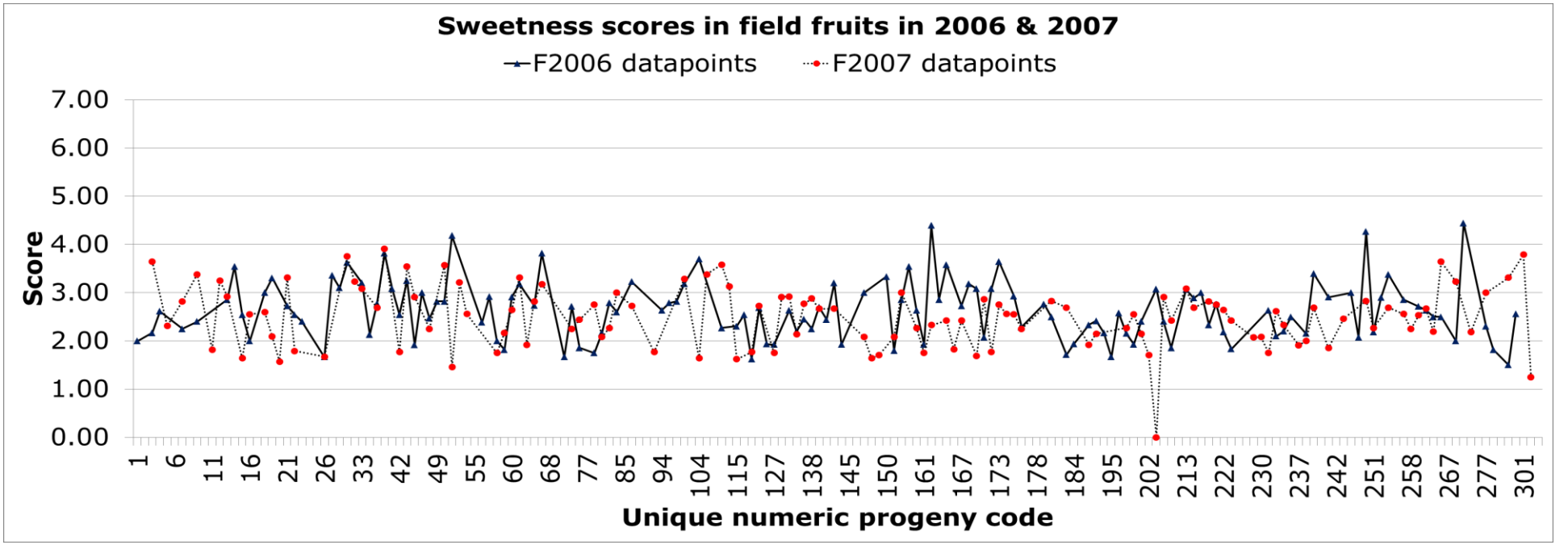


Figure 3.3 (a) Mean sweetness scores in field fruits of 2006 (—◆—) and 2007 (- ●-). X-axis denotes unique numeric code of each progeny and Y-axis denotes mean sweetness score. Mean scores (mean±SD) were: 2006 (131) = 2.59±0.54 and 2007 (118) = 2.51±0.59.

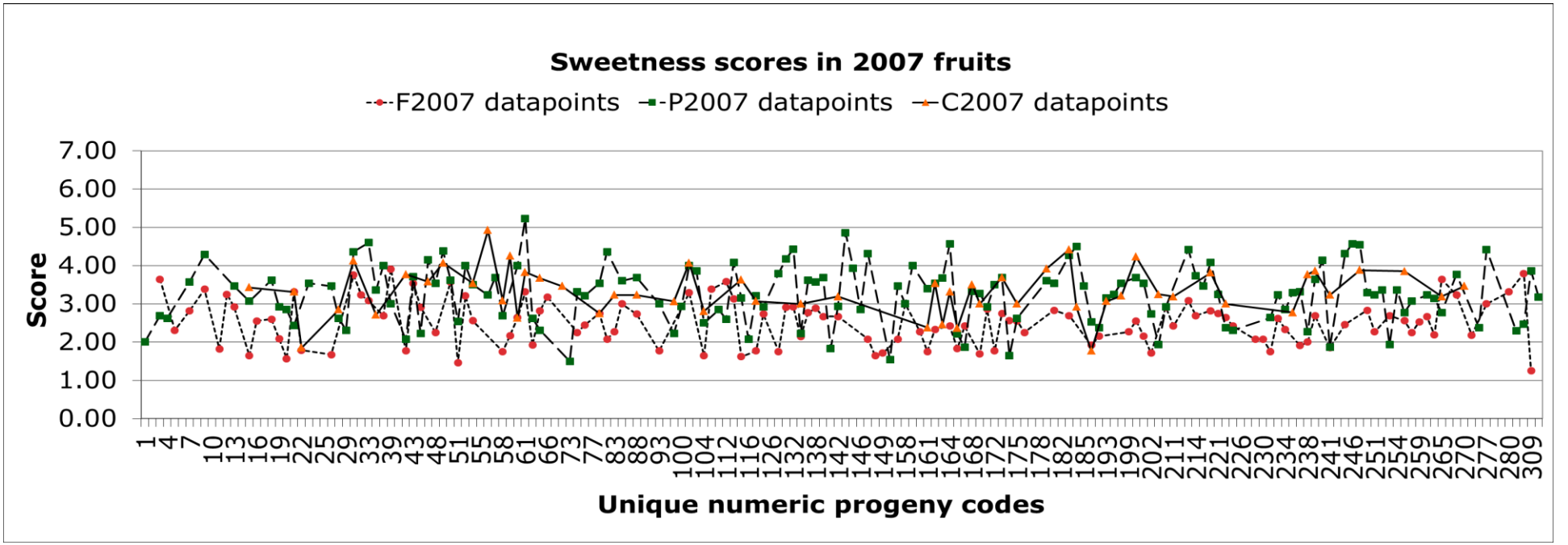
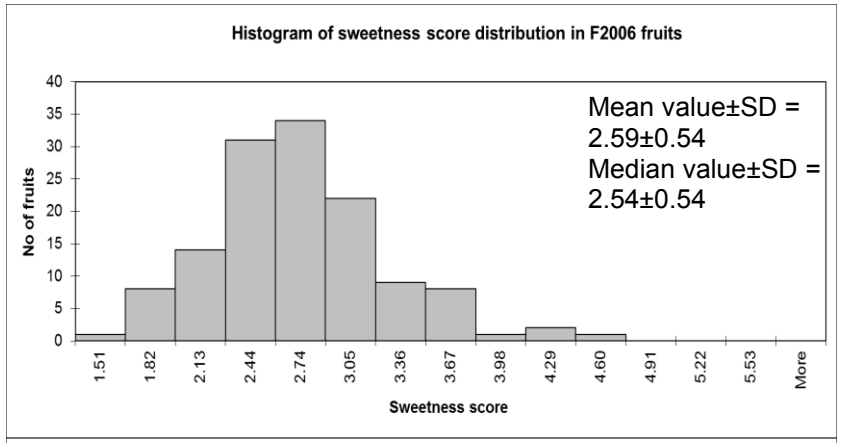
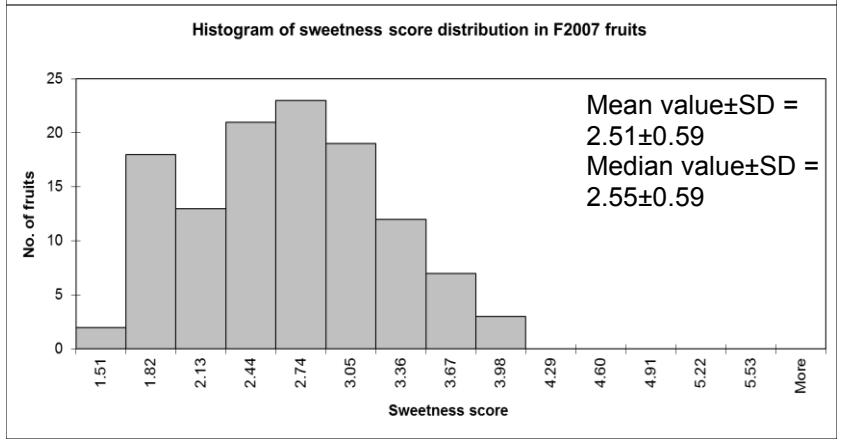


Figure 3.3 (b) Mean sweetness score in all 2007 fruits; Field (F 2007 = -●-), SCRI Polytunnel (P 2007 = - ■- -) and Commercial Polytunnel (C 2007 = —▲—). Mean values (mean±SED g/ml) were: F 2007 (118) = 2.51±0.59, P2007 (138) = 3.25±0.77 and C2007 (65) = 3.33±0.61.

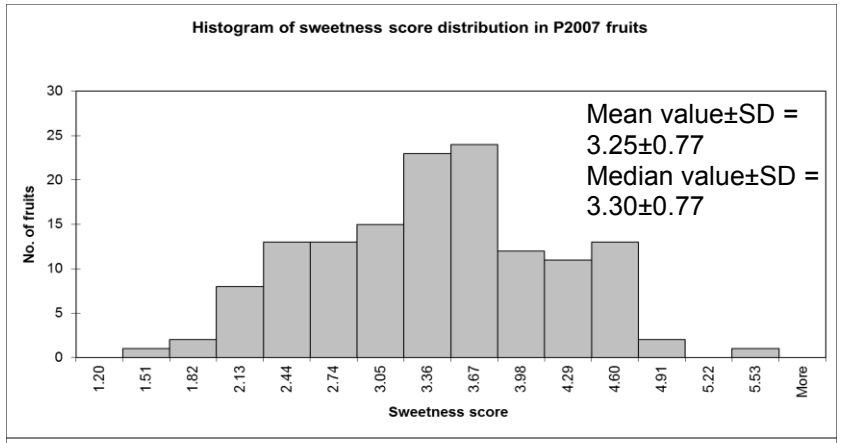


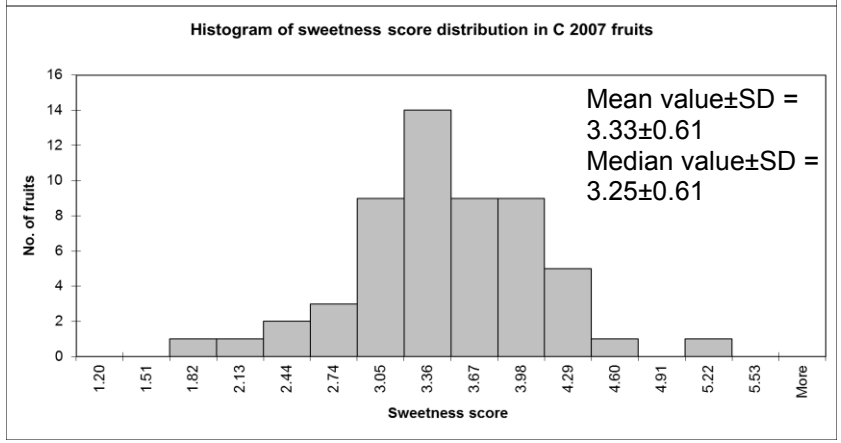
Sweetness scores in parent fruit (mean value±5%LSD)
Glen Moy = 3.92±1.46
Latham = 2.60±1.46



Sweetness scores in parent fruit (mean value±SD)
Glen Moy = 3.09±0.59

Figure 3.4 (a) Sweetness score distributions in field fruits of 2006 (131)(F 2006) and 2007 (117)(F 2007).





Sweetness scores in parent fruit (mean value±SD)
Glen Moy = 2.69±0.77
Latham = 3.07±0.77

Figure 3.4 (b) Sweetness score distributions in polytunnel fruits in 2007 from SCRI (138)(P 2007) and Commercial (65) (C 2007) sites. Parent fruits were unavailable in 2007 Commercial polytunnel due to disease.

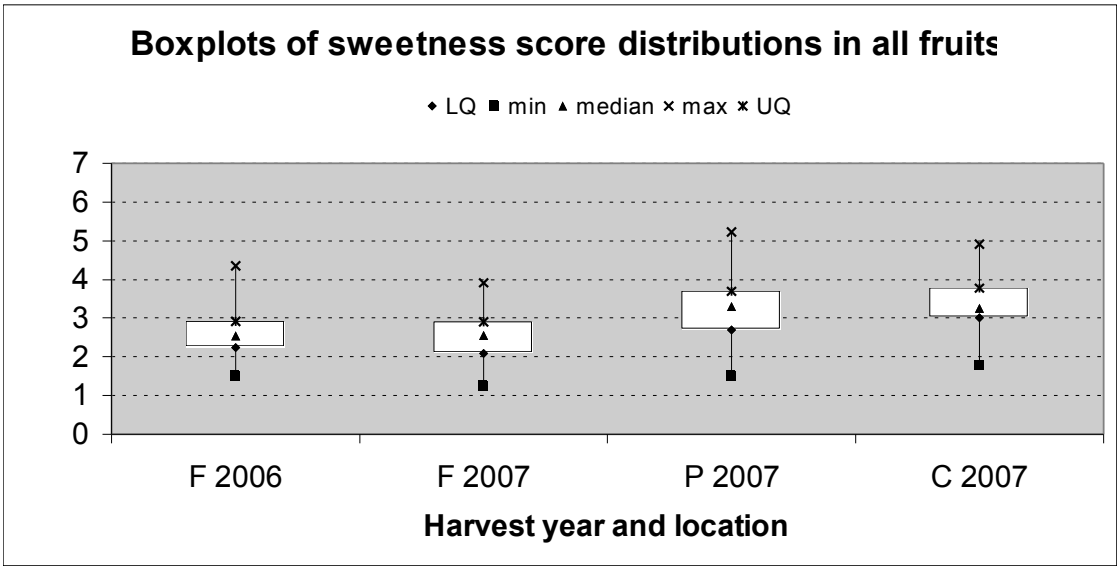


Figure 3.5 Sweetness score distributions in all fruits. Legend: F 2006 - open field fruit 2006, F 2007 - open field fruit 2007, P 2007 - SCRI polytunnel fruit 2007, C 2007 - Commercial polytunnel fruit 2007, LQ = 1st quartile, UQ = 3rd quartile.

Table 3.5 Pearson correlation coefficients and *p*-values for sweetness score in progeny fruit: field crops (F2006 & F2007) and 2 polytunnel in 2007 (P2007 = SCRI; C2007 = Commercial). Values in bold are significant.

Environment/ Year	F 2006	F 2007	P 2007
F 2007	p = n/s *r* = 0.177		
P 2007	p = n/s *r* = 0.100	***p* < 0.001** ***r* = 0.269**	
C 2007	p = n/s *r* = 0.058	***p* < 0.001** ***r* = 0.421**	***p* = 0.02** ***r* = 0.341**

Table 3.6 Significance in variation of sweetness scores across all fruits. Significance was calculated through independent two sample *t*-test with 95% confidence interval. Results highlighted in bold indicate significant difference in sweetness scores between 2 fruit crops. Legend: F 2006 = 2006 field, F 2007 = 2007 field, P 2007 = 2007 SCRI polytunnel and C 2007 = 2007 commercial polytunnel.

Environment/ Year	F 2006	F 2007	P 2007
F 2007	*p* = 0.26 T = 1.13		
P 2007	***p* < 0.001** T = -8.24	***p* < 0.001** T = -8.73	
C 2007	***p* < 0.001** T = -7.81	***p* < 0.001** **T = -8.32**	*p* = 0.47 T = -0.72

3.4.5 Sourness in progeny and parent fruit

Mean sourness score in field crop was 23.2% higher in 2007, with most fruit scoring in the higher score range (3.7 – 4.5) than in 2006 (2.7 – 3.7) (Figure 3.6 a). However, highest score (5.19) was in a 2006 crop progeny, which scored 3.7% lower in 2007. Score distributions in the 2 field crops were different (Figure 3.7 a), right-skewed with a wider score range in 2006 and left-skewed distributed in 2007 (Figure 3.8). Mean progeny score (3.18) in 2006 field crop score (3.18) was more similar to Latham score (3.92) than to Glen Moy (2.50). In 2007, Glen Moy scored 23.6% higher than 2006 score average but 2.5% lower in 2007. No 2007 field Latham fruit was available for scoring. From *t*-test, scores significantly differed in the two field crops (Table 3.8) with significant correlations between 2006 field crop with all 2007 crops indicated by Pearson's correlation analyses (Table 3.7).

In 2007, field crop had highest mean score, with 6.9% and 1.5% lower mean scores in SCRI and Commercial polytunnel crops respectively. Score distribution in field crop was narrower than in polytunnel crops (Figure 3.6 b), but all distributions were similar (Figure 3.7b) except for a slight right-skewed distribution in Commercial polytunnel crop (Figure 3.8). The highest score in Commercial polytunnel crop was 5.23, with 4.4% and 7.1% lower highest scores in field and SCRI polytunnel crops respectively. In SCRI polytunnel, parent varieties scored similarly to mean progeny score, but 6.0 – 6.3% higher, and for Glen Moy, polytunnel score was 1.3% higher than in field. Sourness in SCRI crops was not significantly correlated, but significant correlation was found between open field and Commercial polytunnel crop ($p < 0.01$, $r = 0.516$) (Table 3.7). Scores in polytunnel crops were significantly correlated. Although correlations were weak ($p = 0.02$, $r = 0.33$), it was confirmed by *t*-tests ($T = -2.32$, $p = 0.03$)(Table 3.8).

Keypoints:

- 2006 and 2007 field crops differed significantly in sourness, but 2007 crop had similar sourness score to SCRI polytunnel crop.
- Different growing condition between 2006 and 2007 had a significant impact.

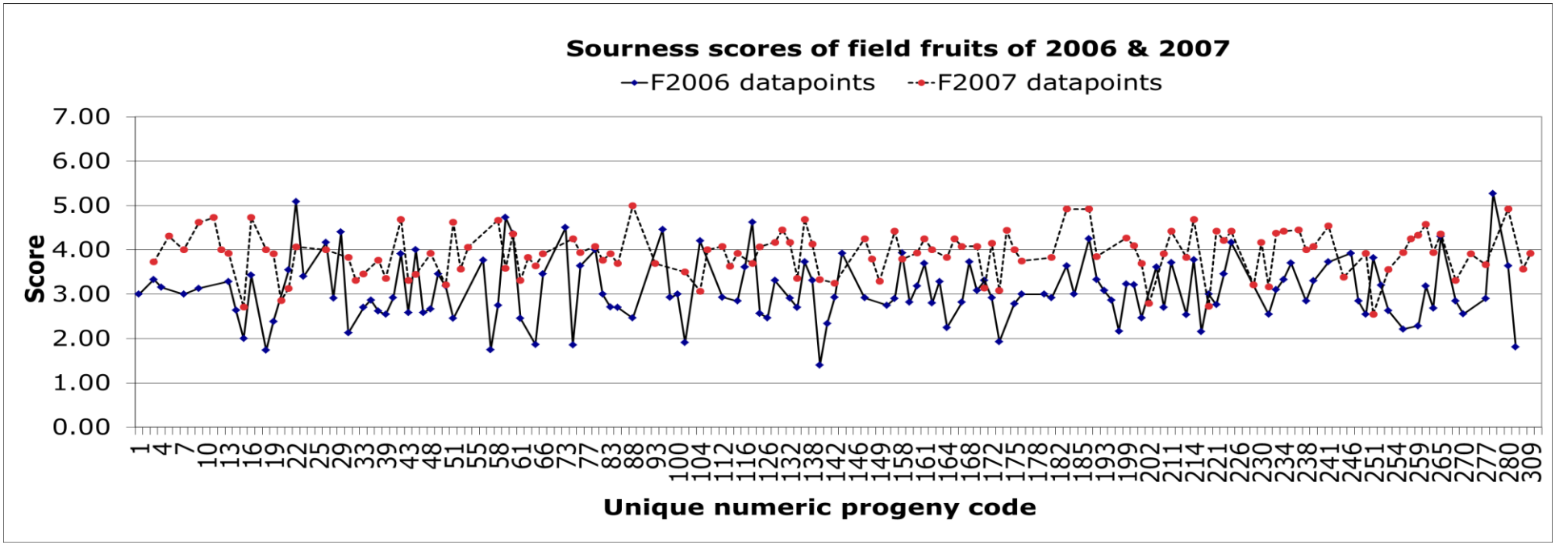


Figure 3.6 (a) Mean sourness scores in field fruits of 2006 (—◆—) and 2007 (- ● -). X-axis denotes unique numeric code of each progeny and Y-axis mean sourness score. Mean scores (mean±SD) were: 2006 (131) = 3.18±0.67 and 2007 (118) = 3.92±0.51.

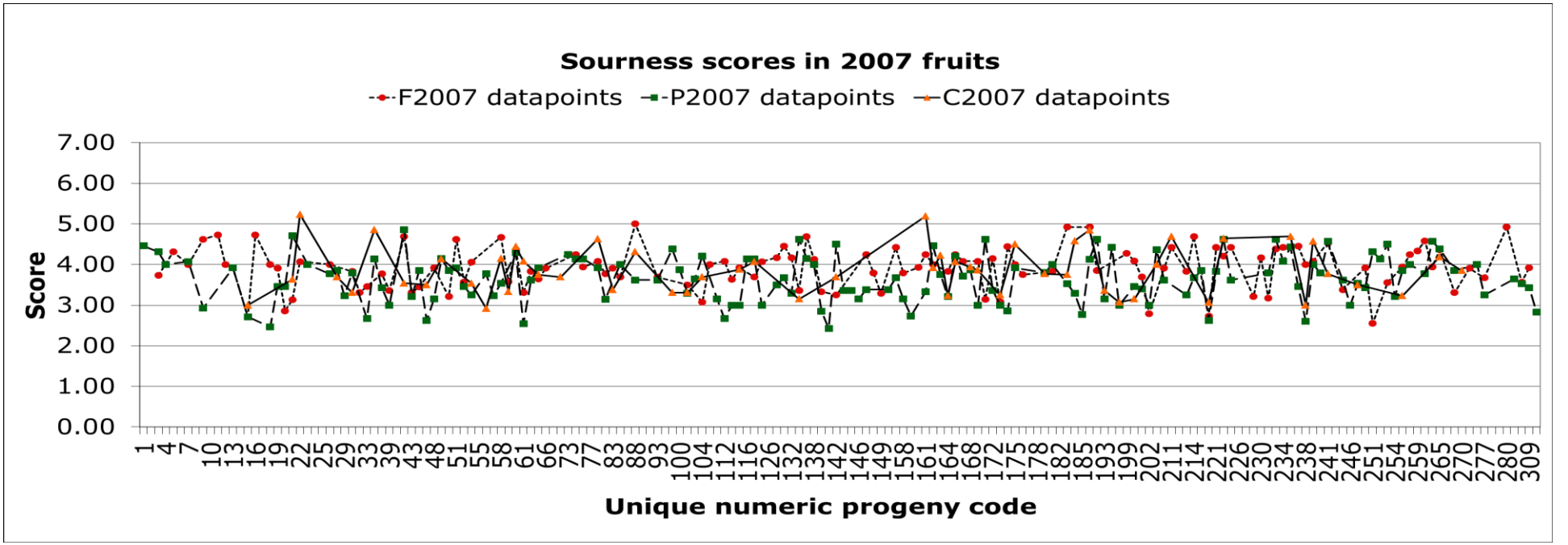
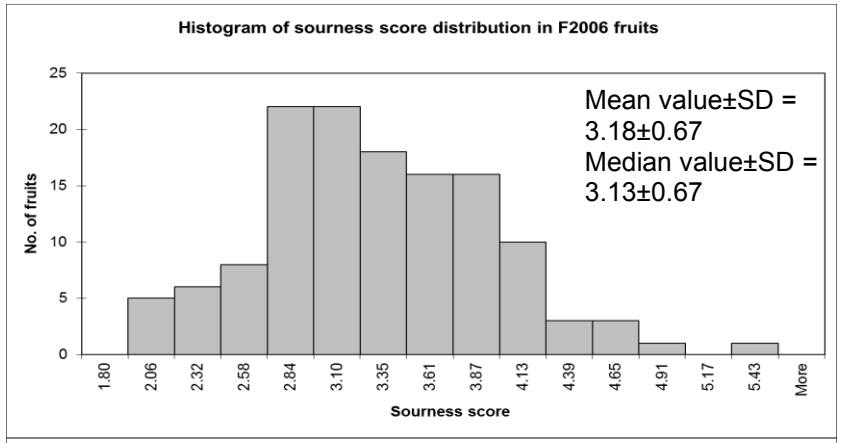
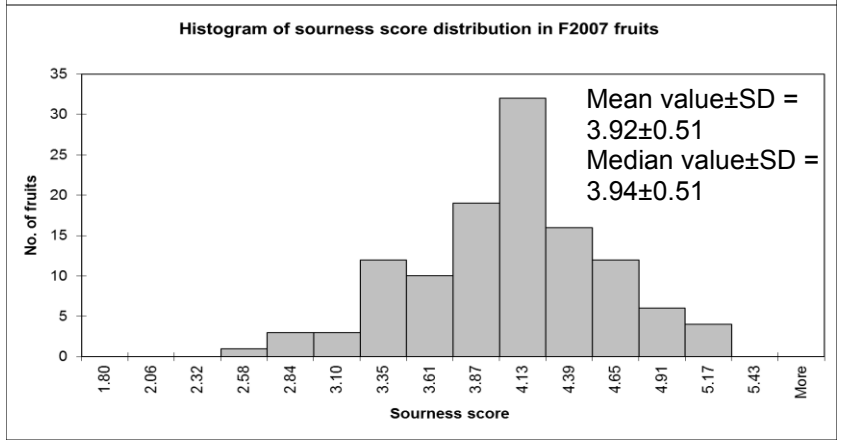


Figure 3.6 (b) Mean sourness score in all 2007 fruits; Field (F 2007 = -●-), SCRI Polytunnel (P 2007 = - ■- -) and Commercial Polytunnel (C 2007 = —▲—). X-axis denotes unique numeric code of each progeny and Y-axis mean sourness score. Mean values (mean±SD g/ml) were: F 2007 (118) = 3.92±0.51, P2007 (138) = 3.65±0.56 and C2007 (65) = 3.86±0.59.

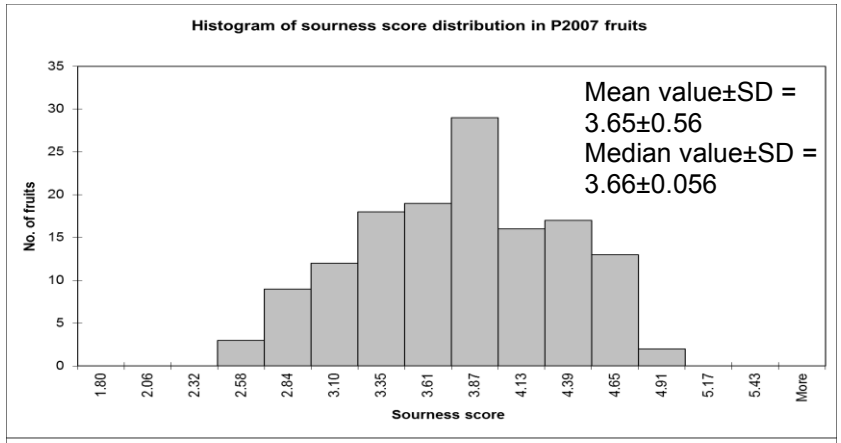


Sourness scores in parent fruit (mean value±5%LSD)
Glen Moy = 2.50±1.41
Latham = 3.90±1.41



Sourness scores in parent fruit (mean value±SD)
Glen Moy = 3.09±0.51

Figure 3.7 (a) Sourness score distributions in field fruits of 2006 (131)(F 2006) and 2007 (118)(F 2007).



Sourness scores in parent fruit (mean value±SD)
Glen Moy = 3.88±0.56
Latham = 3.87±0.56

Figure 3.7 (b) Sourness score distributions in polytunnel fruits in 2007 from SCRI (138)(P 2007) and Commercial (65)(C 2007)

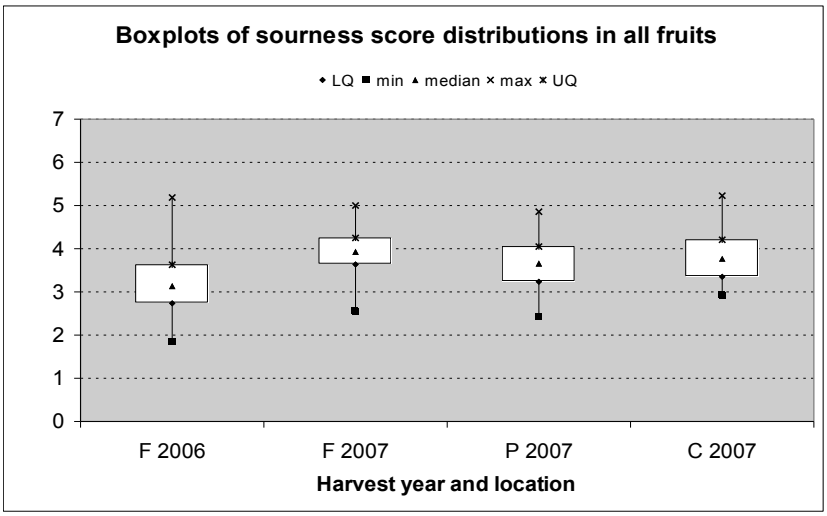


Figure 3.8 Sourness score distributions in all fruits. Legend: F 2006 - field 2006, F 2007 - field 2007, P 2007 - SCRI polytunnel 2007, C 2007 - Commercial polytunnel 2007, LQ = 1st quartile, UQ = 3rd quartile.

Table 3.7 Pearson correlation coefficients and *p*-values for sourness score in progeny fruit: field crops (F2006 & F2007) and 2 polytunnel in 2007 (P2007=SCRI; C2007=Commercial). Values in bold are significant.

Environment/ Season	F 2006	F 2007	P 2007
F 2007	***p* < 0.001** ***r* = 0.345**		
P 2007	***p* < 0.001** ***r* = 0.340**	*p* = n/s r = 0.156	
C 2007	***p* < 0.001** ***r* = 0.446**	***p* < 0.001** ***r* = 0.516**	***p* = 0.02** ***r* = 0.331**

Table 3.8 Significance in variation of sourness scores across all fruits. Significance was calculated through independent two sample *t*-test with 95% confidence interval. Results highlighted in bold indicate significant difference in sourness scores between 2 fruit crops. Legend: F 2006 = 2006 field, F 2007 = 2007 field, P 2007 = 2007 SCRI polytunnel and C 2007 = 2007 Commercial polytunnel.

Environment/ Year	**F 2006**	**F 2007**	**P 2007**
F 2007	*p* < 0.001 T = -10.32		
P 2007	*p* < 0.001 T = -6.53	*p* < 0.001 T = 4.01	
C 2007	*p* < 0.001 T = -7.06	*p* = 0.50 T = 0.67	*p* = 0.03 T = -2.32

3.4.6 Flavour intensity in progeny and parent fruit

Difference in mean scores of field crops in the 2 harvest years was 4.4%, significant from *t*-tests (Table 3.9) but score distributions were similar in both years, within range of 2.2 – 5.0 (Figure 3.9 a). Scores in 2006 field crop were normally distributed across mean and median values (Figure 3.10 a). In 2007, field crop scores were also normally distributed but with higher minimum score. In 2006, mean progeny score was 18.1% and 32.3% less than Latham and Glen Moy scores respectively and in 2007, mean progeny score was 4.2% higher than Glen Moy. No significant correlation was between 2006 and 2007 field crops but there were significant correlations between 2007 field and polytunnel crops but not between field and Commercial polytunnel crops (Table 3.9). Despite significant correlations, mean scores were significantly different between all crops of the 2 harvest years (Table 3.10) but from boxplots, 2007 score distributions in field crops appear similar (Figure 3.11).

Mean score in 2007 Commercial polytunnel crop was 12.6% and 5.2% higher than 2007 field and SCRI polytunnel crops respectively. However, score distribution range for all was similar (1.9 – 5.5), right-skewed and narrower in polytunnel crops (Figure 3.9 b) in score range of 2.4 – 5.0, and in field crop, normally distributed (Figure 3.10 a, b). In SCRI polytunnel crop, mean score was 7.1% and 16.2% higher than Latham and Glen Moy scores respectively. This was also found for mean sweetness scores, but with higher percent difference between mean progeny and Glen Moy scores (20.8%). Score distributions for polytunnel crops appear different to score distribution for field crop, but field crop mean score was significantly correlated to SCRI polytunnel but not Commercial crop (Table 3.9). However, all mean scores were significantly different in all crops of both harvest years, even if mean scores between 2007 polytunnel crops significantly correlated (Table 3.10). Therefore flavour intensity profile is possibly affected by many factors and determined strongly by genetic x environmental interactions.

Key points:

- In field crops flavour intensity was significantly higher in 2007 than 2006.
- In 2007, polytunnel crops scored higher in flavour intensity than field.

- Both polytunnel crops in 2007 scored significantly different for flavour intensity

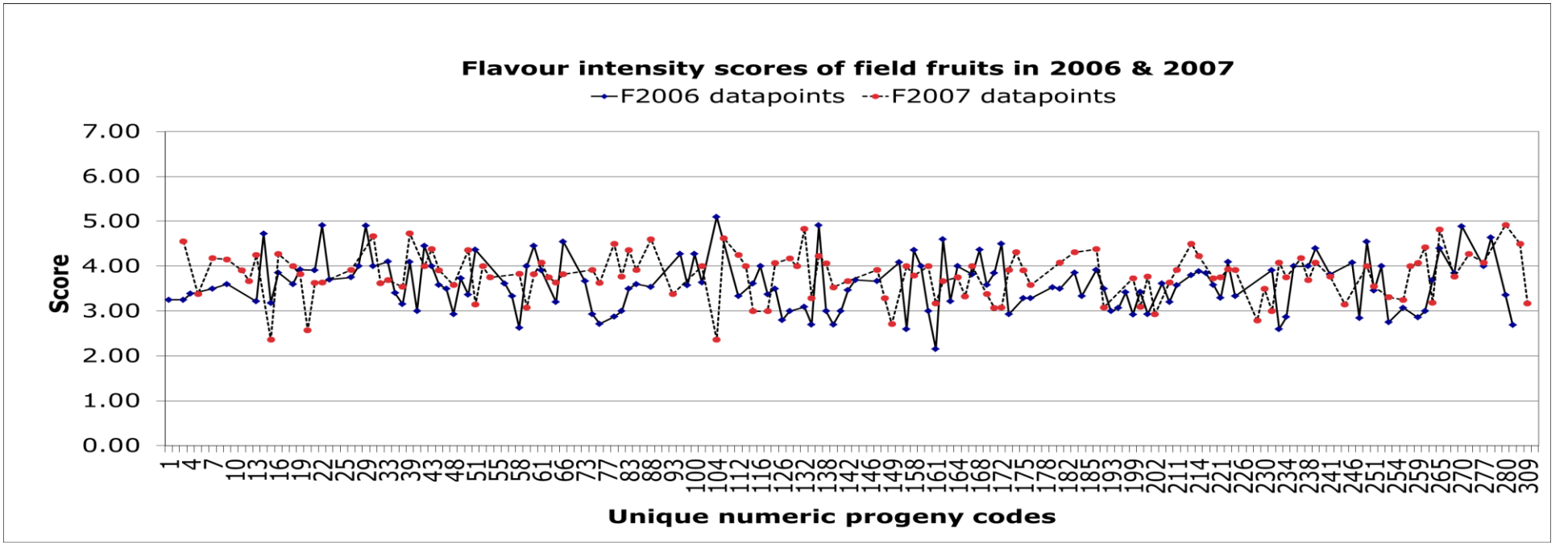


Figure 3.9 (a) Mean flavour intensity scores in field fruits of 2006 (—◆—) and 2007 (- ● -). X-axis denotes unique numeric code of each progeny and Y-axis mean score. Mean scores (mean±SD) were: 2006 (131) = 3.64±0.51 and 2007 (118) = 3.80±0.52.

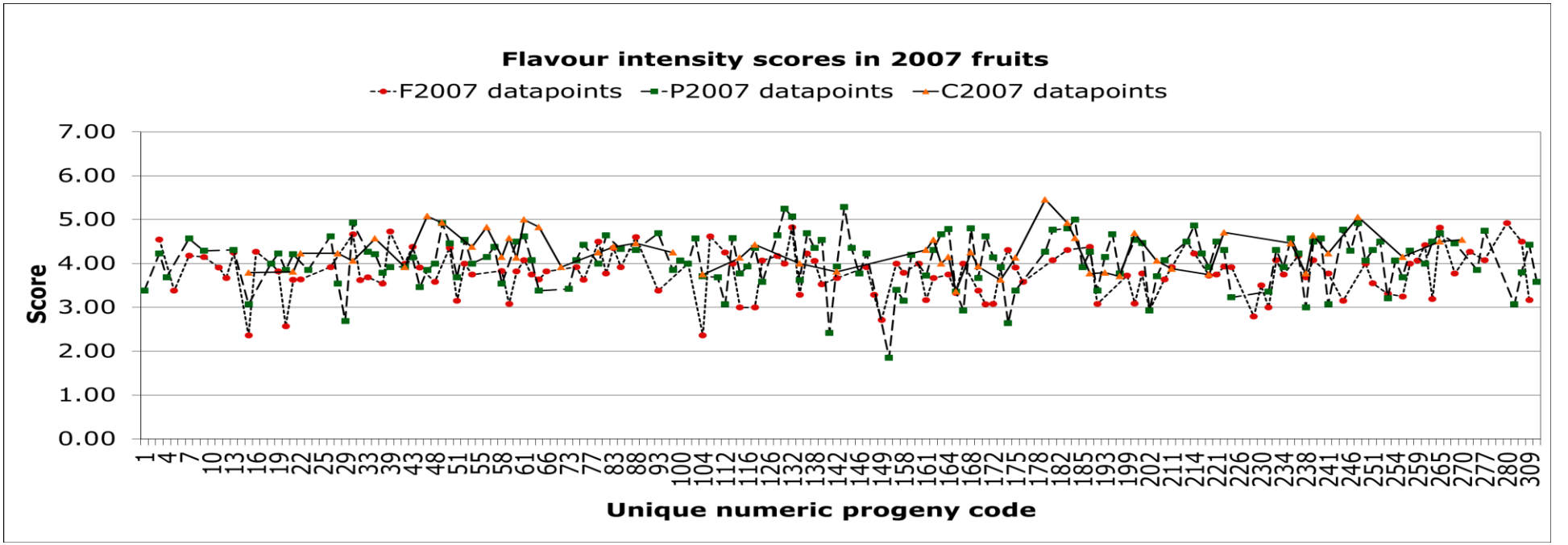
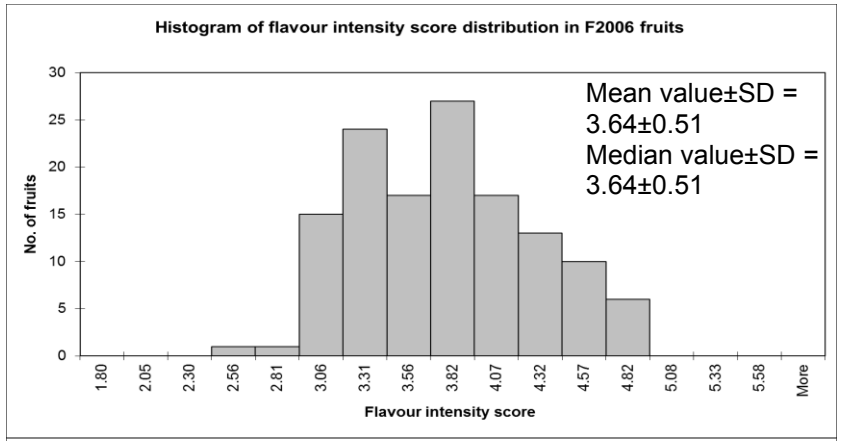
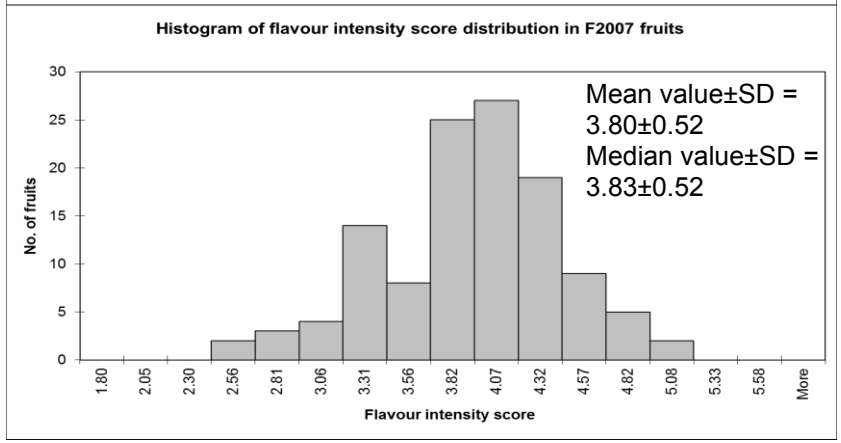


Figure 3.9 (b) Mean flavour intensity score in 2007 fruits; Field (F 2007 = -●-), SCRI Polytunnel (P 2007 = -■- -) and Commercial Polytunnel (C 2007 = —▲—). X-axis denotes unique numeric code of each progeny and Y-axis mean score. Mean values (mean±SD g/ml) were: F 2007 (118) = 3.80±0.52, P2007 (138) = 4.07±0.59 and C2007 (65) = 4.28±0.43.

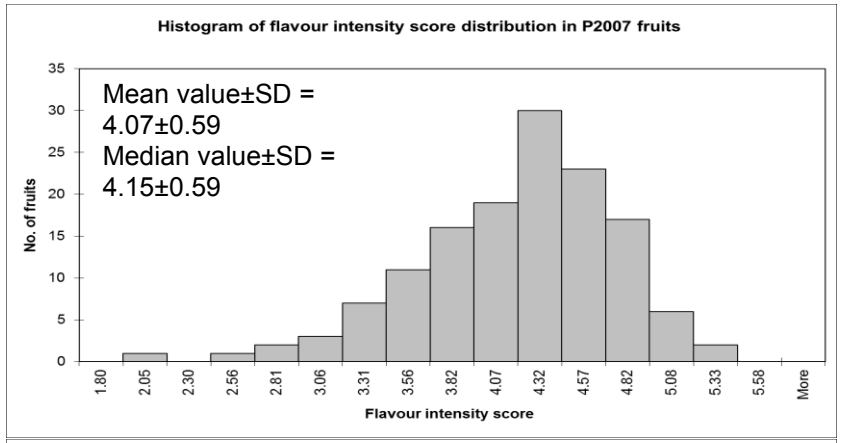


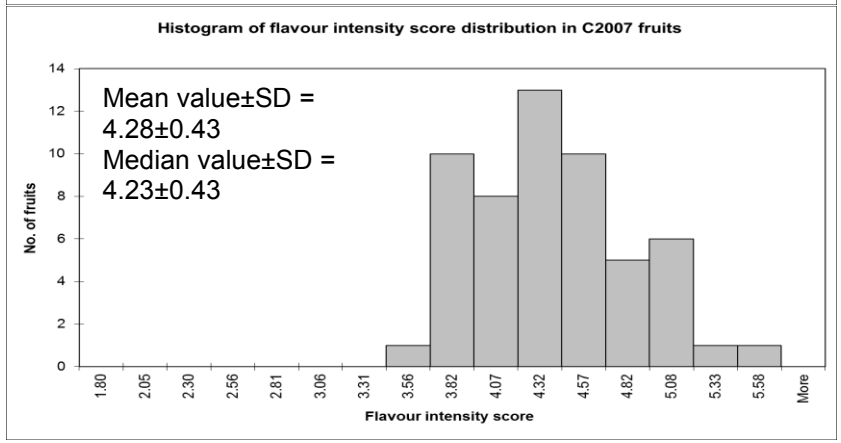
Flavour intensity scores in parent fruit (mean value±5%LSD)
Glen Moy = 4.83±1.17
Latham = 4.30±1.17



Flavour intensity scores in parent fruit (mean value±SD)
Glen Moy = 3.64±0.52

Figure 3.10 (a) Flavour intensity score distributions in field fruits of 2006 (131) (F 2006) and 2007 (118) (F 2007).





Flavour intensity scores in parent fruit (mean value±SD)
Glen Moy = 3.50±0.59
Latham = 3.80±0.59

Figure 3.10 (b)Flavour intensity score distributions in fruits in 2007 from SCRI (138)(P 2007) and Commercial (65) (C 2007) polytunnels.

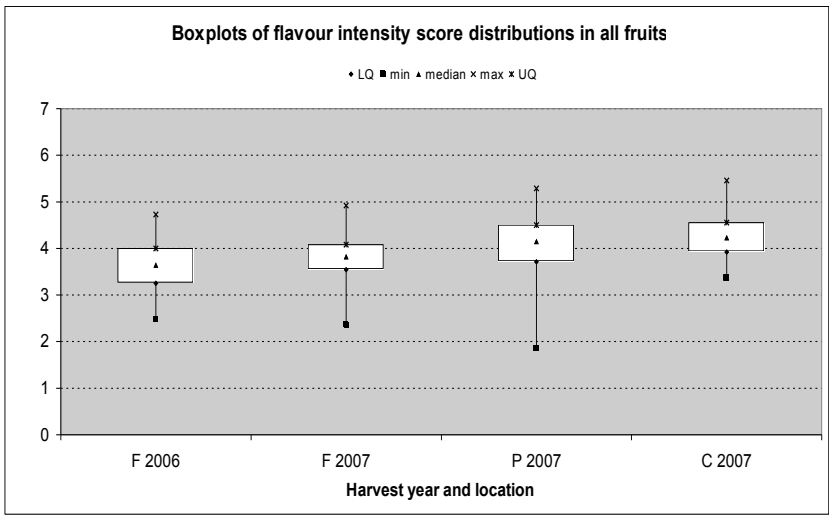


Figure 3.11 Flavour intensity score distributions in all fruits. Legend: F 2006 - field 2006, F 2007 - field 2007, P 2007 - SCRI polytunnel 2007, C 2007 - Commercial polytunnel 2007, LQ = 1st quartile, UQ = 3rd quartile.

Table 3.9 Pearson correlation coefficients and *p*-values for flavour intensity score in progeny fruit: field crops (F2006 & F2007) and 2 polytunnel in 2007 (P2007=SCRI; C2007=Commercial). Values in bold are significant.

Environment/ Season	F 2006	F 2007	P 2007
F 2007	*p* = n/s r = 0.029		
P 2007	*p* = n/s r = 0.079	***p* < 0.001** **r = 0.303**	
C 2007	*p* = n/s r = -0.010	*p* = n/s r = 0.254	***p* < 0.001** **r = 0.457**

Table 3.10 Significance in variation of flavour intensity scores across all fruits. Significance was calculated through independent two sample *t*-test with 95% confidence interval. Results highlighted in bold indicate significant difference in sweetness scores between 2 crops. Legend: F 2006 = 2006 field, F 2007 = 2007 field, P 2007 = 2007 SCRI polytunnel and C 2007 = 2007 commercial polytunnel.

Environment/ Year	**F 2006**	**F 2007**	**P 2007**
F 2007	***p* = 0.02** **T = -2.41**		
P 2007	***p* < 0.001** **T = -6.35**	***p* < 0.001** **T = -3.89**	
C 2007	***p* < 0.001** **T = -8.70**	***p* < 0.001** **T = -6.40**	***p* = 0.01** **T = -2.76**

3.4.7 Sweet: Sour ratios in progeny and parent fruit

Balance of sweetness and sourness is often used as flavour quality index in the soft-fruit industry and is often a central aim in breeding.

In field crops, 2006 mean ratio (range 0.4 – 2.0) was 24.6% higher than in 2007 (0.3 – 1.2) (Figure 3.12 a) with a normal distribution in 2006 and right-skewed in 2007 (Figure 3.13 a, Figure 3.14). In 2006 field crop, mean progeny ratio was 94% lower than Glen Moy, but 17.3% higher than Latham. Under SCRI polytunnel cultivation in 2007, mean Glen Moy ratio was 94% lower compared to in 2006 field crop and mean progeny ratio was 19.8% lower than this parent. Field crop mean ratio values were significantly correlated in the 2 harvest years ($p < 0.01$, r = 0.308) and *t*-test results implied significant seasonal effects on mean scores (T = 5.72, $p < 0.01$) (Table 3.11, 3.12).

Mean ratios in 2007 were highest in SCRI polytunnel crop (range 0.3 – 1.5), with ratios in SCRI field (range 0.3 – 1.2) and Commercial polytunnel (range 0.3 – 1.7) crops lower by 30.1% and 3.2%, respectively (Figure 3.12 b). Distributions were right-skewed in field and normal in polytunnel crops (Figure 3.13 b, Figure 3.14). In 2007 SCRI polytunnel crop, mean ratio was 34.8% and 17.7 % higher than Glen Moy and Latham. All 2007 crop ratios were significantly correlated, strongest between field and Commercial polytunnel crops ($p = 0.01$, r = 0.432), weakest between polytunnel crops ($p = 0.03$, r = 0.309) (Table 3.11). Despite significant correlations, mean ratio value of 2007 field crop was significantly different compared to mean ratios of 2007 crops, from *t*-test analyses ($p < 0.01$, T = -8.71, -6.23) (Table 3.12). Despite significant correlation between mean ratio of 2006 field and 2007 SCRI polytunnel crops ($p < 0.01$, r = 0.326) there was no significant difference in mean values (Table 3.12). Therefore, both harvest year and cultivation method had significant influences on sweet : sour ratio values, and polytunnel cultivation increased ratio values generally.

Key points:

- Different harvest years yielded significantly different sweet : sour ratio values in field crops.

- Polytunnel cultivation generally increased sweet : sour ratio values in progeny.

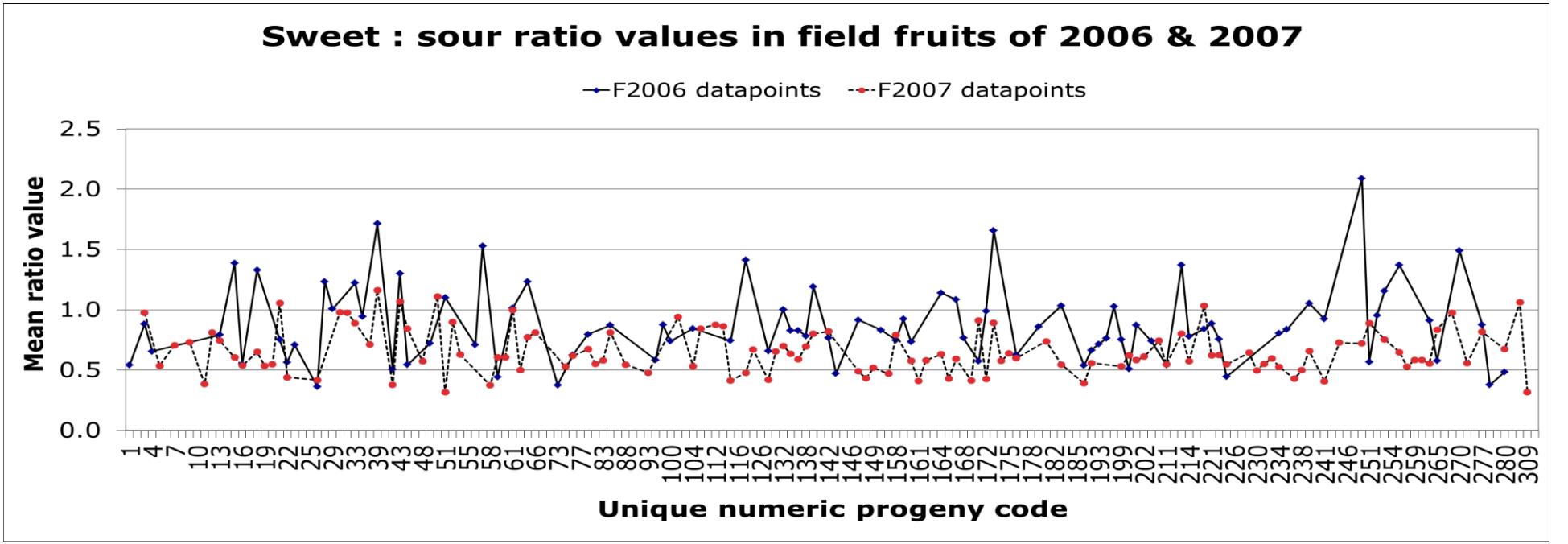


Figure 3.12 (a) Mean sweet : sour ratios in field fruits of 2006 (–◆—) and 2007 (-●- -). X-axis denotes unique numeric code of each progeny and Y-axis mean ratio. Mean ratios (mean±SD) were: 2006 (131) = 0.81±0.00 and 2007 (118) = 0.64±0.02

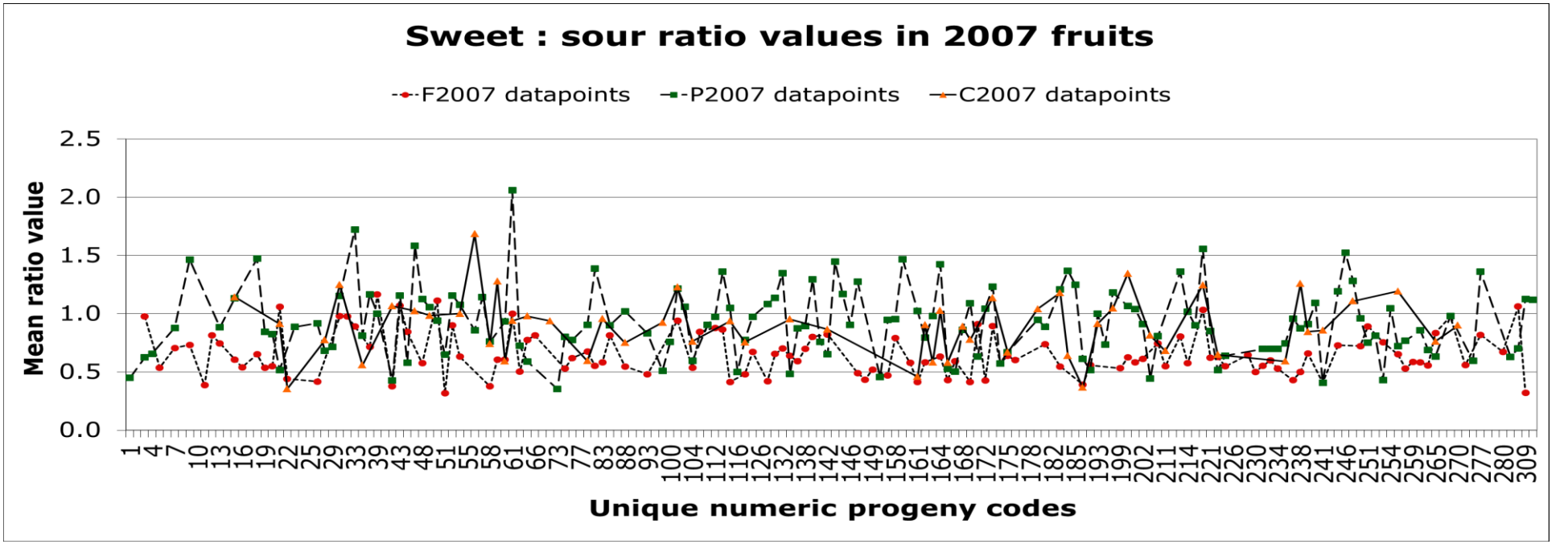
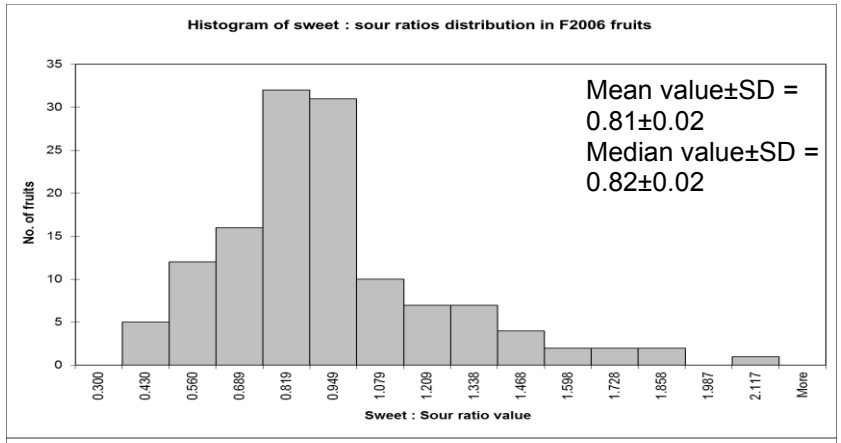
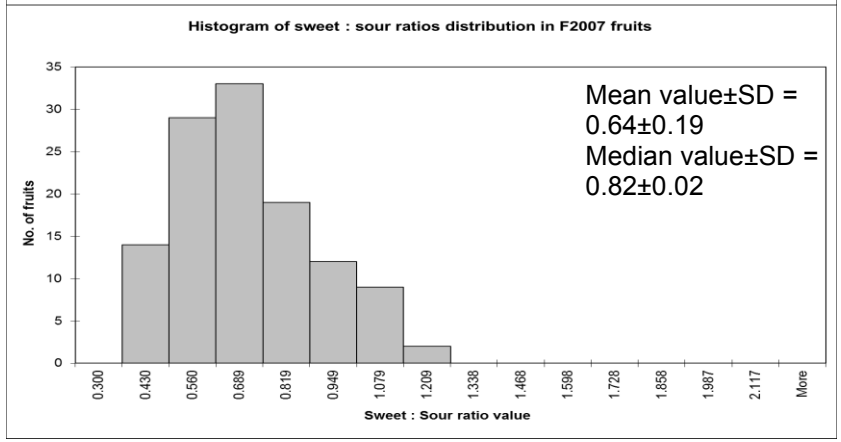


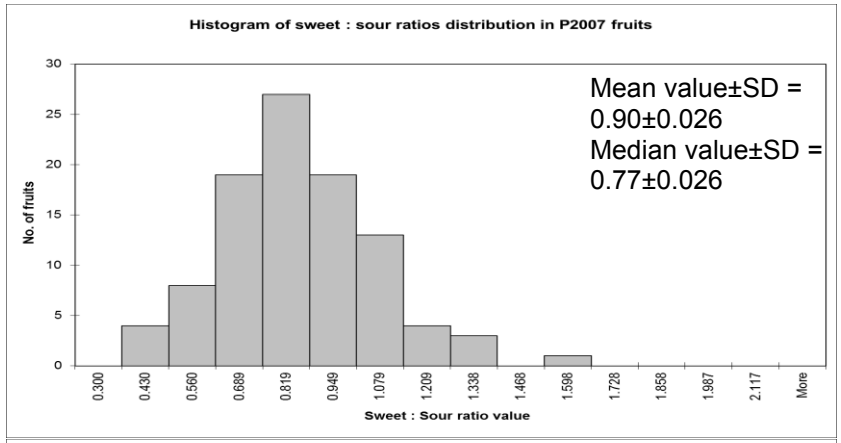
Figure 3.12 (b) Mean sweet : sour ratios in all 2007 fruits; Field (F 2007 = -●-), SCRI Polytunnel (P 2007 = -■- -) and Commercial Polytunnel (C 2007 = —▲—). X-axis denotes unique numeric code of each progeny and Y-axis mean ratio. Mean ratios (mean±SD) were: F 2007 (118) = 0.64±0.02, P2007 (138) = 0.90±0.03 and C2007 (65) = 0.86±0.04.

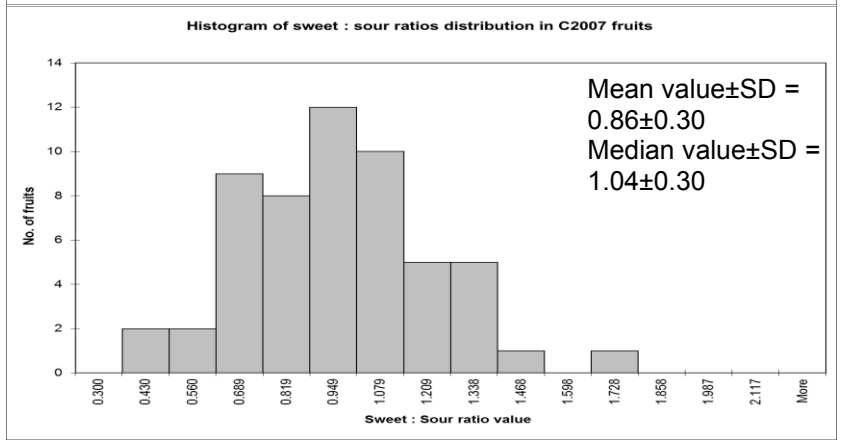




Mean ratio values in parent fruit (mean value±5%LSD)
Glen Moy = 1.57±1.47
Latham = 0.67±1.47

Figure 3.13 (a) Sweet : sour ratios distributions in field fruits of 2006 (131)(F 2006) and 2007 (118)(F 2007).





Mean ratio values in parent fruit (mean value±SD)
Glen Moy = 0.69±0.26
Latham = 0.79±0.26

Figure 3.13 (b) Sweet : sour ratios distributions in polytunnel fruits in 2007 from SCRI (138)(P 2007) and Commercial (65) (C 2007) sites.

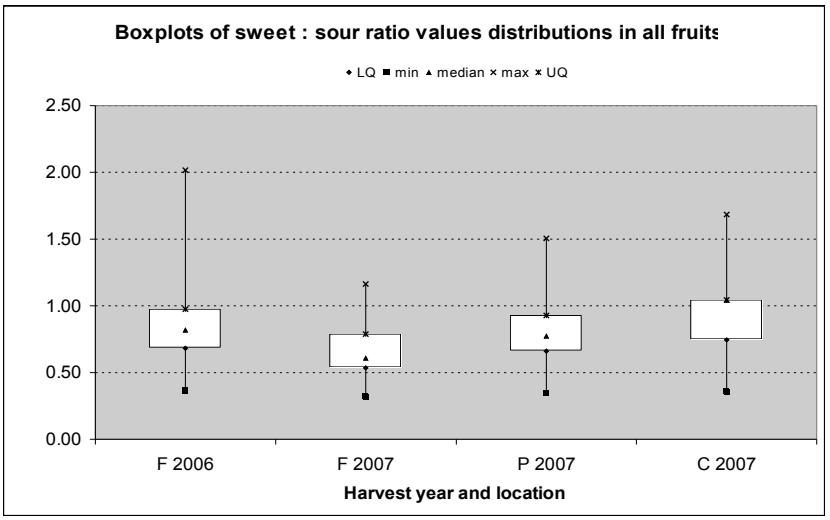


Figure 3.14 Sweet : sour ratios distributions in all fruits. Legend: F 2006 - field 2006, F 2007 - field 2007, P 2007 - SCRI polytunnel 2007, C 2007 - Commercial polytunnel fruit 2007, LQ = 1st quartile, UQ = 3rd quartile.

Table 3.11 Pearson correlation of sweet: sour ratios of progeny fruit in 2 crops (2006 & 2007) and 3 sites (F= Field; P= SCRI & C=Commercial polytunnel). Values in bold significant. *p* = p-value, r = Pearson coefficient value.

Environment/ Season	F 2006	F 2007	P 2007
F 2007	***p* = 0.01** **r = 0.308**		
P 2007	***p* < 0.001** **r = 0.326**	***P* = 0.01** **r = 0.262**	
C 2007	*p* = n/s r = 0.144	***p* = 0.01** **r = 0.432**	***p* = 0.03** **r = 0.309**

Table 3.12 Significance in variation of sweet : sour ratios across all fruits. Significance was calculated through independent two sample *t*-test with 95% confidence interval. Results highlighted in bold indicate significant difference in sweetness scores between 2 fruit crops. Legend: F 2006 = 2006 field, F 2007 = 2007 field, P 2007 = 2007 SCRI polytunnel and C 2007 = 2007 commercial polytunnel.

Environment/ Year	**F 2006**	**F 2007**	**P 2007**
F 2007	***p* < 0.001** **T = 5.72**		
P 2007	*p* = 0.23 T = -1.21	***p* < 0.001** **T = -8.71**	
C 2007	*p* = 0.63 T = -0.48	***p* < 0.001** **T = -6.23**	*p* = 0.52 T = 0.65

3.4.8 Correlations between all scored flavour attributes

The flavour attributes scored by assessors in this study were sweetness, sourness and flavour Intensity. These traits were not independent of each other, shown by results of Pearson's correlation analyses (Table 3.13 and 3.14). From correlation coefficient values, *r*, sweetness was more strongly correlated to flavour intensity than to sourness (Table 3.13). This correlation was strongest in 2007 crop. In field crops, sweetness and sourness both had positive significant contributions to flavour intensity (Table 3.13). But in polytunnel crops, only sweetness significantly affected flavour intensity, possibly due to higher total sugars : total acids ratios compared to field crops (**Chapter 2**). Hence, flavour traits are correlated in all crops, with some correlations becoming non-significant under polytunnel cultivation. Effect of genetic x environment interactions is possibly a strong influencing factor on flavour development and how each flavour trait influences the other.

Key points:

- Sweetness and sourness significantly correlated with flavour intensity in field crops of both harvest years.
- In polytunnel crops only sweetness significantly affected flavour intensity scores.
- Genetic x environment interactions possibly impacted on correlations between flavour traits.

3.4.9 Heritability of flavour traits

Heritability estimates were calculated as percentage trait variance. Estimates were 17.8% – 31.0% higher in 2006 field crops in all flavour traits compared to in 2007 crops. Heritability estimate was highest for 2006 sweetness, with similar estimate values for sourness and flavour intensity. In 2007 crops, the estimate value range was 19.0% - 36.0% for all flavour traits and highest for sourness in SCRI field crop. Generally, estimate values were highest in SCRI polytunnel and least in Commercial polytunnel fruit, notably only 19%, for flavour intensity. Stronger effect by cultivation method on this flavour trait score is possible because flavour intensity was scored highest in Commercial polytunnel crop. Variations in heritability

estimates of all 2007 crops (open field and polytunnel) could arise in part from genotype x environment interactions.

Table 3.13 Pearson's correlation analyses for relationship between sweetness and sourness and score for flavour intensity in field crops in 2006 (F 2006) and 2007 (F 2007). Significant correlations are marked in bold. *p* = significance value at 95% confidence level, *r* = Pearson's correlation coefficient.

	F 2006		**F 2007**	
Variables	Sweetness	Sourness	Sweetness	Sourness
Flavour intensity	***p* < 0.001** ***r* = 0.470**	***p* < 0.001** ***r* = 0.354**	***p* < 0.001** ***r* = 0.627**	***p* = 0.001** ***r* = 0.450**

Table 3.14 Results of Pearson's correlation analyses for all scored flavour attributes in polytunnel crops of SCRI (P 2007) and Commercial (C 2007) sites in 2007. Significant correlations are marked in bold. *p* = significance value at 95% confidence level, *r* = Pearson's correlation coefficient.

	P 2007		**C 2007**	
Variables	Sweetness	Sourness	Sweetness	Sourness
Flavour intensity	***p* < 0.001** ***r* = 0.742**	*p* = n/s ***r* = 0.148**	***p* = 0.001** ***r* = 0.434**	*p* = n/s ***r* = 0.122**

Percent estimates for all flavour trait ranged from 50% - 54% and in 2007 crops, between 19% - 31%, which means variance caused by genotypic factors account for approximately half and even less under polytunnel cultivation. Therefore, from these estimate values and biochemical and sensory profiles of polytunnel crops, control over environmental affects seems effective in developing fruits with maximum metabolite content and flavour trait intensity, which are desirable.

Table 3.15 Variance components and heritability estimates for flavour traits in 2006 and 2007. Heritability was estimated using different equations (explained in 3.3.5) in 2006 and 2007 because of different replications.

Flavour trait	2006 h^2 (%)	2007 σ^2_G			σ^2_{GS}			h^2 (%)		
		F 2007	*P 2007*	*C 2007*	*F 2007*	*P 2007*	*C 2007*	*F 2007*	*P 2007*	*C 2007*
Sweetness	**53.7**	0.3507	0.5896	0.3698		1.3102		**21.1**	**31.0**	**22.0**
Sourness	**50.0**	0.2619	0.3176	0.3499		0.9294		**35.9**	**25.5**	**27.3**
Flavour intensity	**50.0**	0.2659	0.3506	0.1893		0.8058		**24.8**	**30.3**	**19.0**

Key points:

- Flavour trait development has an estimated maximum of 51% heritability contribution
- Genotype x environment interactions is possibly of high impact on flavour trait expression than genotype (i.e. heritability factors) alone.

3.4.10 QTL identification

QTL mapping was performed as described in Section 3.3.5. Sensory QTLs were grouped in 4 out of 7 linkage groups on the genetic linkage map for Latham x Glen Moy (Graham et al., 2009). Discussed are summary results from interval mapping: potential 2007 QTLs with LOD scores > 3 and < 10 permutation rounds and QTLs with LOD > 2.5 for 2006 crop as none had LOD > 3 (Table 3.16).

Linkage group 3 (LG3) had highest number of QTLs with majority mapped from data of 2007 field crop (Figure 3.16). There were four (4) sourness QTLs with two (2) QTLs in LG2 (Figure 3.15). Overlaps of sweetness and flavour intensity QTLs were identified on LG3 and LG2, all from SCRI crops (Figure 3.15 - 3.16). This result as well as significant correlations, from Pearson's analyses, suggests close links between sweetness and flavour intensity. However, as there was a large QTL interval for flavour intensity QTL on LG3, this hypothesis must be viewed with caution. Further fine mapping would be required before substantial genetic correlations of flavour traits are made. Furthermore, from low estimated heritability

in 2007 crops, environmental factors were possibly more influential than genotype and had greater role in flavour trait expression.

Only 2 QTLs were mapped from 2006 field crop data, for sourness and for flavour intensity on LG4 and LG5 respectively (Figure 3.17 - 3.18). Flavour intensity QTLs on LG5 from 2006 field and 2007 SCRI polytunnel crops overlapped, and also to colour development QTLs and molecular markers RiD4R1 and RiD4R2. These overlaps suggests that flavour intensity may share common genetic regulation that also control berry pigmentation.

Other important overlaps were of QTLs and important molecular markers; raspberry TIP (**T**onoplast **I**ntrinsic **P**rotein), important for metabolite and water transport, with sourness QTL for 2007 field crop on LG2 (99.1 cM); all 2007 field crop flavour QTLs and sourness QTL of 2007 SCRI polytunnel crop and to markers for a MYB-transcription factor and 4-coumarate-CoA ligase, which both are implicated in anthocyanin synthesis. Possible links between flavour and physicochemical traits were suggested also by overlap of 2006 field crop sourness QTL with flavonoid 3'-hydroxylase (*F3'H*) gene, important in anthocyanin synthesis in apples (Han et al., 2010). Therefore, these results suggest flavour development might share common genetic regulation pathways as other plant development traits, for e.g. metabolite transportation and pigment development. However, as previously mentioned, QTL intervals are large and at best these genetic associations are simple linkages, which further mapping with higher resolution can verify. However, as a preliminary finding, it is encouraging for breeders in effort produce varieties of premium flavour that flavour trait and metabolite QTLs in this study overlap with a few flavour QTLs, implying control over conditions affecting metabolite contents may also be affective on flavour development.

Key points:

- Preliminary simple genetic associations of flavour traits and candidate genes / markers are:
  - (i) sourness QTL and *F3'H* gene for anthocyanin biosynthesis,
  - (ii) flavour intensity QTL and markers for RiD4R1 and RiD4R2 (transcription factors),

(iii) sourness QTL with marker for raspberry TIP (for metabolite and water transport), MYB-transcription factor and 4-coumarate ligase (anthocyanin biosynthesis).

- At present proposed QTLs for flavour traits are simple linkages, which could be better defined by further QTL mapping of back-cross population.

Table 3.16 Summary of flavour QTLs: year, site, linkage group (LG), location on linkage group (LG), LOD score and percent (%) variance explained. Legend: F = SCRI field, P = SCRI polytunnel and C = Commercial polytunnel. Values in italics are highest in trait explained variance.

Flavour	Year	Site	LG	Location (cM)	LOD score	% variance explained
Sweetness	2007	F	3	25.3- 29.6	3.14	12.6
			3	63.6- 126.9	3.49	13.3
			5	*52.8- 65.3*	*3.06*	*13.5*
		P	2	136.0-136.6	3.05	10.5
Sourness	2006	F	5	0.0- 24.7	2.50	10.1
	2007	F	2	68.8- 70.8	3.44	13.3
			2	99.1- 109.7	3.29	12.4
			3	82.5- 85.6	3.28	14.0
			4	121.9- 128.3	3.03	11.6
		P	3	84.4- 102.2	3.28	15.4
		C	5	*24.1-49.1*	*3.26*	*27.1*
Flavour intensity	2006	F	4	50.8- 56.6	2.79	12.7
	2007	F	3	*52.6- 84.4*	*4.48*	*18.8*
		P	2	12.8- 148.6	3.61	13.2
			4	44.8- 109.7	3.41	15.3

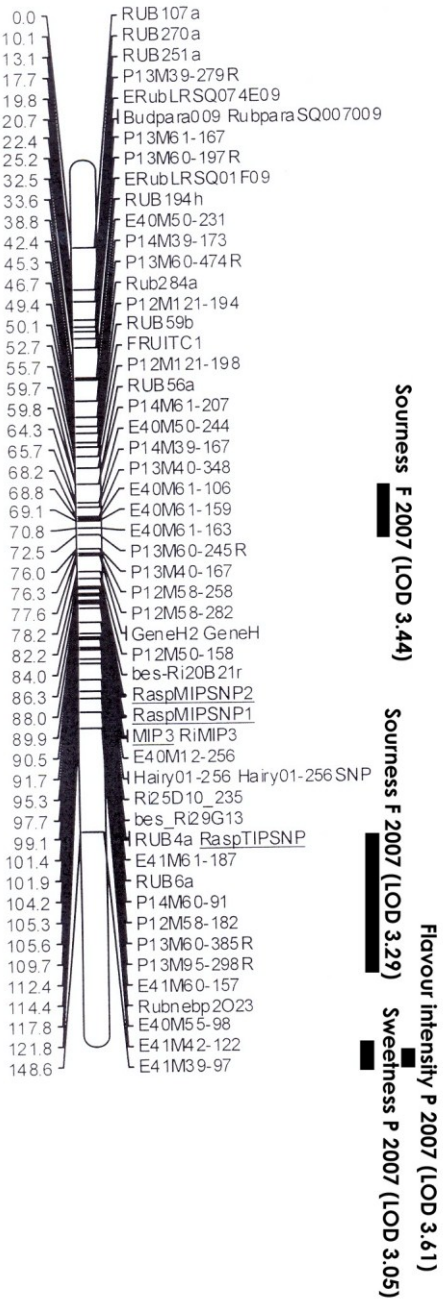


Figure 3.15 Flavour traits QTLs on Linkage Group 2: sourness (2), sweetness and flavour intensity. QTLs are generally placed on the joint map except for LG 4 where the two parental maps do not join. Legend: F 2007 = SCRI field 2007, P 2007 = SCRI polytunnel crop 2007.

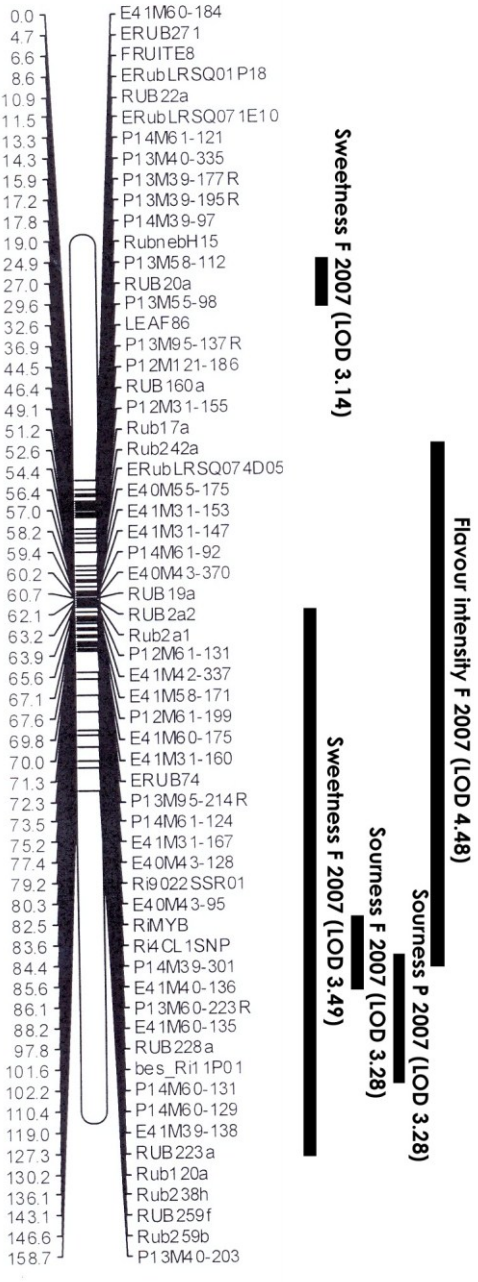


Figure 3.16 Flavour traits QTLs on Linkage Group 3: sourness (2), sweetness (2) and flavour intensity. QTLs are generally placed on the joint map except for LG 4 where the two parental maps do not join. Legend: F 2007 = SCRI field 2007, P 2007 = SCRI polytunnel crop 2007.

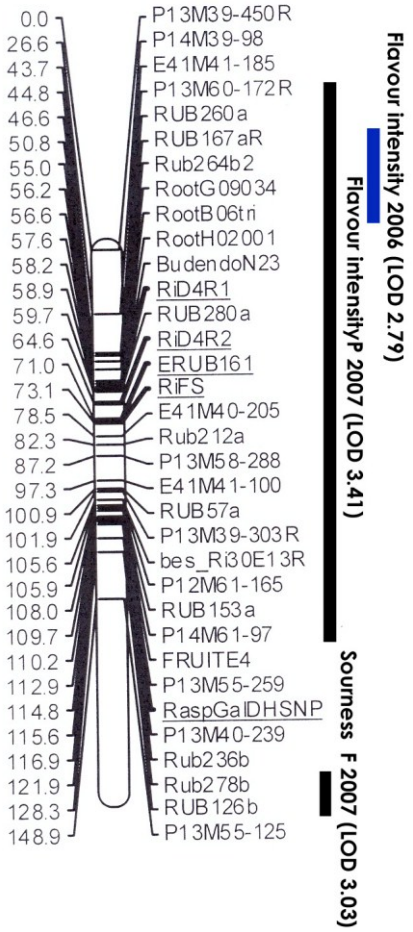


Figure 3.17 Flavour QTLs on Linkage Group 4: sourness and flavour intensity (2). All QTLs are on Latham linkage map. Legend: F 2006 = SCRI field 2006, F 2007 = SCRI field 2007 and P 2007 = SCRI polytunnel crop 2007.

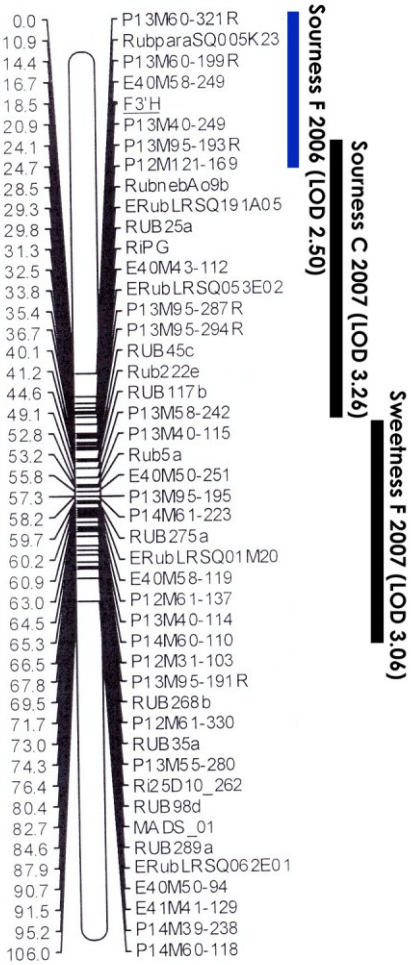


Figure 3.18 Flavour traits QTLs on Linkage Group 5: sourness (2) and sweetness QTLs are generally placed on the joint map except for LG 4 where the two parental maps do not join. Legend: F 2006 = SCRI field 2006, F 2007 = SCRI field 2007 and P 2007 = SCRI polytunnel crop 2007.

3.4.11 Associations of QTLs for metabolite contents and flavour traits

One objective of this study was to relate fruit metabolites QTLs, primarily sugars and organic acids QTLs, to sensory QTLs of flavour traits examined in this study; sweetness, sourness and flavour intensity. Candidate genes and sugar and acid QTLs co-located to transcription factors (TFs) involved in berry pigments and volatile syntheses through anthocyanin and phenylpropanoid pathways (Figure 3.19 - 3.22). Berry pigments and volatiles are important for colour and aroma traits, both important contributors to raspberry flavour quality. Transcription factors are central elements in plant development regulation, which from QTL mapping efforts from this study and that of Graham, McCallum and others (Graham et al., 2006; 2009; McCallum et al., 2010) show that it acts on multiple traits.

Interaction between sugars and acids to other metabolites was demonstrated in strawberries, where acetic acid content affected pigment stability, particularly pelargonidin (Garzón and Wrolstad 2002). In this study, a malic acid QTL for Commercial polytunnel crop was co-located to Gene *H* marker which control cane hair presence (Figure 3.16). Raspberry ketone QTL was also adjacent to Gene *H* locus. This possibly implied that gene *H* expression may be linked to common genetic regulation pathways which also influence other traits than cane hair presence. Other simple genetic associations were ripening, colour and taste development QTLs with markers related to plant development RiMYB (82.5 cM), Ri4CL1SNP (83.6 cM) and RiD4R2 (64.6 cM) on LG3 (Figure 3.17). QTLs for colour traits (i.e. visible colour scores, *Y* colour values and anthocyanin content) co-located to FRUITE4 marker (110.2 cM) on LG4 (Figure 3.21). These markers were determined fairly recently (Graham et al*.,* 2009) and have not been fully annotated, but seemed to have importance on overall fruit quality, demonstrated by its associations to measurable quality traits, such as metabolite quantities and flavour trait scores in this study.

There were also co-locations between sensory and metabolite QTLs in this study. These QTLs were adjacent to polymorphic markers for volatiles and acids contents (McCallum et al., 2010) and plant growth factors. For example, sourness QTLs in 2007 field crop on LG2 were adjacent to QTL for malic acid content and a number of

other QTLs relevant to physical traits, i.e. bud break, open flower, green fruit and late bloom (Graham et al., 2009) (Figure 3.16). On LG3 sweetness and flavour intensity QTLs of 2007 SCRI crops were co-located (Figure 3.20) to QTLs of organic acids content (McCallum et al., 2010) and β-damascenone QTL, an important aroma volatile (Kassim et al., 2008). A flavour intensity QTL on LG4 (Figure 3.21) for 2007 SCRI polytunnel crop co-located with QTLs for instrumental colour values, acids (this study; McCallum et al., 2010) and anthocyanin contents (Kassim et al., 2009). In LG5, sourness QTLs for 2006 and 2007 field crops were linked to acid QTLs (McCallum et al., 2010) and volatiles contents (Kassim et al., 2009) (Figure 3.22). Therefore, sensory traits studied here, mapped onto the genetic linkage map showed co-localisation and simple genetic associations, not only to each other, but also to expression systems of other plant traits, which suggested flavour development could share common environmental cues that trigger genetic expression systems controlling other plant traits. However further phenotypic and expression analyses would be required to confirm this hypothesis.

Key findings:

- Sensory and metabolite QTLs studied here have simple genetic associations between them.
- Co-locations of sensory QTLs to other plant trait QTLs implied possible links to genetic expression pathways controlling these traits, which further study can confirm.

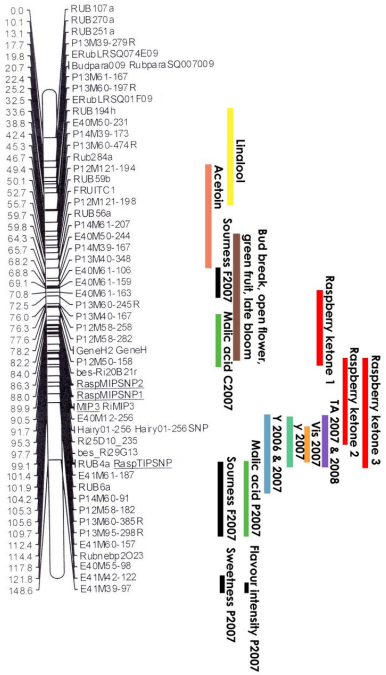


Figure 3.19: Fruit quality QTLs on LG2

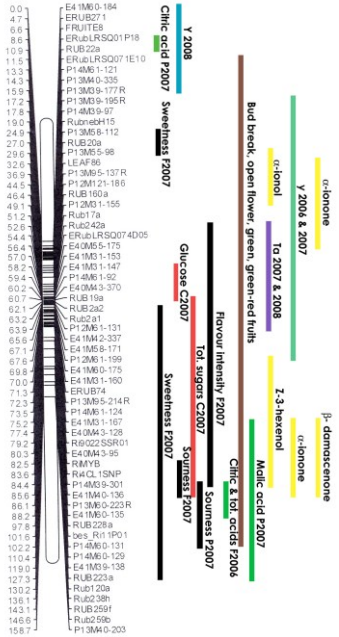


Figure 3.20: Fruit quality QTLs on LG3

Fruit and plant traits	Sources
Flavour, sugars and acids	This study
Plant development	Graham et al., 2009
Colour parameters and titratable acidity (Ta)	McCallum et al., 2010
Volatiles	Kassim, 2009 (PhD thesis)

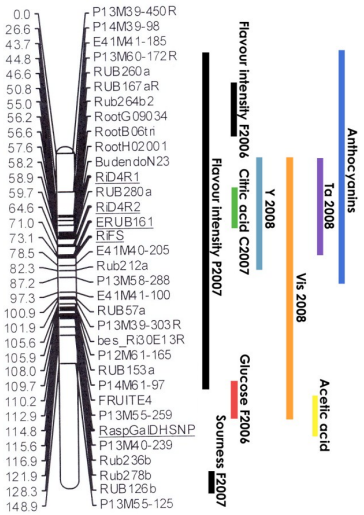


Figure 3.21: Fruit quality QTLs on LG4

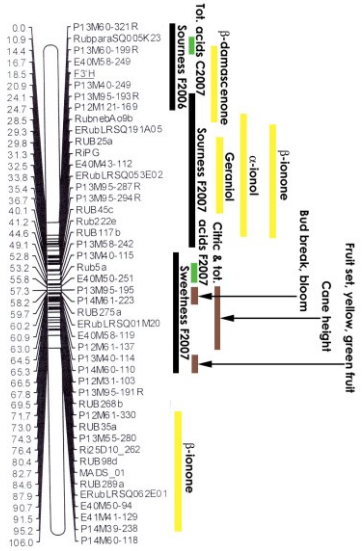


Figure 3.22: Fruit quality QTLs on LG5

Fruit and plant traits	Source
Flavour, sugars and acids	This study
Plant development	Graham et al., 2009
Colour parameters and titratable acidity (Ta)	McCallum et al., 2010
Anthocynins	Kassim et al., 2009
Volatiles	Kassim, 2009 (PhD thesis)

3.5 Discussion

The primary aim of this component of the study was to investigate effects of season, agricultural practice and genotypic factors on three (3) flavour quality traits; sweetness, sourness and flavour intensity, of fruits from a mapping population derived from a varietal cross. Parent varieties differed in geographic origin and other plant and flavour traits. Harvest year and polytunnel cultivation had significant effects on most, if not all, scored flavour traits. Variations arising from different seasonal conditions resulted in 2007 field crop scoring higher for sourness and flavour intensity but lower in sweetness compared to 2006 crop. From meteorological data, in 2007 there was a rise in water availability and reduced light intensity and temperatures, which yielded fruits more tart and less sweet, but scored higher in flavour intensity. Plants cultivated under controlled environments in polytunnels produced berries that scored higher for all flavour traits, highest of all in Commercial polytunnel crop. Therefore, environmental effect on flavour quality development in berries is assumed less under polytunnels, which produced berries of good flavour quality, a conclusion derived from higher sensory scores. Variation from different panels used to score fruits was less pronounced as standard deviations of field crops scores were similar in both years.

Key points:

- Increased water availability in 2007 yielded field fruits more tart and less sweet, but intensely flavoured.
- Polytunnel cultivation increased intensity of all flavour traits.

3.5.1 Univariate correlations of flavour attributes

Key findings:

- All flavour traits were significantly correlated in field crops.
- No significant correlations between sourness and flavour intensity was observed in polytunnel crops.

- Significant correlations between sweetness and flavour intensity remained in

polytunnel crops.

- Sourness did not significantly correlate to flavour intensity perceptions in polytunnel crops.

Pearson's correlations analyses showed significant links between sweetness and flavour intensity traits in field crops, stronger than links between flavour intensity and sourness (Table 3.13). This was also demonstrated by non-significant difference in flavour intensity scores despite significantly higher sourness scores in 2007 field crop. Simple genetic links, in QTL mapping efforts, of sweetness and flavour intensity QTLs on LG3 further substantiated these links. Although genetic predisposition to develop good flavour quality through enhanced metabolite accumulation is desirable, environmental effects still exert a greater effect, yielding differences in flavour. This was indicated by lower heritability estimates in 2007 crops of different sites compared to estimates in 2006 field crop. Therefore, seedlings could be selected based on higher genetic predilection to accumulate more flavour metabolites, but ultimately agricultural conditions facilitate / enhance genetic control on metabolite accumulation and final flavour quality. It could be genetic x environment interactions are the most impacting of all factors on flavour development.

3.5.2 Importance of ratio values

Key findings:

- Different harvest years yielded significantly different sweet : sour ratio values in field crops.
- Polytunnel cultivation generally increased sweet : sour ratio values in progeny

Sweet: sour ratios were significantly different between field and polytunnel crops with higher ratio values in polytunnel crop. Coincidentally, this corresponded to higher sugars and acids contents, and total sugars : total acids ratio in these crops compared to values in field crop. Balance of sweetness and sourness and its interactions has been studied in champagne (Martin, 2002), where sweetness had stronger muting effect on

sourness, from addition of sucrose compared to sourness, with addition of tartaric acid. In contrast, from an orange flavour jelly character study, sourness had a stronger effect on sweetness and enhanced perception of 'fruitiness', suggesting an importance of sourness in orange flavour character (Bonnans and Noble, 1993). In kiwifruit pulp, suppression of sweetness by organic acids was more dependent on acid type than acid quantity. Pulps with high total soluble solid content (SSC%) were less susceptible to change in character with addition of sugars and organic acids, which suggested threshold / saturation levels for flavour-active compounds (Marsh et al*.,* 2003, 2006). In mango, addition of citric acid influenced scoring of 'sweet', 'sour', 'peachy', 'pine/terpentine', 'astringent', and 'biting' scores, whereas addition of sugar enhanced scores for all flavour attributes (Malundo et al., 2001). Other than correlations and interactions of sweetness and sourness, these flavour traits also impact on flavour intensity perception, shown by Schifferstein and Frijters (1990) to be an addition of sweetness and sourness traits represented in vector form. They showed total taste intensity scoring of citric acid / sucrose mix solution was well correlated to combined relative sweetness and sourness scores (i.e. actual score x constant, *x*) from only sucrose and citric acid solutions respectively, with muting effects of sucrose and citric acid on sweetness and sourness perceptions. Thus, to create premium flavour in fresh fruit requires balance of sweetness and sourness, which impacts on flavour intensities. From this study, it appears increased sensory scores could be controlled through increased quantities of sugars and acids, achieved by polytunnel cultivation, presumably through reduced environmental effects on berry development and consequent flavour quality.

3.5.3 Environmental factors: Effects of cultivation conditions on flavour quality

Key findings:

- Polytunnel cultivation resulted in crops higher in flavour scores and metabolite contents; high sugars but lower acids content.

Most research on cultivation conditions and its effects on fruit crops examine its impact on fruit quality traits (e.g. yield, fruit set, flavour quality). As fruit ripening contributes to flavour development, cultivation practices or conditions, which accelerate or enhance this process is desirable. To minimise variance caused by environmental effects on ripening, polytunnel cultivation offers a potential solution. In this study, it had positive effects on flavour quality with overall increased flavour and increased sugar and acids contents (**Chapter 2**). Lack of significant correlation between sourness and flavour intensity scores in polytunnel crops could be due to increased sugars contents and its muting effects on sourness perceptions, as shown by Schifferstein and Frijters (1990). Total acids contents in polytunnel crops were similar to contents in field crop, but total sugars were 51% - 115% higher in polytunnel crops in 2007.

Effects of environmental factors on sugar and acid contents in fruits and its consequent final flavour quality have also been demonstrated in other fruit crops (Johansson et al., 1999; Thybo et al., 2000; Sinesio et al., 2000; Causse et al., 2002; Žnidarčič et al., 2003; Molina et al., 2008; Voća et al., 2009).

3.5.4 Genotype Associations of QTLs for metabolite contents and flavour traits

Key points:

- Co-location of metabolite QTLs to markers of fruit development factors implied common external stimuli, which trigger common expression pathways.
- Co-location of sensory with sugars and acids QTLs to other QTLs for physical plant traits suggested flavour quality to be a complex trait, only partly determined by metabolite contents.

Flavour trait QTLs identified in this study co-located to polymorphic markers for fruiting and ripening development in raspberry, specifically for pigment, colour development and metabolite transport systems. Correlations between sensory to sugar and acid QTLs were also observed, suggestive of common regulatory mechanisms for fruit metabolite contents and berry flavour character. However, from sensory, biochemical data and heritability estimates from this study, impact of environmental factors on flavour quality could be more pronounced than genotype factors. But it is likely interactions between environment and genotype has the most dominating influence. There are QTLs that co-locate to different LGs for different environments. This can be addressed through generation of BC (back-crossing to a parent variety) populations repeatedly and producing near-isogenic lines (NILs). Gene epistasis and undesirable phenotypes are avoided through **A**dvanced **B**ackcross **QTL** (AB-QTL), where QTL mapping is performed on later generations and these issues are out-bred and stabilised (Wang and Chee, 2010).

3.6 Conclusion

Flavour traits in raspberries originate partly from non-volatile metabolite contents (i.e. sugars and acids content) at harvest and point of consumption. Two environmental factors (harvest year and cultivation method) and genotype had varying effects on scorings of three flavour traits, sweetness, sourness and flavour intensity. Cultivation method had greater impact than genotype in producing berries with high sensory scores. Higher flavour scores in polytunnel crops indicated this, in a season with less favourable weather compared to previous harvest year. Higher flavour scores in polytunnel crops can be partially explained by increased berry sugars and acids content (**Chapter 2**). Preliminary identification of candidate genes underlying QTLs suggested transcription factors (e.g. MYB) could play a major role in determining metabolite contents and final berry flavour quality. This could be tested further by correlating sugar and acids to anthocyanin contents of berries in this mapping population. These qualities however could share common regulation pathways with other plant and / fruit trait development; pigment biosynthesis and consequent colour development and metabolite transportation

of possibly sucrose and other metabolites. Co-location of berry sugars and acids QTLs to sensory QTLs also imply simple genetic links and possibly common regulatory pathways. This link strengthened previously found univariate correlations between these flavour traits. Further study into metabolite regulation pathways and the factors that influence its accumulation during ripening would be useful in expanding our knowledge of flavour character development in red raspberry. Identification of correlations and simple genetic links of factors studied here may serve as a starting point for further study into red raspberry flavour development.

CHAPTER 4

Modelling Relationships Between Compositional and Sensory Data

Chapter 4 Modelling relationships between compositional and sensory data

4.1 Introduction

Statistical models relate datasets; suggests correlations with variables most likely responsible for quantitative variations in traits, expressed as trait qualitative differences. In modelling, effects from different measurement methods and measurement units which cause wide data variation are minimised, through the process of normalising data; datasets are weighted and centred before analyses. In models extent of influences by independent variables to dependent variables are gauged from correlation coefficient values, numerical and directional in nature and expressed in linear equations. Statistical models also predict future responses of dependent variables to effects by independent variables, where values for dependent variables are extrapolated from calibration models with existing data sets. Modelling strategies relevant to fruit quality breeding research include identifying variables most associated to flavour trait, through preliminary univariate analysis (e.g. Pearson's correlations) and modelling these variables to continuous trait intensity by multi-linear correlations analyses (e.g. PLSR) (Meullenet, Xiong and Findlay (2007).

4.1.1 Multivariate statistical analyses in fruit and vegetable crops

This data correlation method has been applied extensively in the flavour quality research of many fruit and vegetable crops. Regression modelling correlated sensory data with other flavour-associated datasets in chicory (François et al., 2008), wine (Blackman et al., 2010) and strawberry (Gunnes et al., 2009). Crop texture has also been extensively modelled to sensory data; in potato, texture variables (e.g. *hardness*, *juiciness*, *mealiness*) correlated to acceptability scorings by assessors (Thybo and Martens, 2000; Thybo et al., 2006), also found in kiwifruit (Harker et al., 2009; Ragni et al., 2010). Texture was demonstrated to affect sweetness scorings in modelling of rheology parameters to sweetness perception as a function of sugar release from an artificial food matrix (Holm et al., 2009). Other than its use in correlating sensory and other flavour datasets, multivariate regression analyses enabled quality assessment of fruits high in juice content without using invasive techniques; by correlating firmness measured by a dynamometer to degrees of flesh softening, often the cause of fruits not fit for sale (Menesatti et al., 2009). This

technology was also applied similarly to satsumas (Gómez et al., 2006), apples (Zude et al., 2006) and kiwifruit (McGlone and Kawano 1998; Ragni et al., 2010). Fruits high in juice content often suffer from bruising, which necessitates non-invasive firmness measurement to determine fruits fit for market sales (Ragni et al., 2010; Menesatti et al., 2009). These examples demonstrate how multivariate regression analyses enable mechanisation of quality assessment in replacement of human inspectors. This is often more time and cost efficient. Other examples of quality monitoring without human inspection were also demonstrated in apple and peach; volatile profiles produced by these fruits stored in different conditions (modified vs. ambient) measured by GC-MS correlated to consumer acceptability scores (Lara et al., 2006; Echeverría et al., 2008; Ortiz et al., 2009). PCA and **P**artial **L**east **S**quares - **D**iscriminant **A**nalysis (PLS-DA) differentiated climacteric and non-climacteric genotypes of melon near-isogenic lines from its volatile profiles during ripening (Obando-Ulloa et al., 2008, 2009). Spectral measurements of crop trait (e.g. firmness) correlated to metabolite data (e.g. total soluble solids, ^{o}Brix, pH) has been suggested as a replacement to trained sensory assessors for quality because it is robust with high throughput and have reduced susceptibility to experimental variation (Blackman et al., 2010). Indeed in the examples mentioned here, multivariate data regression enabled data deconstruction and identified variables most influential in effecting significant changes in dependent variables.

4.2 Aims of research

This component of the study aimed to model dependent sensory variables in progeny fruits; sweetness, sourness and flavour intensity to independent variables related to fruit metabolite contents and other fruit quality traits; individual, total and ratios of sugars and acids contents, volatiles, anthocyanins and raspberry ketone contents, ^{o}Brix and 10-berry weight. Sugars, acids and raspberry ketone contents were quantified in this study, other datasets were procured from parallel research (Kassim, 2009; McCallum, 2009).

4.3 Material and Methods

4.3.1 Data collection

Sugars, acids and raspberry ketone data were collected as described in **Chapter 2**. Other datasets were provided by members of the research group; individual anthocyanins and volatiles content from Kassim (2009); colourmeter parameter, ^{o}Brix and weight values from McCallum (2009). Dependent variable sensory scores were acquired as described in **Chapter 3**. Data sets were collected from field crops of 2 years (2006 & 2007) and in 2007 from 3 sites: SCRI field, SCRI polytunnels and Commercial polytunnels near Blairgowrie.

4.3.2 Statistical analyses

Univariate analyses (ANOVA and Pearson's correlation analysis) performed using Minitab v.15 (Minitab Inc. USA) software, measured significance and magnitude of linear influence of independent to dependent variables. Partial least square regression (PLS-1) analyses modelled relationships between multiple independent X (compositional) variables to single dependent Y (flavour scoring), analysed by Unscrambler 9.7 (CAMO A/S, Norway) software. PLS-1 model reliability was assessed through calibrated (RMSEC) and predicted (RMSEP) regression values; with a good model fit being an overlap of these values. Other analyses outputs were sample groupings (from scores spaces) and β-coefficient values, numerical and directional in nature, that identified variables most associated to traits in a loadings plot.

Univariate correlation analyses were performed on raw datasets and normalised data was used for multivariate regression analyses. Due to missing data year-to-year and site-to-site, a complete dataset on 26 progeny seedlings that possessed values for all input variables was used in PLS-1 modelling. consistently produced enough fruits for analyses each year and in each site in 2007. Although the sample size (26) is small, PLSR is able to yield representative multivariate models using small sample sizes (Haenlein and Kaplan, 2004), also demonstrated by Chin and Newsted (1999) with a low sample size of 50. A comparison of different statistical approaches (PLSR, multiple regression (MR) and PCA and MR) to multivariate data deconstruction of

sample sizes 15, 30, 60 and 120 showed PLSR to be the best method that produced a validated model close to a 'true model'; even with a sample size as small as 15Normalised datasets were used for multivariate regression analyses; datasets were weighted and centred to overcome variability from different quantification methods and quantification units. A raspberry ketone model was constructed using data from only the SCRI polytunnel crop in 2007, consisting of 138 members.

4.4 Results

4.4.1 Univariate analyses: One-way ANOVA and Pearson correlations of flavour characters and metabolite contents with other variables

Variance in sweetness, sourness and flavour intensity in 2007 were significantly affected by cultivation methods (p-value < 0.05) (Table 4.1). Harvest year and different polytunnel sites also significantly affected variance in sourness and flavour intensity. Sweetness variance however was only significantly affected by different cultivation methods of open field and polytunnel in 2007.

Linear correlations in field crop flavour traits were more significant in 2006 than in 2007; sweetness and sourness significantly correlated to flavour intensity scoring, with an increase in r-value in 2007 (Table 4.2). Sourness inversely affected sweetness in 2006 field crop but not in 2007 crop. In polytunnel crops (SCRI and Commercial), sweetness significantly correlated to flavour intensity, with s in SCRI crop (Table 4.3). Sourness inversely correlated to sweetness, more strongly in Commercial than in SCRI polytunnel crop. However, sourness did not significantly influence flavour intensity in polytunnel crops, found in field crops.

Table 4.1 One-way ANOVA analyses of flavour character scores in progeny fruit crops in: (i) 2006; Field 2006 (F2006) and Field 2007 (F2007); (ii) 2007 = Field (F2007), SCRI polytunnel (P2007) and Commercial polytunnel (C2007). *p* = significance value at 95% confidence level. *p*-values highlighted in bold are significant.

Variable	*p*-value (i)		*p*-value (ii)			
	F 2006	F 2007	F 2007	P 2007	P 2007	C 2007
Sweetness	0.10		**<0.001**		0.49	
Sourness	**<0.001**		**<0.001**		**0.02**	
Flavour intensity	**0.01**		**<0.001**		**0.02**	

Table 4.2 Pearson's correlation analyses for flavour attributes in field crops in 2006 & 2007. Significant correlations are marked in bold. *p* = significance value at 95% confidence level, r = Pearson's correlation coefficient.

	F 2006		**F 2007**	
Variables	Sweetness	Sourness	Sweetness	Sourness
Flavour intensity	***p* < 0.001** **r = 0.470**	***p* < 0.001** **r = 0.354**	***p* < 0.001** **r = 0.627**	***p* = 0.001** **r = 0.450**
Sweetness		***p* < 0.001** **r = -0.379**		*p* = 0.32 r = -0.092

Table 4.3 Pearson's correlation analyses for flavour attributes in polytunnel crops in 2007. Significant correlations are marked in bold. *p* = significance value at 95% confidence level, r = Pearson's correlation coefficient.

	P 2007		**C 2007**	
Variables	Sweetness	Sourness	Sweetness	Sourness
Flavour intensity	***p* < 0.001** **r = 0.742**	*p* = 0.08 r = 0.148	***p* = 0.001** **r = 0.434**	*p* = 0.38 r = 0.122
Sweetness		***p* < 0.001** **r = -0.363**		***p* < 0.001** **r = -0.638**

From Pearson correlation analyses of fruit sugar contents, harvest year had most significant influence on individual and total sugars contents, hexose ratios and ^{o}Brix values but not in total sugars : total acids ratios (Table 4.4). This ratio was also not affected by cultivation method in 2007. Polytunnel cultivation in 2007 significantly affected individual, total sugars contents and ^{o}Brix, but not metabolite ratios between SCRI field and polytunnel crops (Table 4.4). In 2007 polytunnel crops, all sugar contents and ^{o}Brix, with exception of total sugars : total acids ratio, were significantly different between sites.

In acids contents, harvest year was also the most significant factor that affected individual contents and its ratios but not total content (Table 4.5). In 2007, significant variations in acids contents were found between all planting sites. However, citric : malic acid ratios did not significantly differ between polytunnel sites, but was significantly different between field and polytunnel site (Table 4.5). Interestingly in 2007, total acid contents were similar in field and polytunnel crops of SCRI, but different between polytunnel sites. This suggested that under polytunnel cultivation different agronomic practices in both locations produced different acid and sugar profiles in fruits; Commercial polytunnel site producing highest contents of both sugars and acids contents.

In 2006 field crop, sweetness and flavour intensity traits better correlated with sugar contents and total sugars : total acids ratios than to acids contents (Table 4.6 and 4.7). Sourness did not significantly correlate to any metabolite contents or ^{o}Brix values, but to flavour intensity trait (Table 4.7). Therefore metabolite parameters that affected flavour intensity could possibly affect sourness also. Sweetness, flavour intensity, sugars parameters and total sugars : total acids ratio were all significantly correlated to ^{o}Brix.

Table 4.4 One-way ANOVA analysis of metabolites content in progeny fruit: individual and total sugars contents, hexose ratios and °Brix. *p* = significance value at 95% confidence level. *p*-values highlighted in bold are significant. Crops as in Table 4.1.

Variable	*p*-value				*p*-value			
	F 2006	F 2007	F 2006	P 2007	F 2007	P 2007	P 2007	C 2007
Fructose	**0.01**		**<0.001**		**<0.001**		**<0.01**	
Glucose	0.40		**<0.001**		**<0.001**		**0.05**	
Fructose : Glucose	**<0.001**		**<0.001**		0.91		**0.01**	
Total sugars: Total acids	0.14		**<0.001**		0.45		0.08	
Total Sugars	**0.03**		**0.03**		**<0.001**		**<0.001**	
°Brix	**<0.001**		0.19		**<0.001**		**0.03**	

Table 4.5 One-way ANOVA of metabolites: individual and total acid, malic : citric acid ratios and °Brix of progeny fruit. *p* = significance value at 95% confidence level. *p*-values highlighted in bold are significant. Crops as in Table 4.1.

Variable	*p*-value				*p*-value			
	F 2006	F 2007	F 2006	P 2007	F 2007	P 2007	P 2007	C 2007
Malic Acid	**<0.001**		**<0.001**		0.19		**0.002**	
Citric Acid	**<0.001**		**<0.001**		0.93		**< 0.001**	
Malic Acid: Citric Acid	**<0.001**		**<0.001**		**0.02**		0.26	
Total Acids	**< 0.001**		0.10		0.67		**< 0.001**	

In 2007 field crop, sweetness significantly correlated to flavour intensity (Table 4.8), and both flavour traits with ^{o}Brix, with stronger correlations compared to 2006 field crop (Table 4.8 and 4.9). Individual sugars correlated significantly, but negatively, to flavour intensity, notably fructose (Table 4.9). Sourness significantly correlated to individual and total acids contents, notably to citric acid but not to ^{o}Brix values (Table 4.9). Total sugars and acids were higher in 2006 (**Chapter 2**), and more strongly correlated to flavour traits inferred from higher r-values than in 2007. Also lower sugar contents in 2007 field crop did not significantly correlate to ^{o}Brix, contrary to significant correlations with higher sugars content in 2006 (Table 4.6). From this result, a possible explanation could be sugars content was a substantial portion representing total soluble solids measured by ^{o}Brix, a situation not found in 2007 field crop. In summary, ^{o}Brix was a robust indicator of sweetness and flavour intensity traits in field crops, consistent over the two harvest years, despite inconsistent correlations with different levels of sugars and acids 2006 and 2007 field crops (Table 4.5). These varying levels could also have varied its influence on flavour traits variance, indicated from Pearson's correlation analyses (Table 4.6 – 4.9).

In 2007 polytunnel crops, sweetness and flavour intensity traits did not significantly correlate to ^{o}Brix values. These flavour traits were significantly correlated to individual and total sugars contents. Unlike 2006 field crop, sugars contents did not significantly correlate to ^{o}Brix, with the exception of hexose ratio (Table 4.10). Lack of significant correlation to ^{o}Brix maybe stemmed from lower representation of sugars contents in total soluble solids measured by ^{o}Brix, and therefore it is again a reliable indicator of sweetness and flavour intensity traits, but not of sugars and acids contents. In other pigmented soft fruits, like cherries and blueberries, anthocyanin pigments have been similarly correlated to soluble solids content measured by ^{o}Brix (Krupa and Tomala, 2007). The edible portion highest in anthocyanin content in 4 cherry cultivars was also highest in ^{o}Brix (Chaovanalikit and Wrolstad, 2003). Therefore, ^{o}Brix values measured in this study could also represent pigments and metabolites other than sugars and acids content.

Sourness in 2007 polytunnel crops was not convincingly correlated to any other flavour traits or metabolite variables, contrary to field crop, which it significantly affected flavour intensity and correlated to acids contents (Table 4.9 – 4.11). This was unusual because Commercial polytunnel crop had highest total acids contents, however it also had highest total sugars contents, which could minimise or diminish sourness effects caused by acids contents, previously shown by Schifferstein and Frijters (1990). This was possibly indicated by significant inverse correlations of sourness to sweetness in polytunnel crops.

In 2007 Commercial polytunnel crop, flavour traits did not significantly correlate to any metabolite variable (Table 4.12, 4.13). Sourness inversely affected sweetness and sweetness had positive significant effects on flavour intensity. ^{o}Brix values only had significant correlations with flavour intensity trait and glucose contents, therefore assumed here that no substantial representations by sugars and acids in total soluble solids contents in crops was measured by ^{o}Brix values. However, although there are not significant links between sweetness and ^{o}Brix, variables affecting flavour intensity perceptions are assumed to be similarly affecting sweetness. It was evident in Commercial polytunnel crop, metabolite variables other than sugars and acids contents may have more influence in flavour trait variance.

In summary, flavour traits in the studied crops had linear correlations with sugars and acids contents, which varied dependent on cultivation year and site. However, ^{o}Brix values can be used, to a degree of confidence, as an indicator of sweetness and flavour intensity traits in crops, more reliable for flavour intensity than for sweetness.

Key points:

- All flavour traits were significantly affected by cultivation practice.
- Sourness and flavour intensity were affected by both harvest years and different cultivation practices, sweetness only by harvest years.
- In field crops, flavour intensity significantly correlated to sweetness but inversely correlated to sugars content.

- In field crops flavour intensity had significant links with sourness, which correlated to acids content in 2007.
- Sweetness and flavour intensity were correlated in polytunnel crops, linked to sugars content in SCRI site.
- Sugars positively affected flavour intensity in polytunnel crops, but negatively affected it in field.
- Sourness did not correlate well with any metabolite variable in polytunnel crops.
- Sourness had inverse correlations with sweetness in all crops.
- ^{o}Brix was a robust predictor of sweetness and flavour intensity in field crops but only for flavour intensity in Commercial polytunnel crop.
- ^{o}Brix correlated with metabolites content inconsistently through harvest years and cultivation sites.

4.4.2 Univariate analyses: Impact of raspberry ketone on flavour traits

From restricted SCRI polytunnel crop sample dataset, raspberry ketone (RK) content did not significantly correlate with any flavour traits (Table 4.14), sugars or acid contents (Table 4.15). Of physicochemical measurements, brightness (L*), a colourmeter parameter, was inversely linked to raspberry ketone contents, implying darker berries could have higher contents (Table 4.16). Specific volatiles (α-ionol, hexanoic acid, benzyl alcohol and acetoin) were also significantly correlated to raspberry keton contents, notably α-ionol ($p < 0.001$, r = 0.377) (Table 4.17). These volatiles are thought important for plant survival, as it is responsible for attracting fruit fly *Batrocera latifrons*, an important pollinator (Diptera: Tephritidae; Flath et al.1994; McQuate and Peck 2001). The volatile α-ionol was identified as an aroma-active volatile, which contributed to difference in aroma profiles of Meeker variety raspberries cultivated in two North American locations (Klesk et al., 2004). In another raspberry study, benzyl alcohol and hexanoic acid contents were found to contribute to sweet flavour notes, together with raspberry ketone and acetoin contents (Larsen and Poll, 1990).

Key points:

- Darker berries could possibly have higher raspberry ketone content, inferred from inverse correlation with instrumental brightness (L*) measurements.
- Specific volatiles that correlated with raspberry ketone contents are shown to impart 'sweet' flavour notes in other raspberry studies.

4.4.3 Results: Multivariate analyses: PLS-1 Modelling

4.4.3.1 Hypothesis and strategy

Raspberry sensory attribute scores (sweetness, sourness, flavour intensity) can be thought of as flavour trait intensities that behave as dependent variables explained by combinations and interactions of independent metabolite variables in fruit composition. Univariate analyses of sugars, acids and specific volatiles content datasets suggested metabolite variables correlated with (sensory) flavour trait, but these singular linear correlations only provide partial explanations of sensory trait variance in fruits. To correlate a number of metabolite variables to sensory trait at once, multivariate regression technique partial least squares regression (PLS1) was performed. Available metabolite variables were: non-volatiles contents; sugars and organic acids parameters, volatiles content; 12 volatile compounds and pigment content variables. Physicochemical related variables were: 10-berry weight, ^{o}Brix and colour measurement values. From multivariate regression analyses, expected output was combinations of independent variables most comprehensive in explaining and predicting each dependant sensory variable, i.e. flavour traits; sweetness, sourness, and flavour intensity.

Table 4.6 Pearson's correlations (significance - *p*-value; coefficient value, r) of flavour traits in 2006 field crop with individual, and total sugars contents, hexose ratio, total acids and °Brix. *p* = significance value at 95% confidence level. *p*-values highlighted in bold are significant.

Field 2006							
Variables	Fructose	Glucose	Fructose: Glucose	Total sugars	Total sugars: Total acids	Total acids	°Brix
Sweetness	***p* < 0.001 0.343**	***p* < 0.001 0.368**	*p* = 0.081 -0.155	***p* < 0.001 0.370**	***p* < 0.001 0.347**	*p* = 0.604 -0.047	***p* = 0.003 0.259**
Sourness	*p* = 0.117 -0.138	*p* = 0.173 -0.121	*p* = 0.775 -0.026	*p* = 0.154 -0.127	*p* = 0.061 -0.169	*p* = 0.243 0.105	*p* = 0.804 0.022
Flavour intensity	*p* = 0.366 0.080	*p* = 0.076 0.157	*p* = 0.058 -0.168	*p* = 0.091 0.150	**p = 0.043 0.182**	p = 0.689 -0.036	**p = 0.021 0.202**
Fructose		**p < 0.001 0.928**	**p = 0.009 -0.215**	**p < 0.001 0.942**	**p < 0.001 0.725**	p = 0.477 0.060	**p < 0.001 0.333**
Glucose			**p < 0.001 -0.500**	**p < 0.001 0.999**	**p < 0.001 0.766**	p = 0.445 0.065	**p < 0.001 0.332**
Fructose: Glucose				**p < 0.001 -0.472**	**p < 0.001 -0.364**	p = 0.953 0.005	**p = 0.012 -0.208**
Total sugars					**p < 0.001 0.767**	p = 0.444 0.065	**p < 0.001 0.344**
Total sugars: Total acids						**p < 0.001 -0.486**	**p = 0.001 0.289**
Total acids							p = 0.459 -0.062

Table 4.7 Pearson's correlations (significance - *p*-value; coefficient value, r) of flavour traits in 2006 field crop with individual, and total acids contents, acids ratio, total sugars and ^{o}Brix. *p* = significance value at 95% confidence level. *p*-values highlighted in bold are significant.

Field 2006						
Variables	Citric acid	Malic acid	Citric acid: Malic acid	Total acids	Total sugars	^{o}Brix
Sweetness	p = 0.758 -0.028	p = 0.385 -0.078	p = 0.484 0.063	p = 0.604 -0.047	**p < 0.001** **0.370**	**p = 0.003** **0.259**
Sourness	p = 0.326 0.088	p = 0.200 0.114	p = 0.665 -0.039	p = 0.243 0.105	p = 0.154 -0.127	p = 0.804 0.022
Flavour intensity	p = 0.942 0.007	p = 0.163 -0.124	p = 0.349 0.084	p = 0.689 -0.036	p = 0.091 0.150	**p = 0.021** **0.202**
Citric acid		**p < 0.001** **0.633**	**p < 0.001** **0.711**	**p < 0.001** **0.967**	p = 0.605 0.044	p = 0.550 -0.050
Malic acid			p = 0.641 -0.039	**p < 0.001** **0.810**	p = 0.257 0.096	p = 0.384 -0.073
Citric acid: Malic acid				**p < 0.001** **0.525**	p = 0.792 0.023	p = 0.694 0.033
Total acids					p = 0.444 0.065	p = 0.459 -0.062

Table 4.8 Pearson's correlations (significance - *p*-value; coefficient value, *r*) of flavour traits in 2007 field crop with individual, and total sugars contents, hexose ratio, total acids and °Brix. *p* = significance value at 95% confidence level. *p*-values highlighted in bold are significant.

Field 2007							
Variables	Fructose	Glucose	Fructose: Glucose	Total sugars	Total sugars: Total acids	Total acids	°Brix
Sweetness	p = 0.415 -0.163	p = 0.586 -0.110	p = 0.507 -0.134	p = 0.390 -0.172	p = 0.785 -0.055	p = 0.500 -0.079	**p < 0.001** **0.465**
Sourness	p = 0.155 -0.281	p = 0.840 -0.041	p = 0.160 -0.278	p = 0.207 -0.251	p = 0.067 -0.358	**p = 0.001** **0.362**	p = 0.341 0.088
Flavour intensity	**p = 0.017** **-0.456**	p = 0.446 -0.153	**p = 0.036** **-0.404**	**p = 0.024** **-0.433**	**p = 0.018** **-0.452**	p = 0.342 0.111	**p = 0.001** **0.311**
Fructose		**p = 0.001** **0.485**	**p < 0.001** **0.740**	**p < 0.001** **0.960**	p = 0.213 0.199	p = 0.530 0.101	p = 0.660 0.070
Glucose			p = 0.371 -0.142	**p < 0.001** **0.710**	p = 0.699 0.062	p = 0.340 -0.153	p = 0.861 -0.028
Fructose: Glucose				**p < 0.001** **0.551**	p = 0.565 0.092	p = 0.134 0.238	p = 0.686 0.064
Total sugars					p = 0.258 0.181	p = 0.837 0.033	p = 0.766 0.047
Total sugars: Total acids						**p < 0.001** **-0.542**	p = 0.608 -0.083
Total acids							p = 0.938 0.008

Table 4.9 Pearson's correlations (significance - *p*-value; coefficient value, r) of scores of flavour traits in 2007 field crop with individual, and total acids contents, acids ratio, total sugars and ^{o}Brix. *p* = significance value at 95% confidence level. *p*-values highlighted in bold are significant.

Field 2007						
Variables	Citric acid	Malic acid	Citric acid: Malic acid	Total acids	Total sugars	^{o}Brix
Sweetness	*p* = 0.580 -0.065	p = 0.284 -0.125	p = 0.179 0.156	p = 0.500 -0.079	p = 0.390 -0.172	**p < 0.001** **0.465**
Sourness	**p = 0.001** **0.364**	**p = 0.037** **0.240**	p = 0.185 0.154	**p = 0.001** **0.362**	p = 0.207 -0.251	p = 0.341 0.088
Flavour intensity	p = 0.300 0.120	p = 0.826 0.026	p = 0.068 0.210	p = 0.342 0.111	**p = 0.024** **-0.433**	**p = 0.001** **0.311**
Citric acid		**p < 0.001** **0.694**	p = 0.364 0.089	**p < 0.001** **0.991**	p = 0.805 0.040	p = 0.779 0.027
Malic acid			**p = 0.004** **-0.273**	**p < 0.001** **0.784**	p = 0.964 0.007	p = 0.377 -0.086
Citric acid: Malic acid				p = 0.793 0.026	p = 0.935 -0.013	**p = 0.003** **0.283**
Total acids					p = 0.837 0.033	p = 0.938 0.008

Table 4.10 Pearson's correlations (significance - *p*-value; coefficient value, r) of flavour traits in 2007 SCRI polytunnel crop with individual, and total sugars contents, hexose ratio, total acids and °Brix. *p* = significance value at 95% confidence level. *p*-values highlighted in bold are significant.

SCRI polytunnel 2007							
Variables	Fructose	Glucose	Fructose: Glucose	Total sugars	Total sugars: Total acids	Total acids	°Brix
Sweetness	**p = 0.030 0.237**	**p = 0.002 0.329**	p = 0.271 0.121	**p = 0.013 0.270**	p = 0.087 0.188	p = 0.705 0.033	**p < 0.001 0.560**
Sourness	p = 0.409 -0.091	p = 0.349 -0.103	p = 0.616 -0.055	p = 0.375 -0.098	p = 0.255 -0.126	p = 0.896 0.011	p = 0.541 -0.053
Flavour intensity	**p = 0.049 0.215**	**p = 0.006 0.299**	p = 0.317 0.110	**p = 0.025 0.245**	p = 0.201 0.141	p = 0.499 0.059	**p < 0.001 0.549**
Fructose		**p < 0.001 0.778**	**p < 0.001 0.528**	**p < 0.001 0.988**	**p < 0.001 0.911**	**p = 0.001 0.350**	p = 0.219 0.131
Glucose			p = 0.960 -0.005	**p < 0.001 0.866**	**p < 0.001 0.846**	p = 0.131 0.161	p = 0.119 0.167
Fructose: Glucose				**p < 0.001 0.419**	**p = 0.003 0.315**	**p < 0.001 0.392**	**p = 0.024 0.239**
Total sugars					**p < 0.001 0.933**	**p = 0.002 0.318**	p = 0.173 0.146
Total sugars: Total acids						p = 1.000 0.000	p = 0.442 0.083
Total acids							p = 0.624 -0.041

Table 4.11 Pearson's correlations (significance - *p*-value; coefficient value, r) of flavour traits in 2007 SCRI polytunnel crop with individual, and total acids contents, acids ratio, total sugars and °Brix. *p* = significance value at 95% confidence level. *p*-values highlighted in bold are significant.

SCRI polytunnel 2007						
Variables	Citric acid	Malic acid	Citric acid: Malic acid	Total acids	Total sugars	°Brix
Sweetness	p = 0.926 0.008	p = 0.279 0.095	p = 0.657 -0.039	p = 0.705 0.033	**p = 0.013** **0.270**	**p < 0.001** **0.560**
Sourness	p = 0.489 0.061	p = 0.135 -0.130	p = 0.085 0.150	p = 0.896 0.011	p = 0.375 -0.098	p = 0.541 -0.053
Flavour intensity	p = 0.496 0.060	p = 0.632 0.042	p = 0.898 0.011	p = 0.499 0.059	**p = 0.025** **0.245**	**p < 0.001** **0.549**
Citric acid		**p < 0.001** **0.676**	**p < 0.001** **-0.337**	**p < 0.001** **0.980**	**p = 0.009** **0.275**	p = 0.666 -0.036
Malic acid			**p < 0.001** **-0.488**	**p < 0.001** **0.810**	**p = 0.032** **0.227**	p = 0.590 -0.045
Citric acid: Malic acid				**p < 0.001** **-0.401**	p = 0.409 -0.089	p = 0.797 0.022
Total acids					**p = 0.002** **0.318**	p = 0.624 -0.041

Table 4.12 Pearson's correlations (significance - *p*-value; coefficient value, r) of flavour traits in 2007 Commercial polytunnel crop with individual, and total sugars contents, hexose ratio, total acids and °Brix. *p* = significance value at 95% confidence level. *p*-values highlighted in bold are significant.

Commercial polytunnel 2007							
Variables	Fructose	Glucose	Fructose: Glucose	Total sugars	Total sugars: Total acids	Total acids	°Brix
Sweetness	p = 0.790 -0.042	p = 0.174 0.211	p = 0.290 -0.165	p = 0.946 0.011	p = 0.887 -0.022	p = 0.482 0.099	p = 0.089 0.232
Sourness	p = 0.845 0.031	p = 0.462 -0.115	p = 0.882 0.023	p = 0.995 0.001	p = 0.676 -0.066	p = 0.988 0.002	p = 0.720 0.049
Flavour intensity	p = 0.627 -0.076	p = 0.624 0.077	p = 0.136 -0.231	p = 0.754 -0.049	p = 0.220 -0.191	p = 0.405 0.117	**p = 0.047** **0.269**
Fructose		**p < 0.001** **0.546**	**p < 0.001** **0.630**	**p < 0.001** **0.979**	**p < 0.001** **0.893**	p = 0.113 0.200	p = 0.303 0.131
Glucose			p = 0.115 -0.197	**p < 0.001** **0.704**	**p < 0.001** **0.661**	p = 0.060 0.237	**p = 0.008** **0.329**
Fructose: Glucose				**p < 0.001** **0.486**	**p < 0.001** **0.449**	p = 0.758 0.039	p = 0.300 -0.132
Total sugars					**p < 0.001** **0.917**	p = 0.072 0.227	p = 0.131 0.191
Total sugars: Total acids						p = 0.314 -0.128	p = 0.483 0.089
Total acids							p = 0.205 0.139

Table 4.13 Pearson's correlations (significance - *p*-value; coefficient value, r) of flavour traits in 2007 Commercial polytunnel crop with individual, and total acids contents, acids ratio, total sugars and ^{o}Brix. *p* = significance value at 95% confidence level. *p*-values highlighted in bold are significant.

Commercial polytunnel 2007						
Variables	Citric acid	Malic acid	Citric acid: Malic acid	Total acids	Total sugars	^{o}Brix
Sweetness	p = 0.559 0.082	p = 0.639 0.066	p = 0.578 -0.078	p = 0.482 0.099	p = 0.946 0.011	p = 0.089 0.232
Sourness	p = 0.940 0.011	p = 0.930 -0.012	p = 0.639 -0.066	p = 0.988 0.002	p = 0.995 0.001	p = 0.720 0.049
Flavour intensity	p = 0.401 0.118	p = 0.746 0.046	p = 0.184 -0.185	p = 0.405 0.117	p = 0.754 -0.049	**p = 0.047** **0.269**
Citric acid		**p = 0.011** **0.275**	p = 0.998 0.000	**p < 0.001** **0.896**	**p = 0.002** **0.383**	p = 0.521 0.071
Malic acid			**p < 0.001** **-0.595**	**p < 0.001** **0.672**	p = 0.401 -0.107	p = 0.082 0.190
Citric acid: Malic acid				**p = 0.011** **-0.274**	p = 0.566 0.073	p = 0.081 -0.190
Total acids					p = 0.072 0.227	p = 0.205 0.139

Table 4.14 Pearson's correlations (*p*-value and coefficient value, r) of sweetness, sourness and flavour intensity scores with raspberry ketone (RK) content in 2007 SCRI polytunnel crop. *p* = significance value at 95% confidence level. *p*-values highlighted in bold are significant.

Variables	Sweetness	Sourness	Flavour intensity
Raspberry ketone	p = 0.251 0.109	p = 0.088 -0.162	p = 0.376 0.085

Table 4.15 Pearson's correlations (*p*-value and coefficient value, r) of individual and total sugars and acids, its ratio, total sugars : total acids ratio with RK content in 2007 SCRI polytunnel crop. *p* = significance value at 95% confidence level. *p*-values highlighted in bold are significant.

Variables	Fructose	Glucose	Fructose : Glucose	Total sugars	Citric acid	Malic acid	Citric acid : Malic acid	Total acids	Total sugars : Total acids
Raspberry ketone	p = 0.073 0.219	p = 0.141 0.181	p = 0.193 0.160	p = 0.068 0.223	p = 0.278 -0.102	p = 0.215 -0.118	p = 0.432 0.073	p = 0.260 -0.107	p = 0.162 0.172

Table 4.16 Pearson's correlations (*p*-value and coefficient value, r) of ^{o}Brix, weight and colourmeter values with RK content. Values in bold significant in 2007 SCRI polytunnel crop. *p* = significance value at 95% confidence level. *p*-values highlighted in bold are significant.

Variables	^{o}Brix	Weight	L*	a*	B*	ΔE*
Raspberry ketone	p = 0.595 0.049	p = 0.270 -0.101	**p = 0.039** **-0.187**	p = 0.786 -0.025	p = 0.263 -0.102	p = 0.061 0.170

Table 4.17 Pearson's correlations (*p*-value and coefficient value) of volatiles with RK content in 2007 SCRI polytunnel crop. Values in bold significant. *p* = significance value at 95% confidence level. *p*-values highlighted in bold are significant.

Variables	Linalool	β-damascenone	Geraniol	α-ionone	β-ionone	α-ionol	Benzyl alcohol	Acetic acid	Hexenol	Acetoin	Hexanoic acid
Raspberry ketone	p= 0.921 0.010	p= 0.412 0.081	p= 0.080 0.171	p= 0.619 0.049	p= 0.167 0.135	**p<0.001** **0.377**	**p= 0.022** **0.223**	p= 0.352 -0.091	p= 0.349 0.092	**p= 0.008** **0.256**	**p= 0.001** **0.324**

In summary, variance in dependent Y-flavour trait score was explained most by multiple independent X-metabolite and physicochemical variables. This information is represented graphically in scores and loadings plot, where contributions by each X-variable to dependent Y-variance is listed. An overview of all probable correlations between independent and dependent variables are represented in a bi-plot. The goodness-of-fit of the PLSR-model is assessed from predicted Y-variance numerical value from validation (P_{val}) datasets, i.e. actual datasets. The P_{cal} dataset, derived from theoretical data is used to construct the model. The P_{val} dataset is a subset of experimental data used to assess model performance by sequential exclusion of Y-variable data. Robustness of calibrated and validated models is assessed through plotting of Predicted vs. Measured values of individual samples, and indicate goodness-of-fit of models. An approriate PLS1 model with good fit has similar number of multivariate variables derived from both calibrated and validated datasets, and high regression coefficient values (R^2) for calibration (indicated by red colour in graphs) and validation (indicated by blue colour in graphs) sample sets. Regression coefficient values close to 1 indicate complete model fit. In practice, R^2-value > 0.4 indicate good fit but large differences between calibration and validation R^2-values are undesirable, termed model over-fitting. Contribution by each independent X-variable to the dependent Y-variable is assessed also from its β-coefficient values, numerical and directional (i.e. positive or negative) in nature.

4.4.3.2 PLS-1: Sweetness (Y) from X-variables sugars, acids and volatiles contents

A two-factor (PCs) model (69.6 and 63.5%) was optimal for Y-sweetness with 71% (53 and 18%) variance explained by X-sugars, acids and volatiles content (Figure 4.1). Higher R^2-value was for calibrated (0.72) than validated (0.39) datasets. Samples are grouped into three clusters in the product space; two clusters of field and polytunnel crops and a third cluster of two polytunnel crop outliers; progeny #81 and #60. All independent X-variables correlated to sweetness on Factor 1, except citric and malic acid. From β-coefficient values (Figure 4.2), hexenol most positively correlated to sweetness, then fructose and glucose whilst linalool inversely correlated to sweetness (Figure 4.2). From bi-plot (Figure 4.3), polytunnel crops perceived sweet were due to hexenol, fructose, glucose and acetic acid contents. Volatile

compounds both positively and negatively affected sweetness scoring, but only when modelled with fructose and glucose contents.

Key points:

- Contents of sugars, acids and volatile compounds explained >70% variance in sweetness.
- Hexenol was a positive driver of sweetness while linalool was a negative contributor.
- Volatiles content had both enhanced and reduced effects on sweetness through its association with fructose and glucose.

4.4.3.3 PLS-1: Sweetness (Y) from X-sugars, acids and physicochemical variables

A one-factor model correlated Y-sweetness to X-sugars and acids contents, ^{o}Brix, 10-berry weight and colourmeter values (Figure 4.4). Factor 1 explained 63.1% of variance in sweetness and models had modest R^2-values for both calibrated (0.58) and validated (0.39) datasets. All variables, except malic, citric acid contents and brightness (L*) values positively affected sweetness scoring. X-variables ^{o}Brix and colour difference (ΔE) had most positive influence on sweetness. Sweetness perceptions in polytunnel crops correlated to sugars fructose and glucose, and also to other total soluble solids contents. Positive associations of sweetness with a*, b* and ΔE colourmeter values were also evident (Figure 4.6).

No satisfactory model could explain variance in sweetness to anthocyanin contents because models had low R^2-values with calibrated (0.26) and validated (0.04) datasets (Figure 4.7). These models were not investigated further.

Key points:

- 10-berry weight and colour traits (represented by colourmeter values) were linked to variance in sweetness.
- Fructose, glucose and other total soluble solids (represented by ^{o}Brix), explained a substantial proportion of sweetness variance.
- Brightness negatively correlated to sweetness.
- Anthocyanin contents did not correlate well to sweetness perceptions.

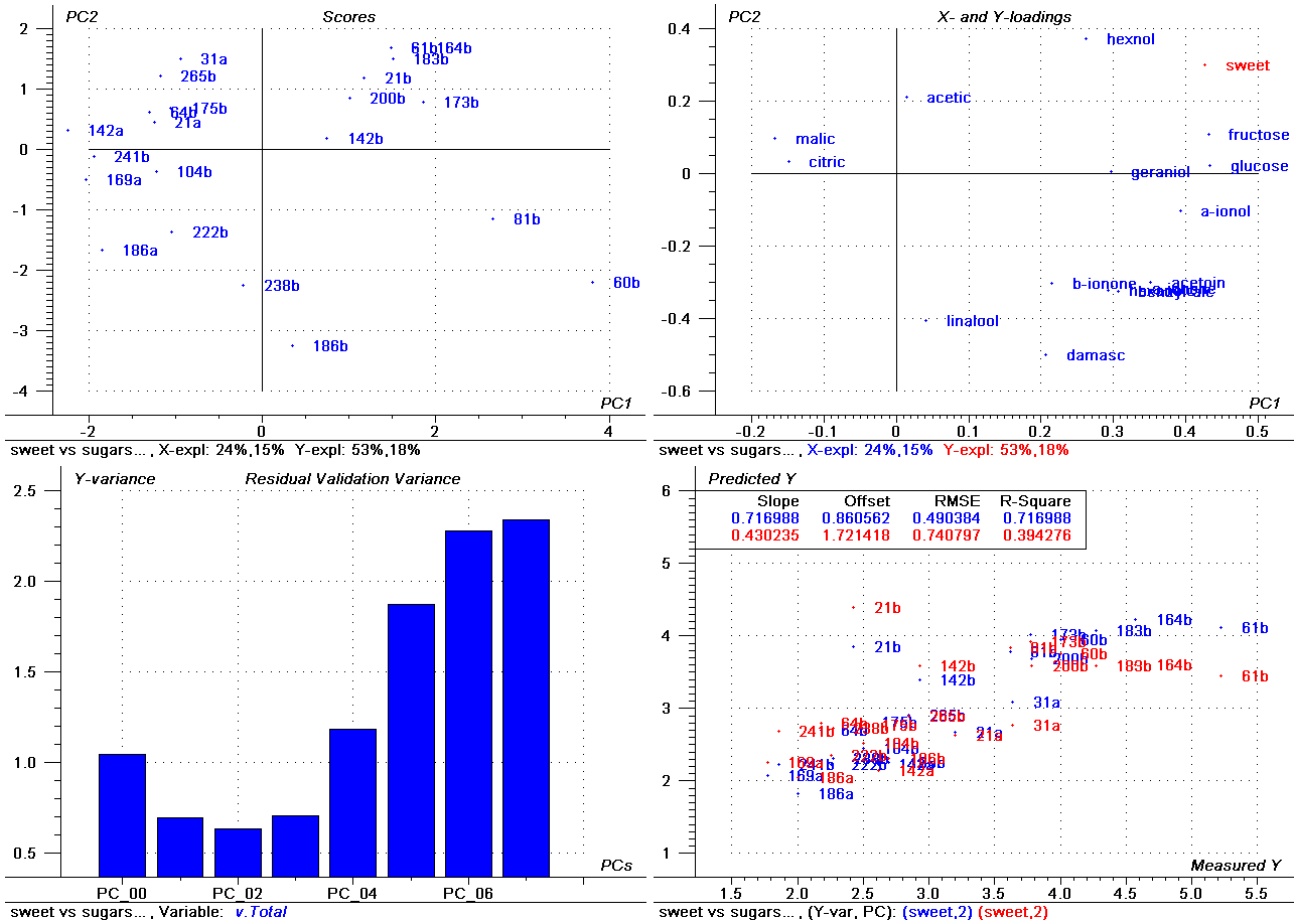


Figure 4.1 PLS-1 prediction of Y sweetness scores from X-variables *sugars, acids and volatiles contents* in fruit a = Field; b = Polytunnel

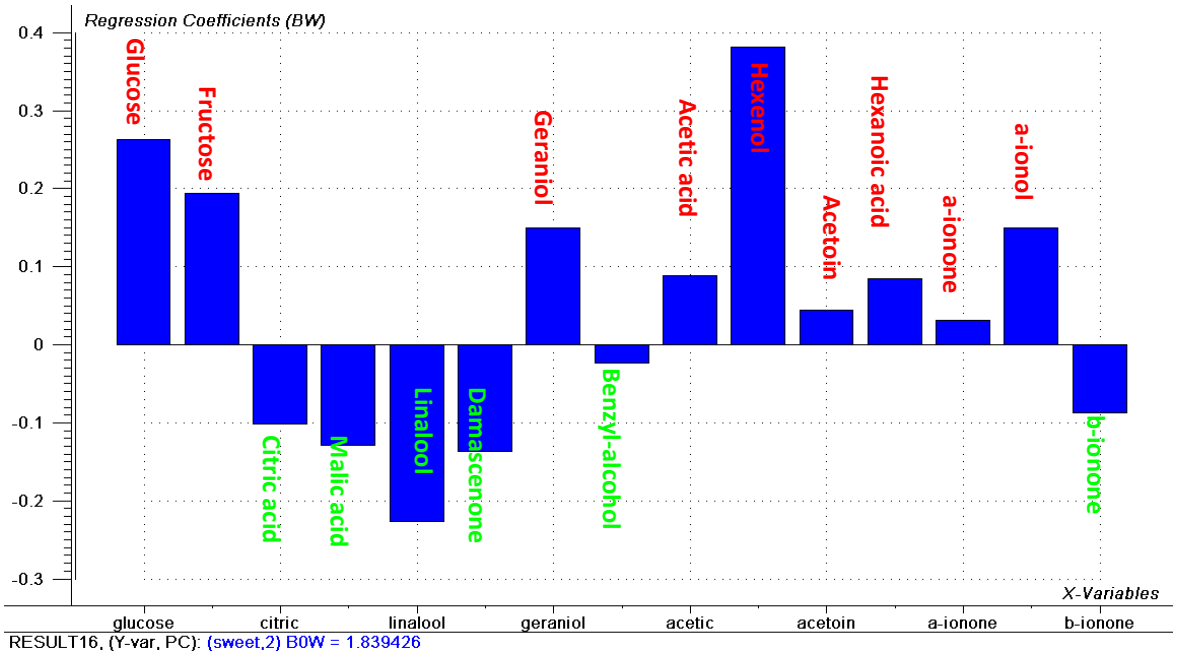


Figure 4.2 PLS-1 β-coefficients for individual X-variable *sugars, acids* and *volatiles* predicting Y sweetness scores. X- variables divided into positive (in red) and negative (in green) effects on sweetness.

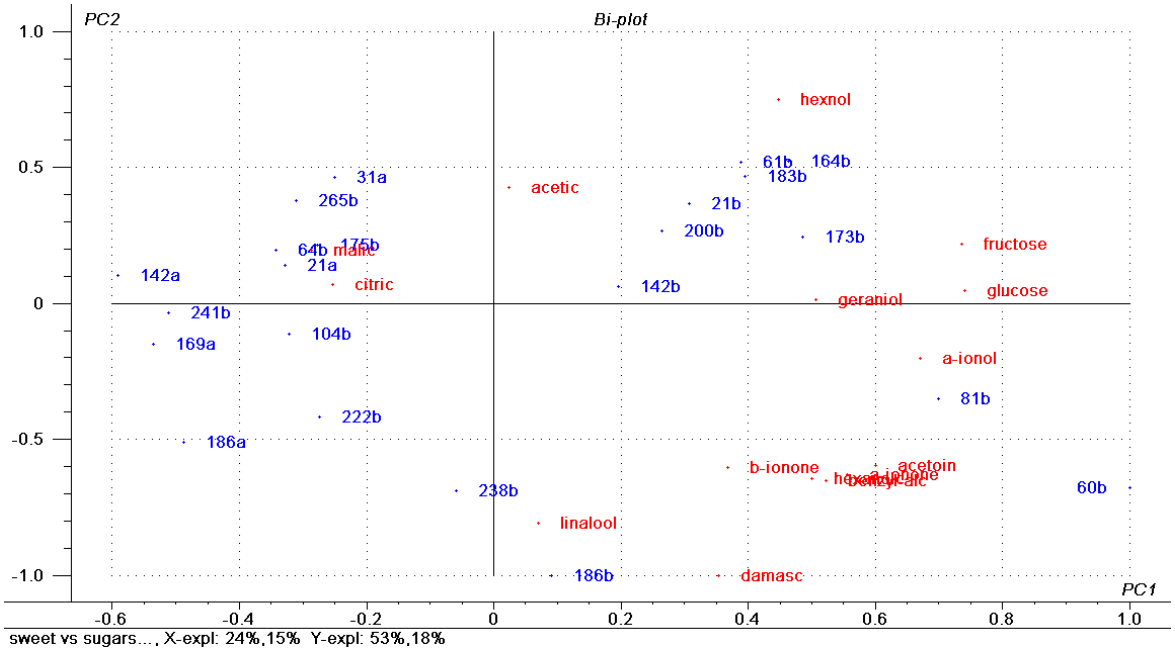


Figure 4.3 PLS-1 space correlating Y sweetness scores with X-variables *sugars, acids and volatiles contents* in crop a = Field; b= Polytunnel.

4.4.3.4 PLS-1: Sourness (Y) from X-variables sugars, acids and other variables

Sourness modelled to sugars, acids and volatiles content yielded unsatisfactory models; low R^2-values with calibrated (0.38) and validated (-0.26) datasets (Figure 4.8). Therefore these models were not explored further.

Sourness modelled to sugars, acids contents and physicochemical variables (^{o}Brix, 10-berry weight and colour readings) (Figure 4.9) produced a two-factor model (Figure 4.6) (60.1%, 16%) better fitted to calibration (0.76) than validation (0.23) datasets. In the multivariate product space, two sample clusters were grouped on Factor 1, to which all independent X-variables were contributors to its variance. On Factor 2, only 10-berry weight, ^{o}Brix and ΔE were positive contributors. Positive drivers of sourness were ^{o}Brix and ΔE (β-coefficient values = 0.382 and 0.383) followed by a* and 10-berry weight (Figure 4.11). Citric acid was the only acids content that was a positive contributor to sourness, although its β-coefficient value was low. Other variables with negative effects on sourness were L* colourmeter value, fructose and malic acid contents. From bi-plot, 2 groups of fruit perceived sour were evident: (a) associated with ^{o}Brix, 10-berry weight and ΔE values and (b) associated with individual sugars and acids contents and, L*, a* and b* colour values (Figure 4.11).

Although ^{o}Brix was positively correlated to sourness in PLS-1 models, it did not have significant linear correlations with sourness in Pearson's correlation analyses. Positive correlations identified in PLS-1 models were possibly multivariate in nature, therefore not apparent from linear correlation analyses. PLS-1 models only yield information on total correlations of all X-variables with most impact, but interactions between independent variables are not considered. However linear correlations help interpret multivariate associations, lending stronger evidence to links between variables.

Inclusion of anthocyanins into X-variables with sugars and acids contents yielded a model with two factors , which explained 59% and 4% sourness variance respectively. The model was better fitted to calibration (R^2_{cal} = 0.63) than validation

(R^2_{val} = 0.11) datasets. Due to low validation R^2-values, this model was not analysed in detail (Figure 4.12) but an influence by anthocyanin contents to sourness perceptions was noted.

Key points:

- Citric acid was the only positive driver of acids to sourness perception; malic acid was negatively correlated.
- Sugars fructose negatively correlated with sourness.
- 10-berry weight and total soluble solids (^{o}Brix) were positive drivers of sourness.
- L*, a* and b* colour values positively correlated to sourness scores, but brightness was most negatively associated.
- Influence by anthocyanins on sourness is possible, but this model was not adequately validated.
- Fruit volatiles content did not significantly affect sourness scores.

4.4.3.5 PLS-1: Flavour intensity (Y) from X-variables sugars, acids and volatiles content

Flavour intensity correlated to X-sugars, acids and volatiles content in a two-factor model, each factor explaining 77.8% and 71.1% of Y-flavour intensity variance. Overall these variables explained 80% (61% and 19%) of total flavour intensity variance (Figure 4.13). Models better fitted to calibration (0.80) than validation (0.34) datasets. Two sample clusters were present in the multivariate product space along Factor 1, with the first cluster consisting of field and polytunnel crops and in the second cluster, only polytunnel crop. All X-variables correlated to flavour intensity along Factor 1, except acids acetic, citric and malic and the volatile linalool. On Factor 2, sugars and acids glucose, fructose, acetic, malic and citric acids and the volatile hexenol significantly correlated to flavour intensity. Of total soluble solids contents, glucose and fructose were the most positive drivers of flavour intensity, with high β-coefficient values 0.37 and 0.28 respectively (Figure 4.14). Hexenol was the most positive driver amongst volatile contents and linalool being the most negative (β-coefficient values = 0.27, -0.25).

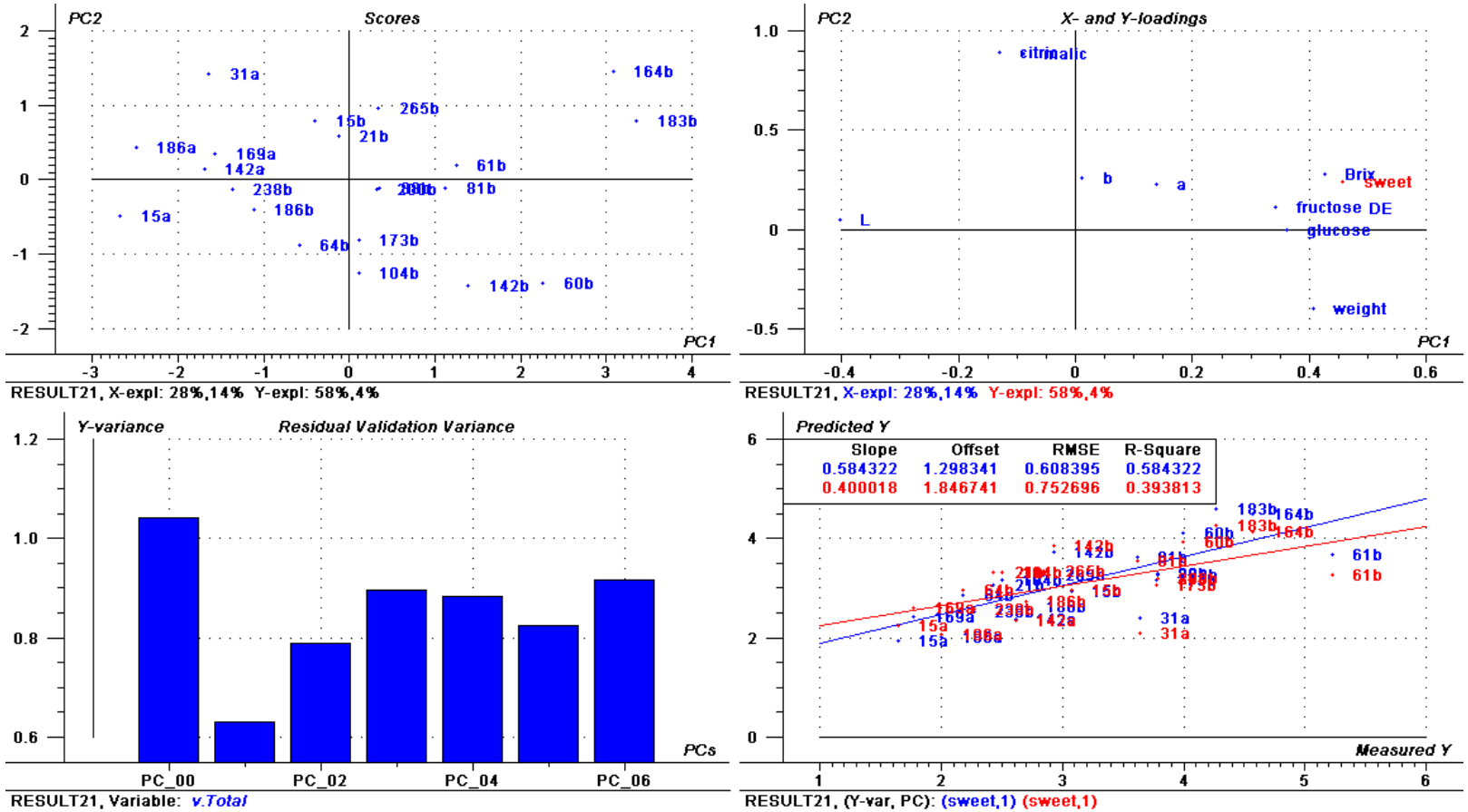


Figure 4.4 PLS-1 predictions of Y sweetness scores from X-variables *sugars, acids, °Brix, weight* and *colour* parameters.

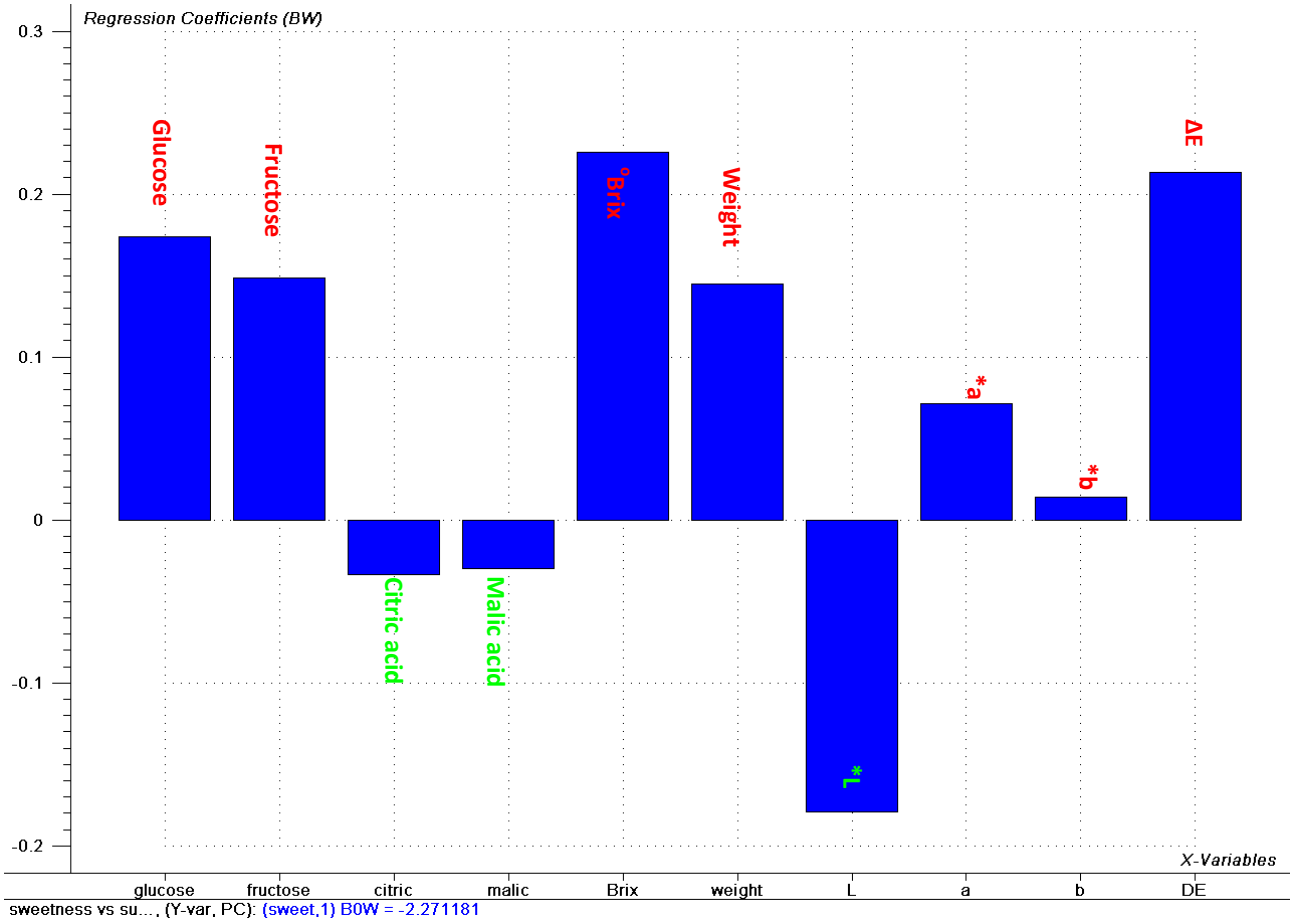


Figure 4.5 β-coefficients for PLS-1: Sweetness (Y) from X-variables *sugars, acids, °Brix, weight* and *colour* parameters.

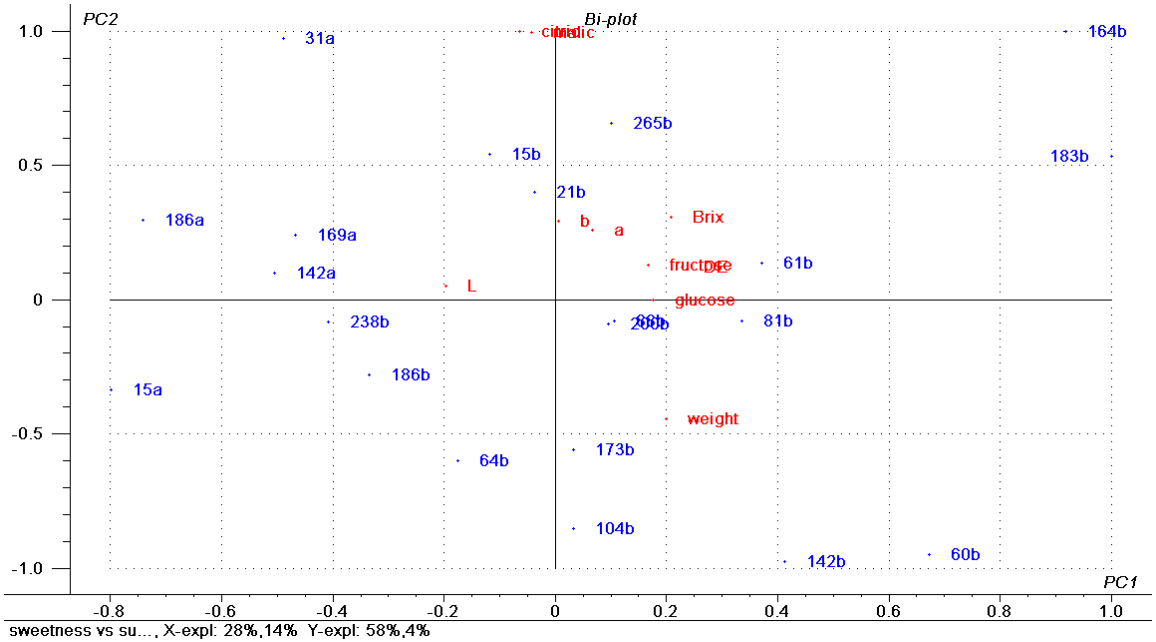


Figure 4.6 PLS-1 bi-plot correlating Y sweetness scores with X-variables *sugars, acids, ^{o}Brix, weight* and *colour* parameters

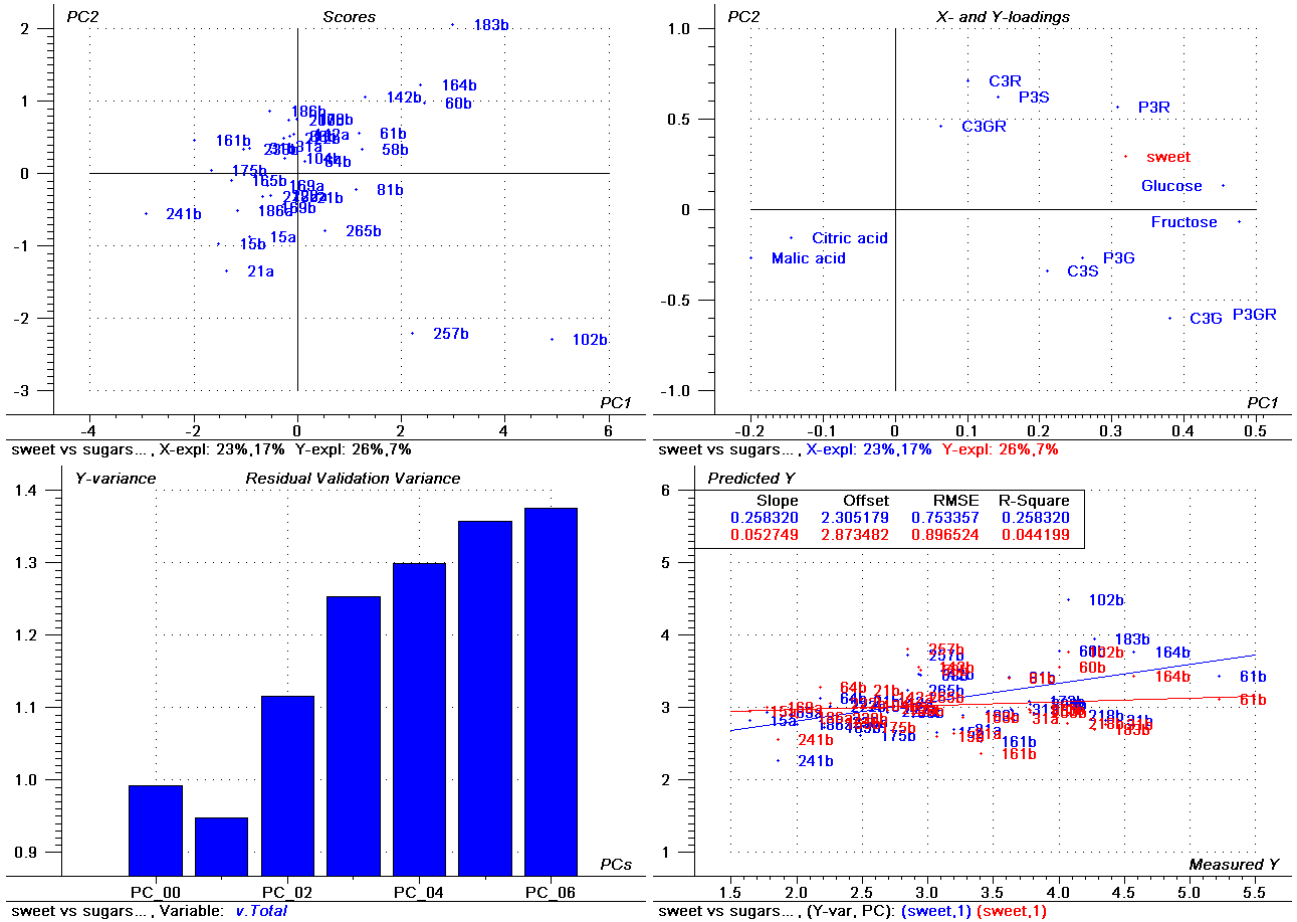


Figure 4.7 PLS-1 predictions of Y sweetness scores from X-variables *anthocyanins* parameters.

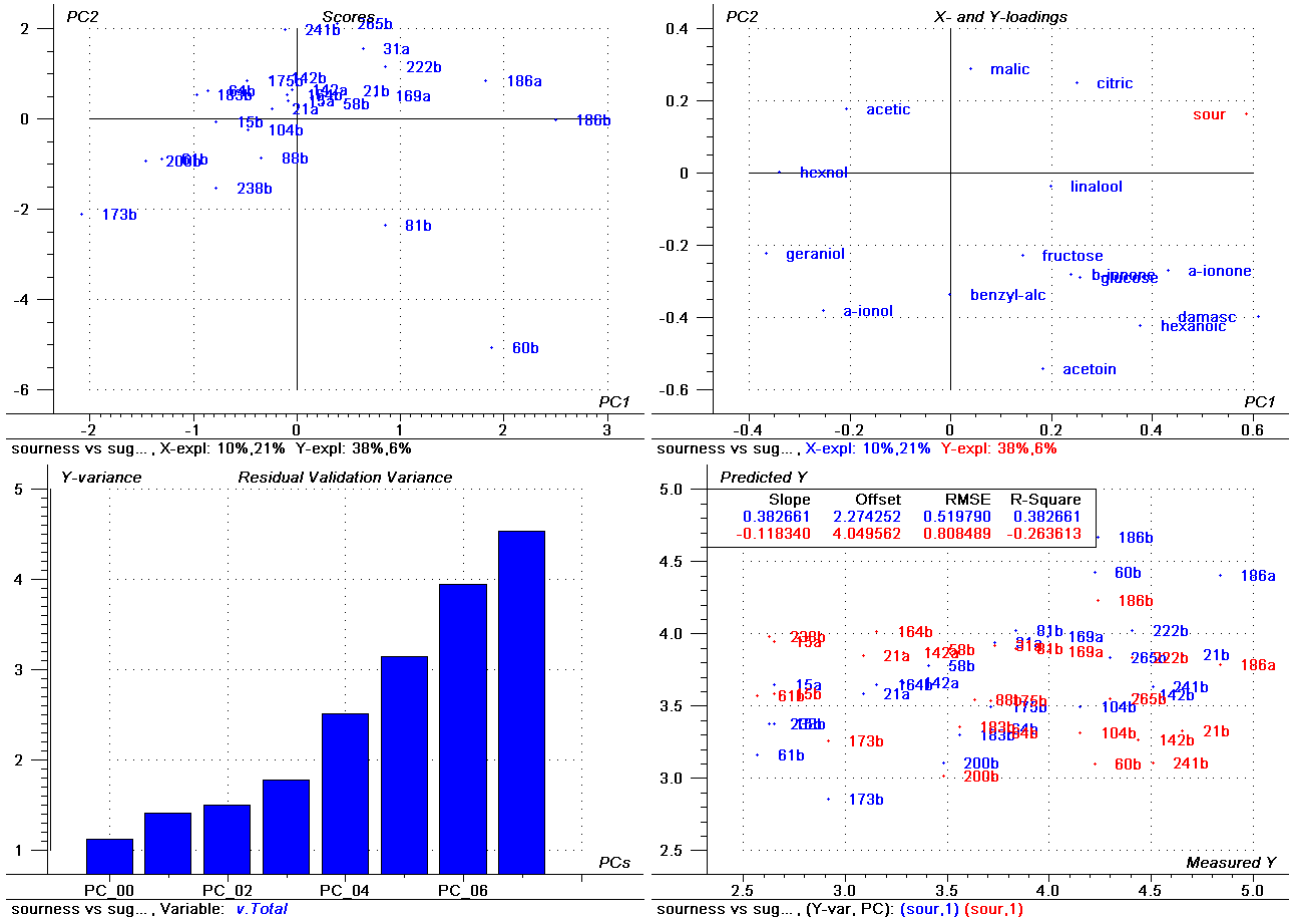


Figure 4.8 PLS-1 predictions of Y sourness scores from X-variables *sugars, acids* and *volatiles* parameters.

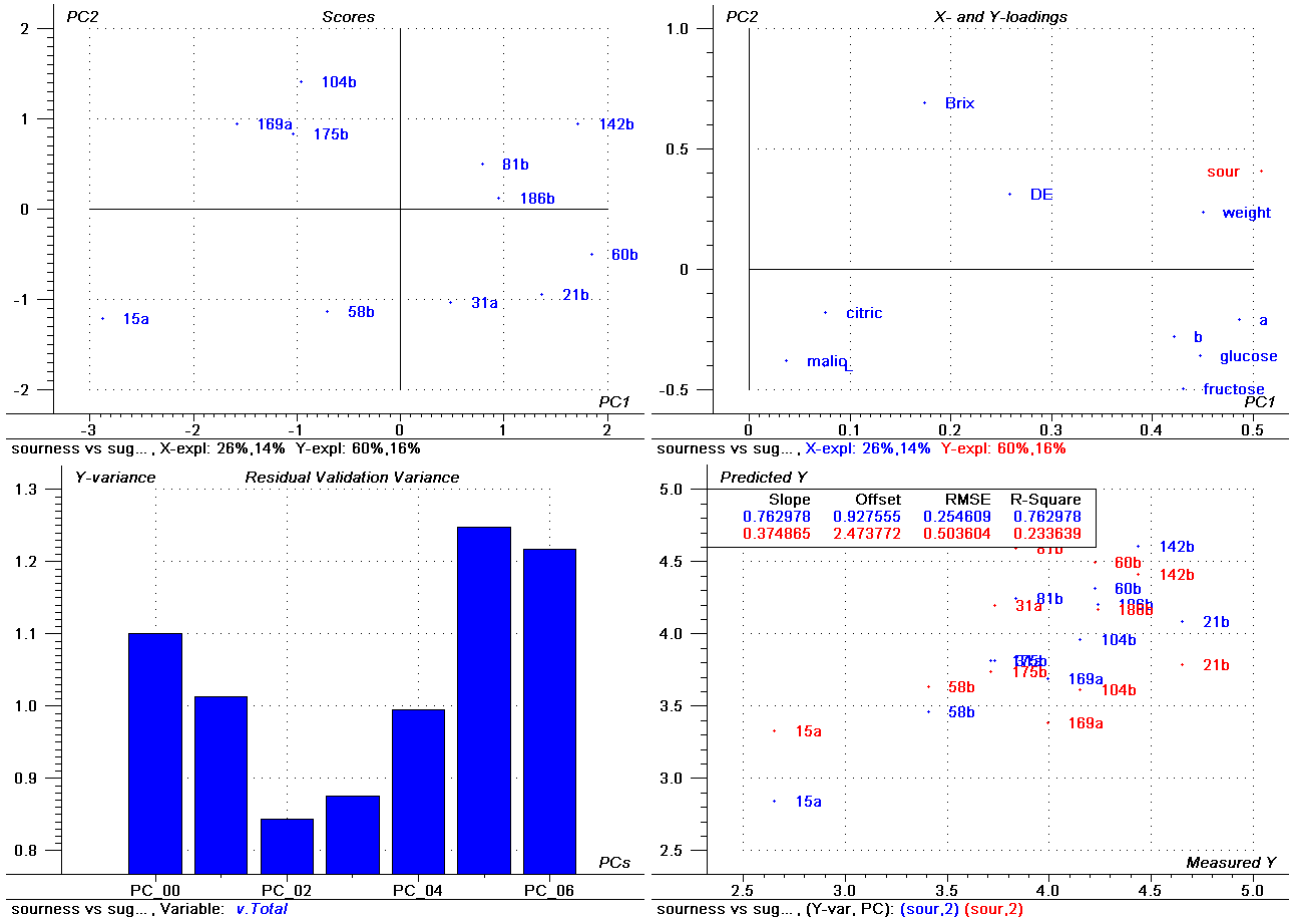


Figure 4.9 PLS-1 predictions of Y sourness scores from X-variables *sugars, acids, °Brix, weight* and *colour* parameters.

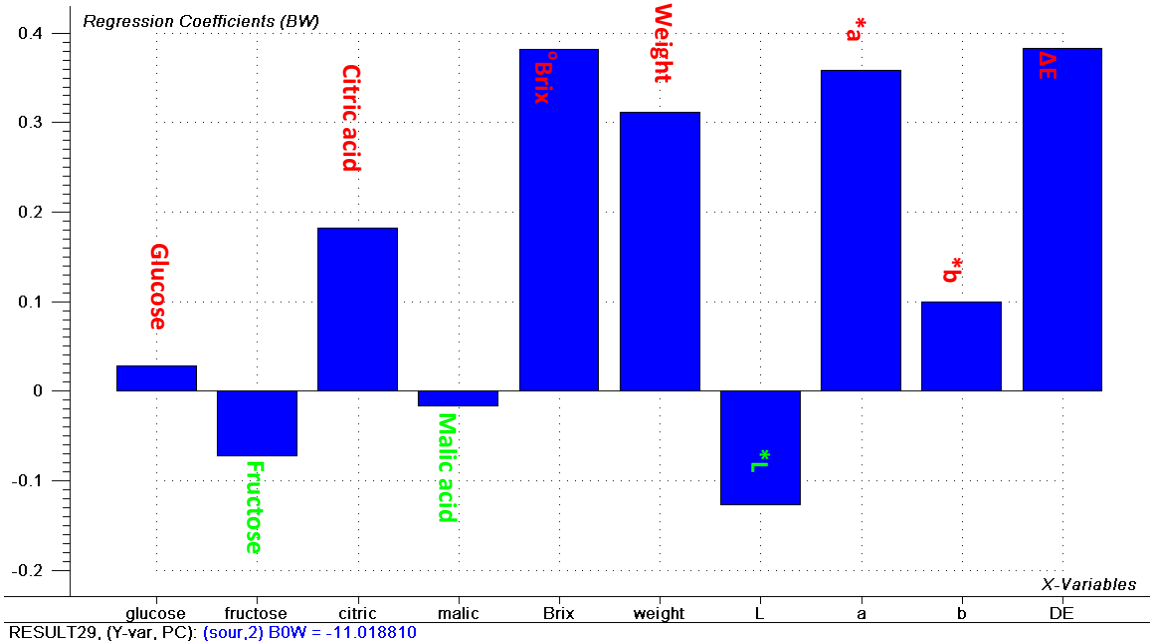


Figure 4.10 β-coefficients for PLS-1: Sourness (Y) from X-variables *sugars, acids, °Brix, weight* and *colour* parameters.

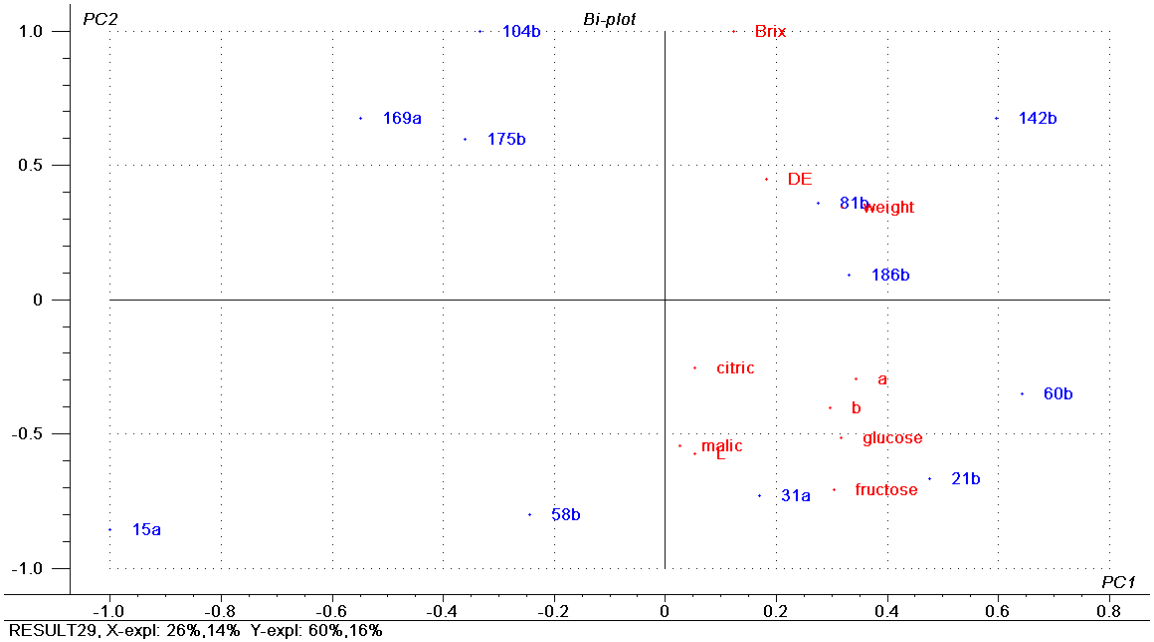


Figure 4.11 PLS-1 bi-plot correlating Y sourness with X-variables: *sugars, acids, °Brix, weight* and *colour* parameters.

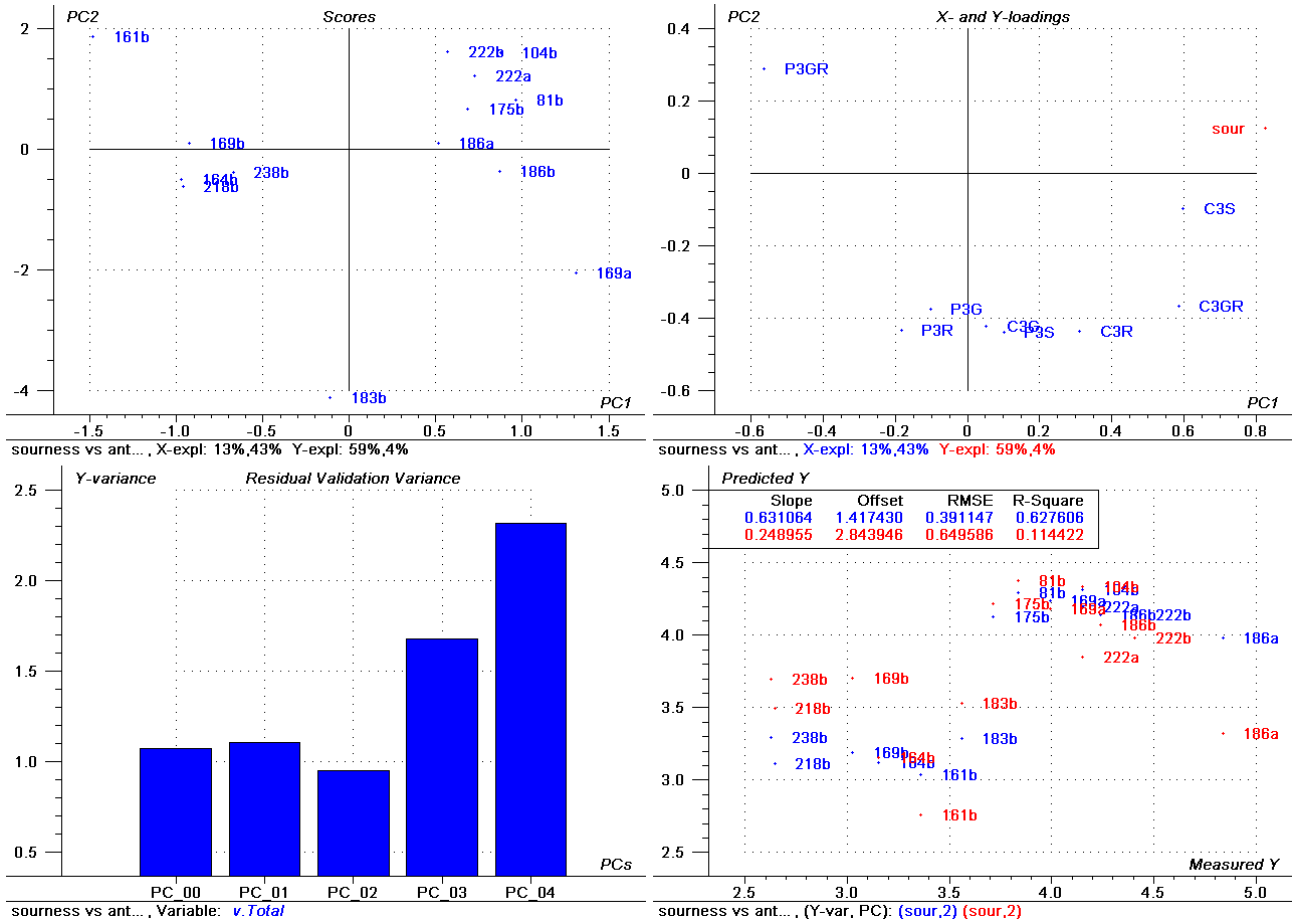


Figure 4.12 PLS-1 prediction of Y sourness scores from X-variables *anthocyanins* parameters.

Ionones (α- and β-ionones) had contrasting effects on flavour intensity scoring; α-ionone positively affected flavour intensity but β-ionone was negatively correlated. Fom β-coefficient values α-ionone also had stronger effects compared to β-ionone. Malic and citric acids negatively affected flavour intensity scoring, with stronger effect by malic acid (β-coefficient values = -0.15 vs. -0.07). From bi-plot, one crop was clustered based on its associations to positive favour intensity drivers; polytunnel crop perceived high in flavour intensity positively correlated to sugars and hexenol contents (Figure 4.15). Crops perceived low in flavour intensity also had high citric and malic acids contents and in linalool.

Key points:

- In total soluble solids, sugars had positive contributions to flavour intensity perceptions but acids content were negative drivers.
- In volatiles content, hexenol enhanced effects of sugars to flavour intensity perceptions, but linalool along with acids content, negatively influenced flavour intensity perceptions.

4.4.3.6 PLS-1: Flavour intensity (Y) from X-variables sugars, acids and physicochemical data

Modelling flavour intensity to X-sugars and acids, 10-berry weight, ^{o}Brix, and colourmeter values produced a two-factor model (71.2%, 71.8%) (Figure 4.16) which better fitted to calibration (R^2_{cal} = 0.55) than validation (R^2_{val} = 0.34) datasets. X-variables explained 66% (55%, 11%) of total flavour intensity variance. On Factor 1 two sample clusters were grouped in the multivariate product space. The first cluster contained field and polytunnel crops and the second cluster had only polytunnel crop. Most positive contributions were by ^{o}Brix and 10-berry weight (Figure 4.17), then sugars content, which is more strongly than acids contents. From bi-plots (Figure 4.18), polytunnel crop high in flavour intensity scores correlated to X-variables in two subcategories; first subcategory based on acids content and ^{o}Brix and second subcategory based on 10-berry weight, glucose, fructose contents and colourmeter values, except *L values (Table 4.18).

Key points:

- Total soluble solids (represented by ^{o}Brix measurements) and 10-berry weight were primary physicochemical variables with positive effects on flavour intensity.
- In total soluble solids, sugars and acids positively contributed to high flavour intensity scores in polytunnel crops.
- Brightness, L*, negatively affected flavour intensity perceptions.

Table 4.18 Subcategories of physicochemical variables with positive contributions to flavour intensity scores in polytunnel crops and individual progeny that met these criteria.

Subcategory	Progeny #
(i)High flavour intensity scores with: High malic, citric acid content High ^{o}Brix values	61, 81, 88, 200, 265
(ii) High flavour intensity scores with: High glucose and fructose content High *a, *b, ΔE values	21, 60, 142, 164

4.4.3.7 PLS-1: Flavour intensity (Y) from X-variables sugars, acids and anthocyanins content

From previous models, colourmeter values appeared to positively contribute to flavour intensity perceptions. However, models correlating flavour intensity variance with X-anthocyanin contents were modest, both in its predictive capacity, with low R^2_{cal} and R^2_{val}-values (0.55 and 0.14 respectively) and in total flavour intensity variance explained (50%) (Figure 4.19). However as this is half of its total variance (100%), its influence on flavour intensity was decided worthy of further study. Inclusion of anthocyanins into X-variables yielded a one-factor (96.0%) model where on Factor 1 samples were grouped based on cultivation sites. Specific anthocyanins namely cyanidin glycosides: 3-sophoroside (C3S) and 3-rutinoside (C3R) had positive contributions to flavour intensity perceptions (β-coefficients

values = 0.33, 0.29 respectively) (Figure 4.20). Other cyanidins, 3-glucoside (C3G) and -3-glucosylrutinoside (C3GR), had negative effects on flavour intensity perceptions (β-coefficient values = -0.40, -0.13 respectively). Total soluble solids were still the most influential factor of flavour intensity namely glucose and citric acid contents (β-coefficient values = 0.57, 0.43) whilst malic acid had negative effects (β-coefficient value = -0.30). From bi-plot (Figure 4.21), field and polytunnel crops perceived high in flavour intensity were high in fructose, glucose, cyanidin and pelargonidin anthocyanin contents (Table 4.19).

Key points:

- Anthocyanin contents had modest contributions to flavour intensity perceptions.
- Flavour intensity perceptions were positively affected by sugars fructose and glucose and specific cyanidin or pelargonidin anthocyanins.

Table 4.19 Subcategories of anthocyanin content variables with positive contributions to flavour intensity scores in polytunnel crops and individual progeny that met these criteria.

Subcategory	Progeny #
(i)High flavour intensity scores with: High glucose content High cyanidin-3-glucosylrutinoside (C3GR), -3-rutinoside (C3R) content High pelargonidin-3-sophoroside (P3S) content	21, 60, 81, 104, 164, 183, 222
(ii) High flavour intensity scores with: High fructose content High cyanidin-3-glucoside (C3G) content High pelargonidin-3-rutinoside (P3R), -3-glucoside (P3G), -3- glucosylrutinoside (P3GR) content	58, 61, 102, 142, 257

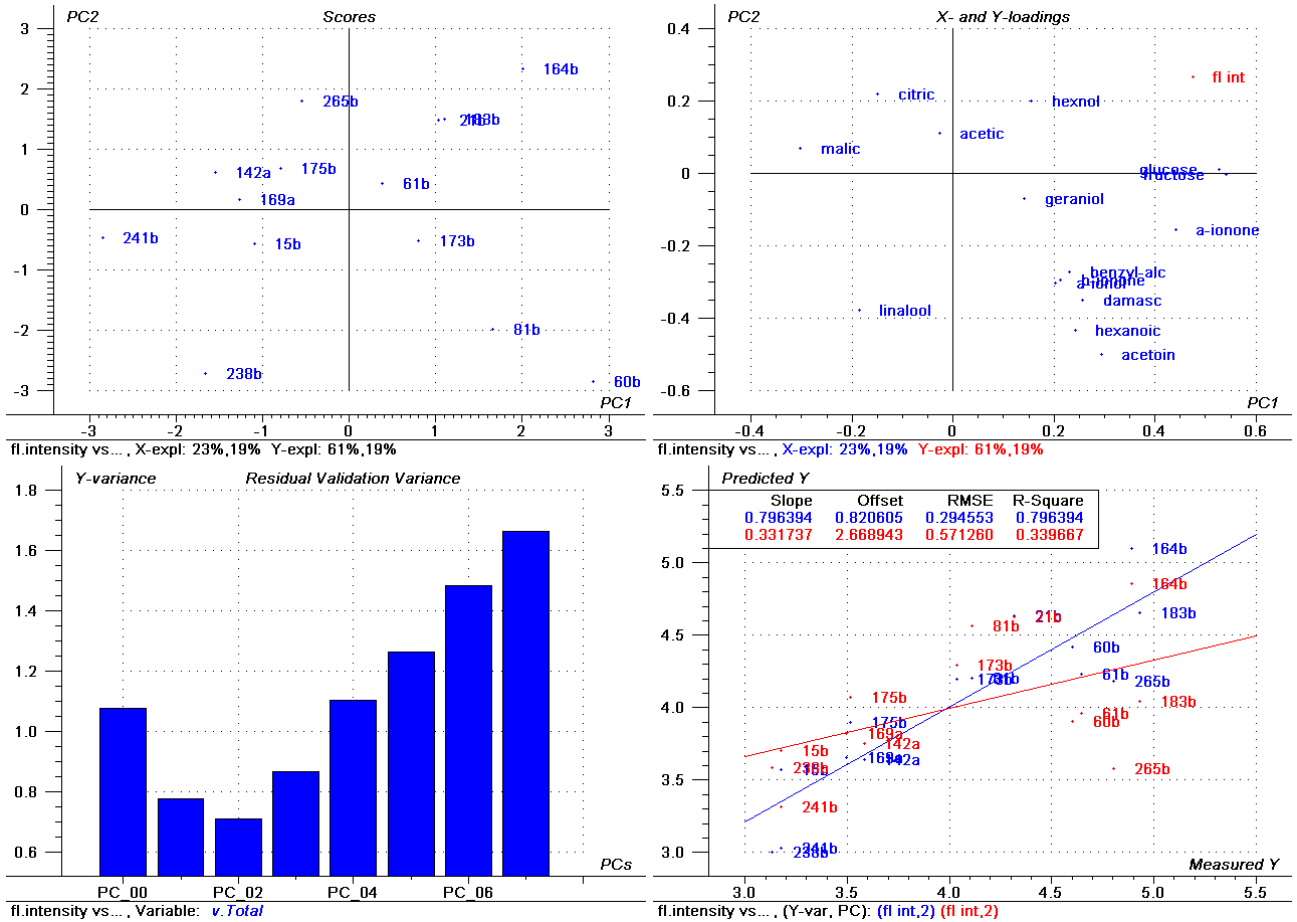


Figure 4.13 PLS-1 prediction of Y flavour intensity scores from X-variables *sugars, acids* and *volatiles contents* in fruit a= field; b= Polytunnel.

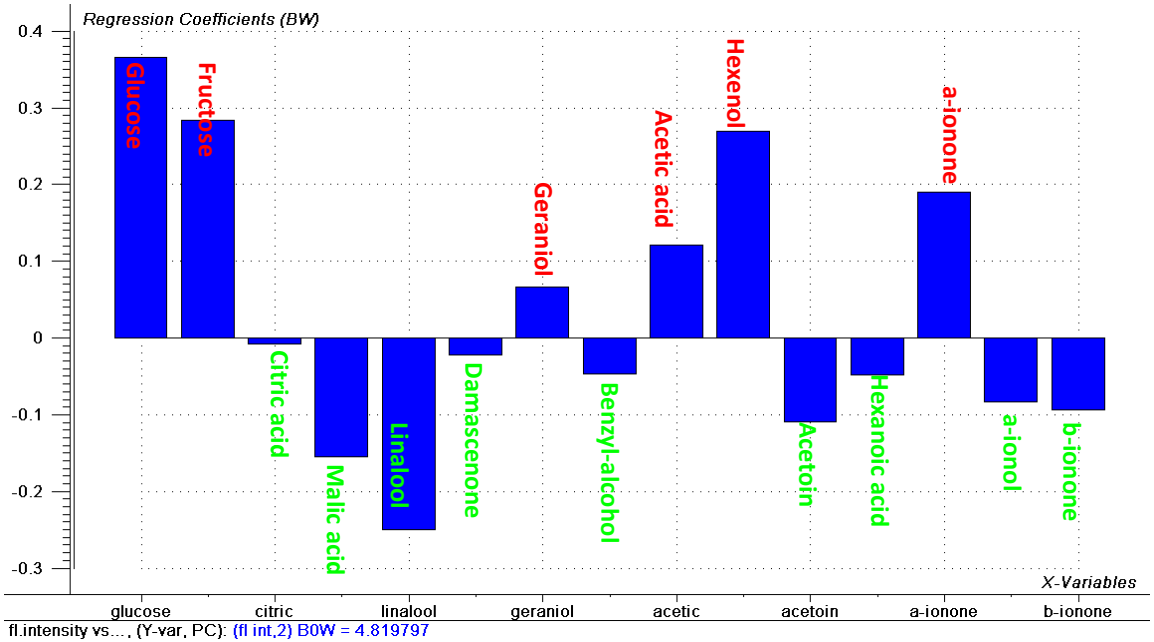


Figure 4.14 β-coefficients for PLS-1*:* Flavour intensity (Y) from X-variables *sugars, acids* and *volatiles* contents.

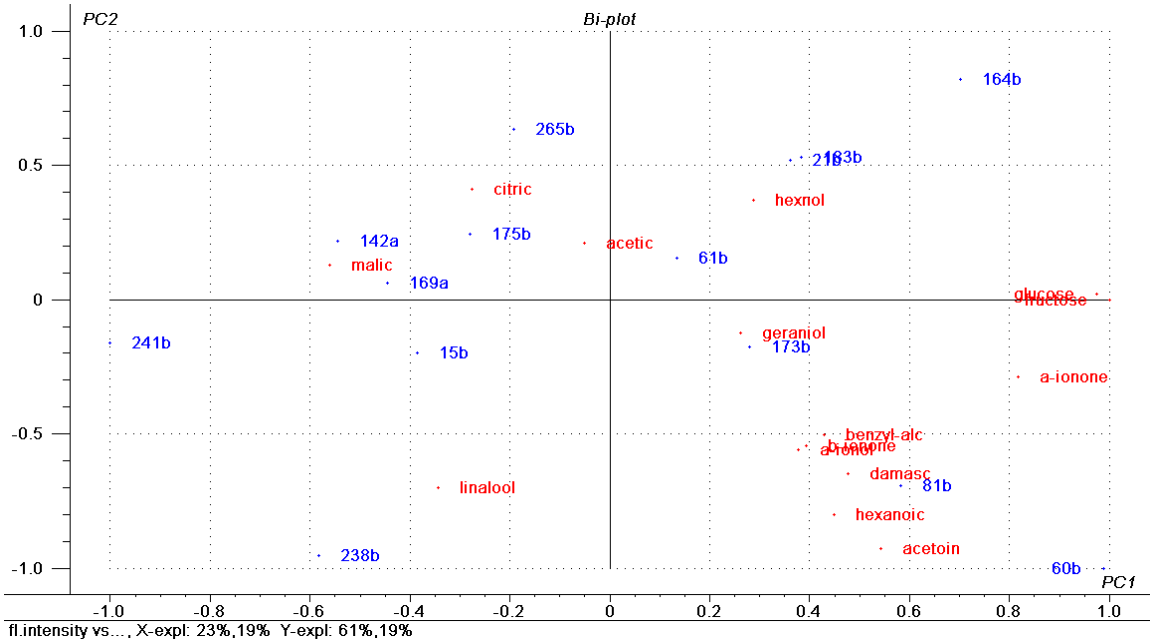


Figure 4.15 PLS-1 bi-plot correlating Y flavour intensity with X-variables *sugars, acids* and *volatiles* contents.

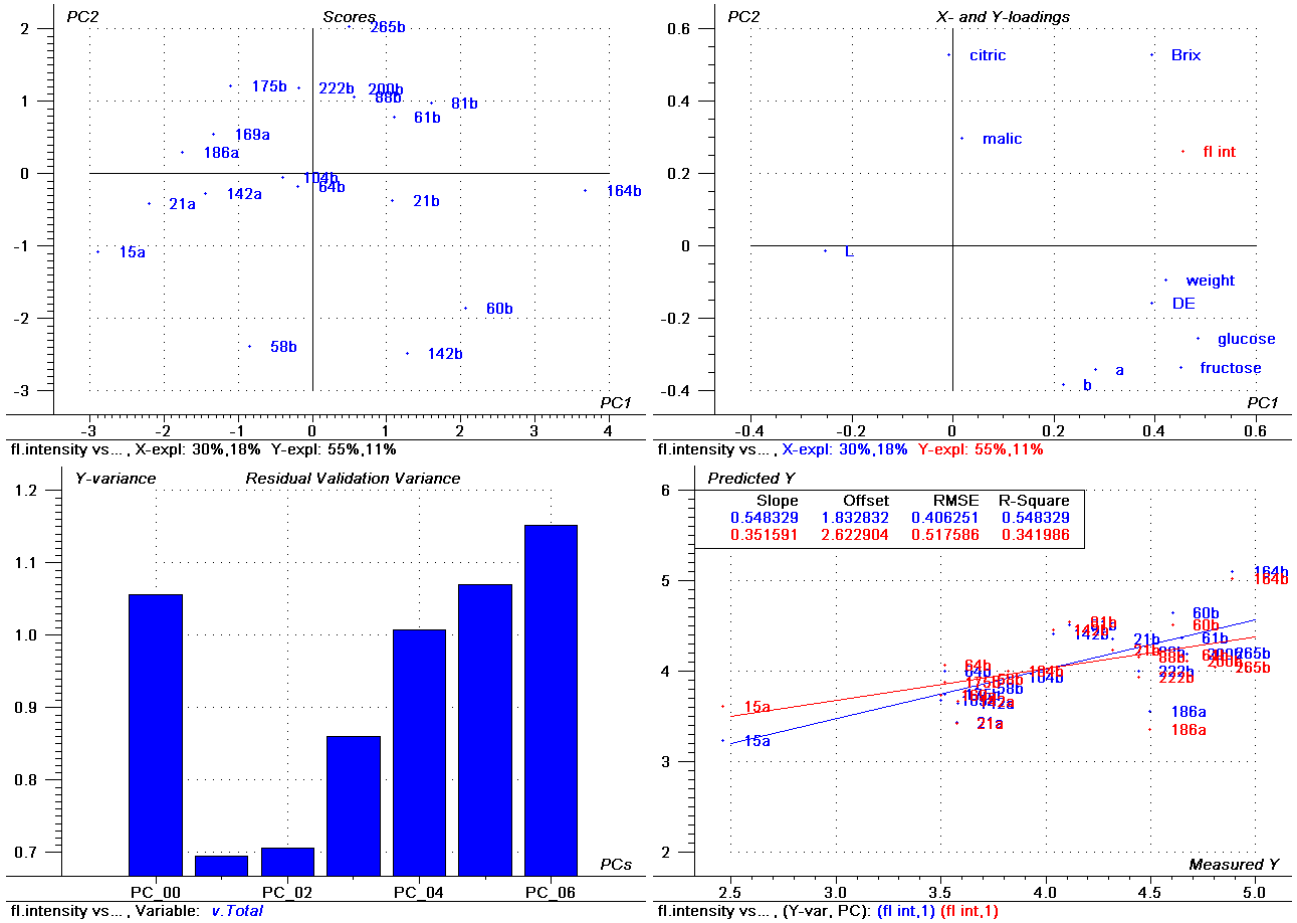


Figure 4.16 PLS-1 predictions of Y flavour intensity scores from X-variables *sugars, acids, °Brix, weight* and *colour* parameters.

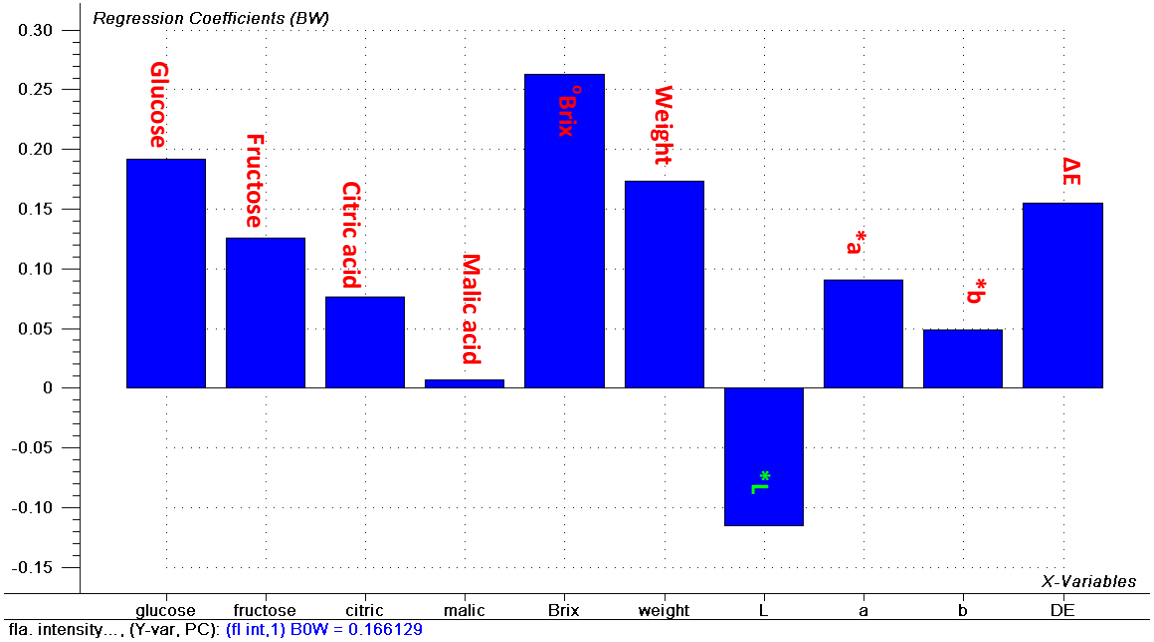


Figure 4.17 β-coefficients for PLS-1*:* Flavour intensity (Y) from X-variables *sugars, acids, °Brix, weight* and *colour* parameters.

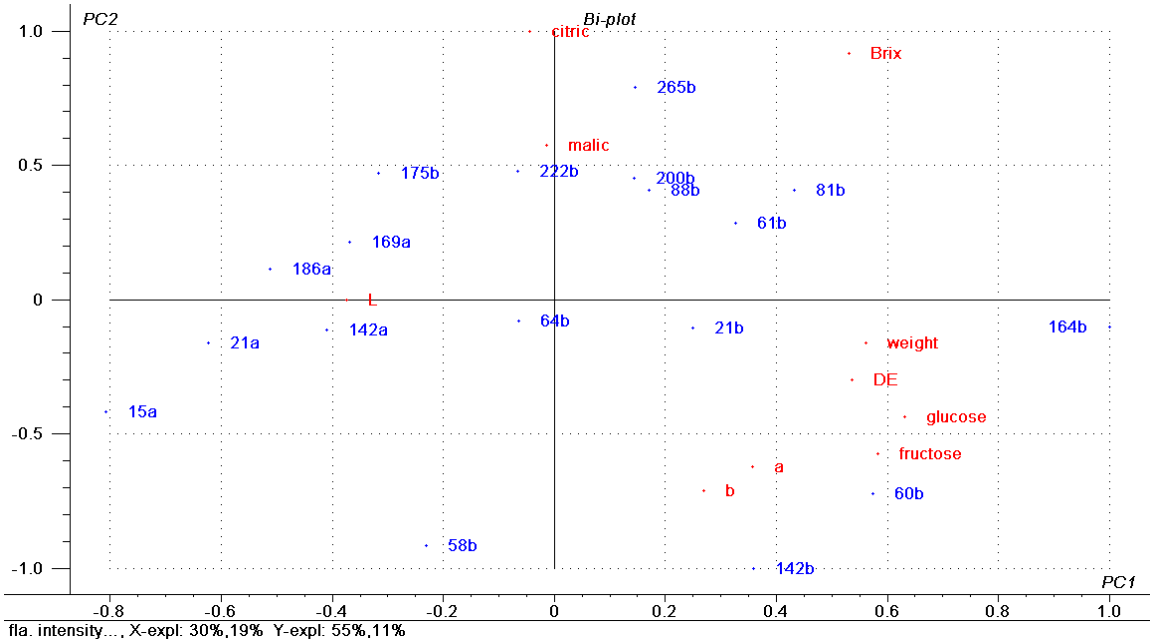


Figure 4.18 PLS-1 bi-plot correlating Y flavour intensity with X-variables *sugars, acids, ^{o}Brix, weight* and *colour* parameters.

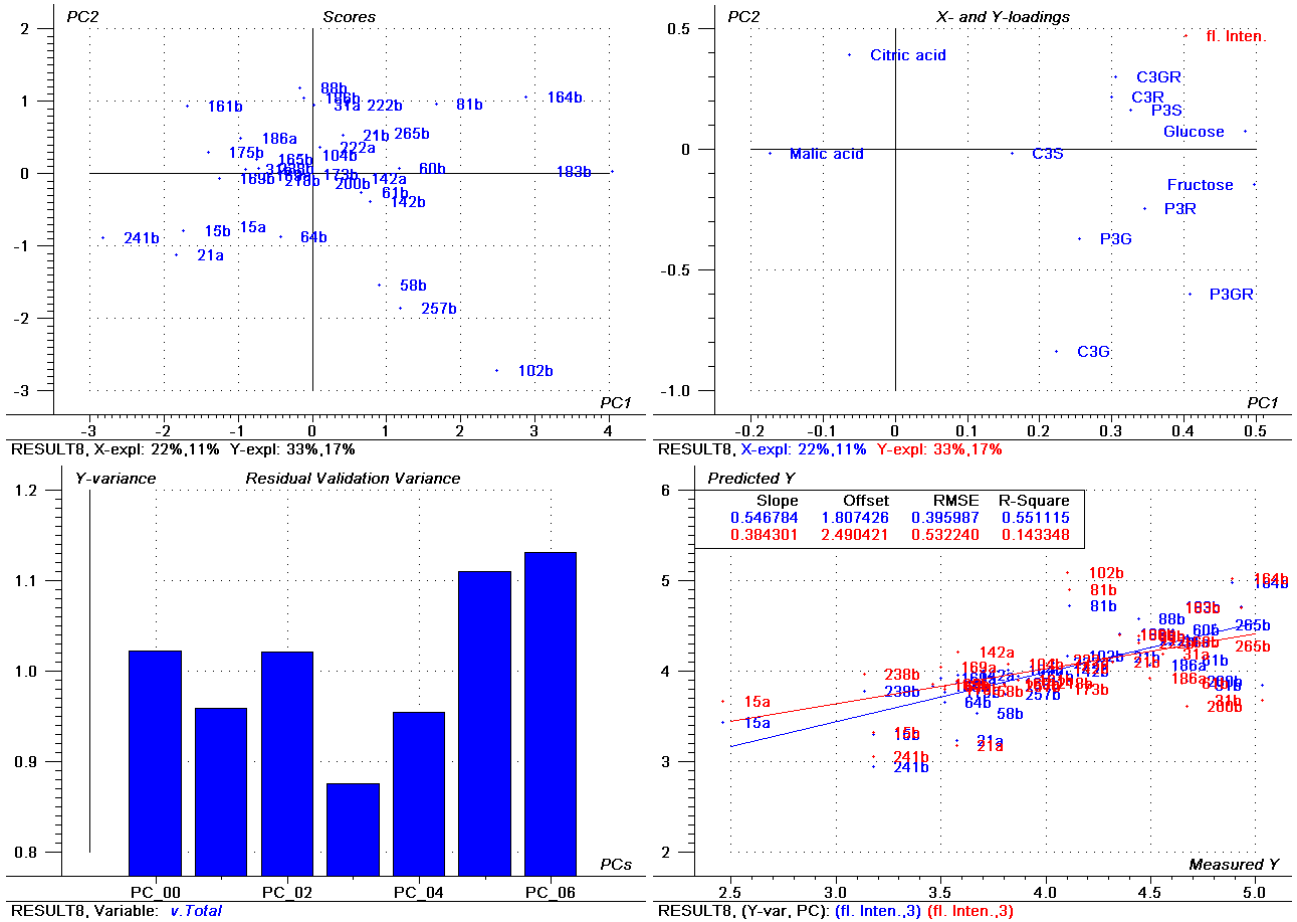


Figure 4.19 PLS-1 predictions of Y flavour intensity scores from X-variable*s sugars, acids* and *anthocyanin* parameters.

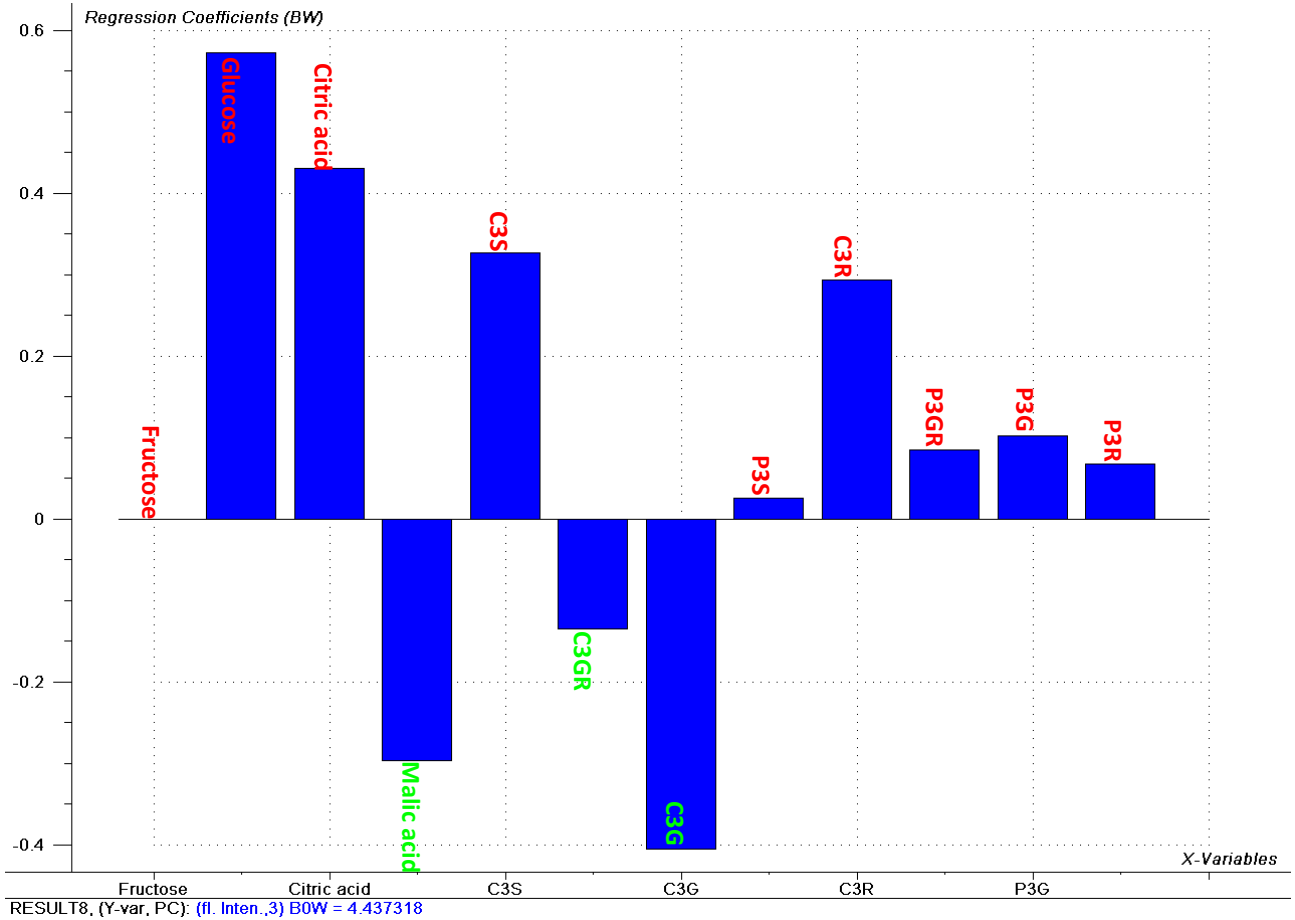


Figure 4.20 β-coefficients for PLS-1*:* Flavour intensity (Y) from X-variables *sugars, acids* and *anthocyanin* parameters.

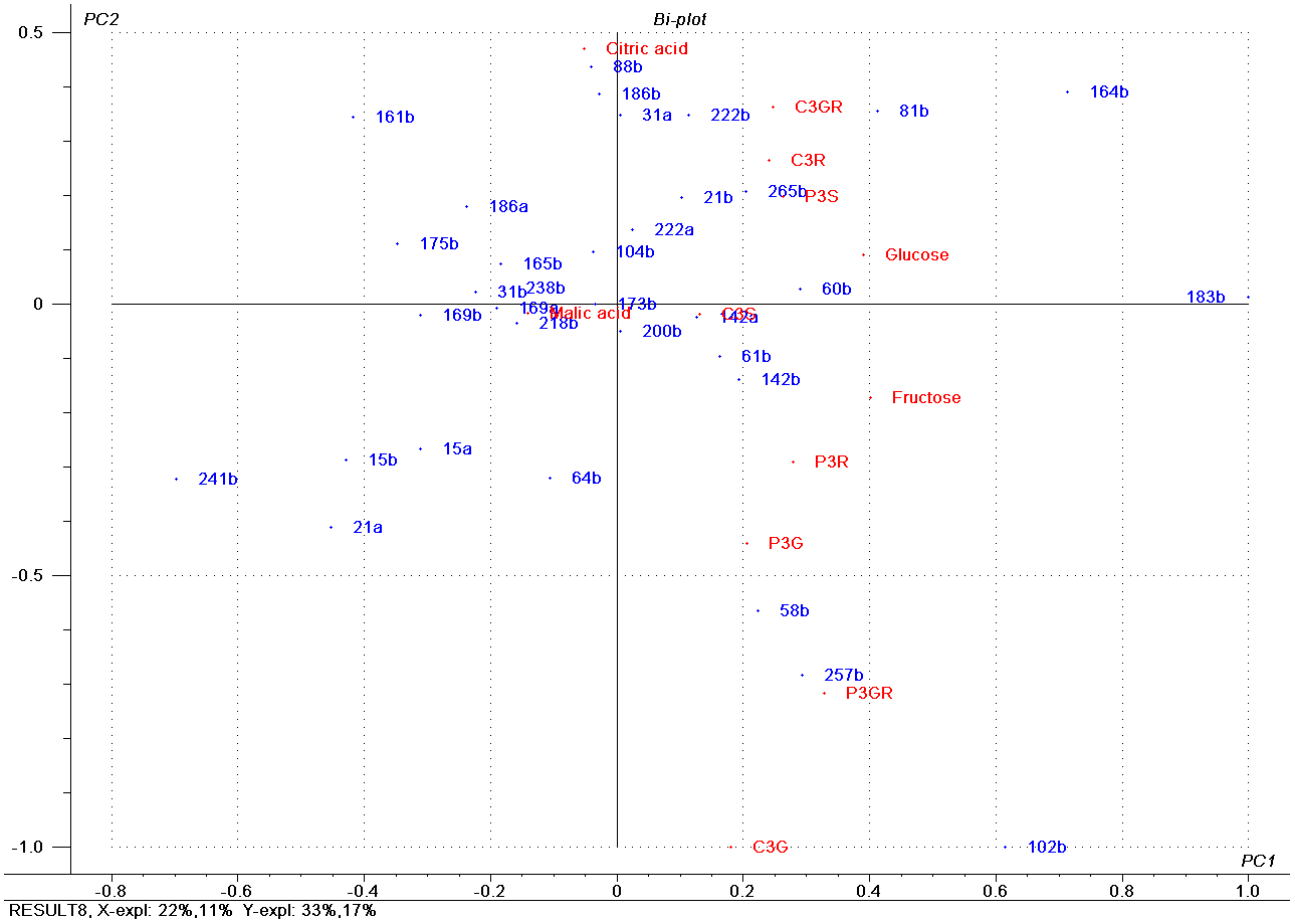


Figure 4.21 PLS-1 bi-plot correlating Y flavour intensity with X-variables *sugars, acids* and *anthocyanin* parameters.

4.4.4 PLS-1 Model: Sweetness (Y) from X sugars and acids contents and ratios

It was apparent from models that effects of sugars and acids content to intensity of flavour traits were compounded with effects of other factors, such as volatiles content and physicochemical variables. Total sugars and acids contents and its ratio in fruits could also affect flavour quality, which in turn affects intensity of flavour traits.

Modelling sweetness with sugars contents (individual, total contents and its ratios) as independent X-variables yielded a one-factor model with low R^2_{cal} and R^2_{val}-values, 0.23 and 0.16 respectively (Figure 4.22). As these R^2-values < 0.4, models were deemed not well-fitted. Furthermore X-sugars contents accounted for only 26% (23% + 3%) of overall sweetness variance. This reinforced findings from previous models where sugars and volatiles contents significantly affected flavour traits, with higher total variance explained (43%) in sweetness than in models presented here. Sugar and acid variables that affected sweetness perceptions the most were sugars glucose, fructose and its total contents, total sugars : total acids ratio, with minor contributions by glucose : fructose and citric acid : malic acid ratios (Figure 4.23). From bi-plot, progeny #58, #142 and #173 of polytunnel crops were grouped together with its sweetness perceptions affected by total sugars : total acids ratios (Figure 4.24).

Key points:

- Sugars and acids content was responsible for only 26% total variance in sweetness, with greatest influences by individual and total sugars contents.
- However models fitted poorly with low R^2-values for calibration and validation datasets.

4.4.5 PLS- 1 Model: Sourness (Y) from X sugars and acids contents and ratios

Models that correlated Y-sourness to X-sugars and acids contents were not well fitted (Figure 4.25) with low and negative R^2-values. Therefore these models were not explored further. This finding was in agreement with previous findings of lack of linear correlations and multivariate regressions of sourness to any X-variables.

4.4.6 PLS- 1 Model: Flavour intensity (Y) from X sugars and acids contents and ratios

A three-factor model (80.5%, 76.9%, 76.2%) correlated Y-flavour intensity to sugars and acids contents (total, individual sugars and acids content and its ratios) and explained 42% of its total trait variance (32%, 10%). The model was of good fit, with high R^2-values for both calibration (R^2_{cal}= 0.42) and validation (R^2_{val}= 0.32) datasets (Figure 4.26). All X-variables correlated to flavour intensity on Factor 1 with exception of individual, total acids contents and hexose ratio values. Individual and total acids contents were positively associated to flavour intensity on Factor 2 with a minor contribution by citric : malic acid ratio, deduced from its low β-coefficient value ($8.309e^{-02}$) (Figure 4.27). Individual and total sugar contents were positive drivers of flavour intensity but hexose ratio had most negative impact on flavour intensity variance (β-coefficient = -0.16), followed by malic acid (β-coefficient = -0.12) and total sugars: total acids ratio (β-coefficient = $-4.862e^{-02}$). From bi-plot, (Figure 4.28) polytunnel crop with high flavour intensity scores were grouped based on its sugars or acids contents: (i) high in individual and total sugars contents and (ii) high in citric: malic acid ratio. Grouping of polytunnel crop progeny #58, #142 and #173 based on total sugars : total acids ratio was also evident here, as was in sweetness model. However, this must correspond to lower flavour intensity scores, as total sugars : total acids ratio had negative effects on flavour intensity perceptions.

Key points:

- Individual and total sugars content had positive influences on flavour intensity perceptions but hexose ratios negatively contributed.
- Acids content mainly negatively influenced flavour intensity except citric acid : malic acid ratio.

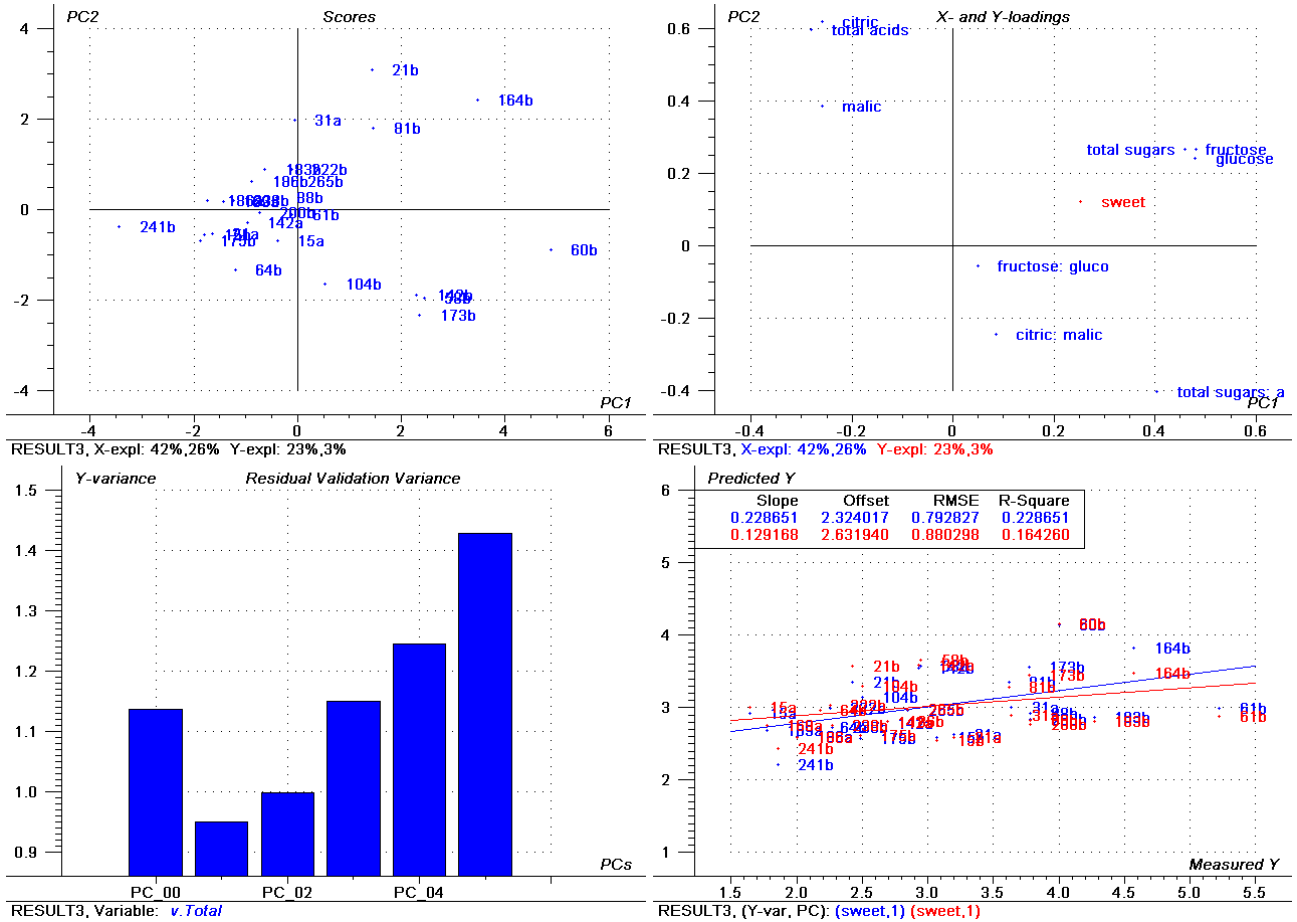


Figure 4.22 PLS1 model for prediction of Y sweetness from X-variables sugars and acids content: *individual, total* and *ratios*.

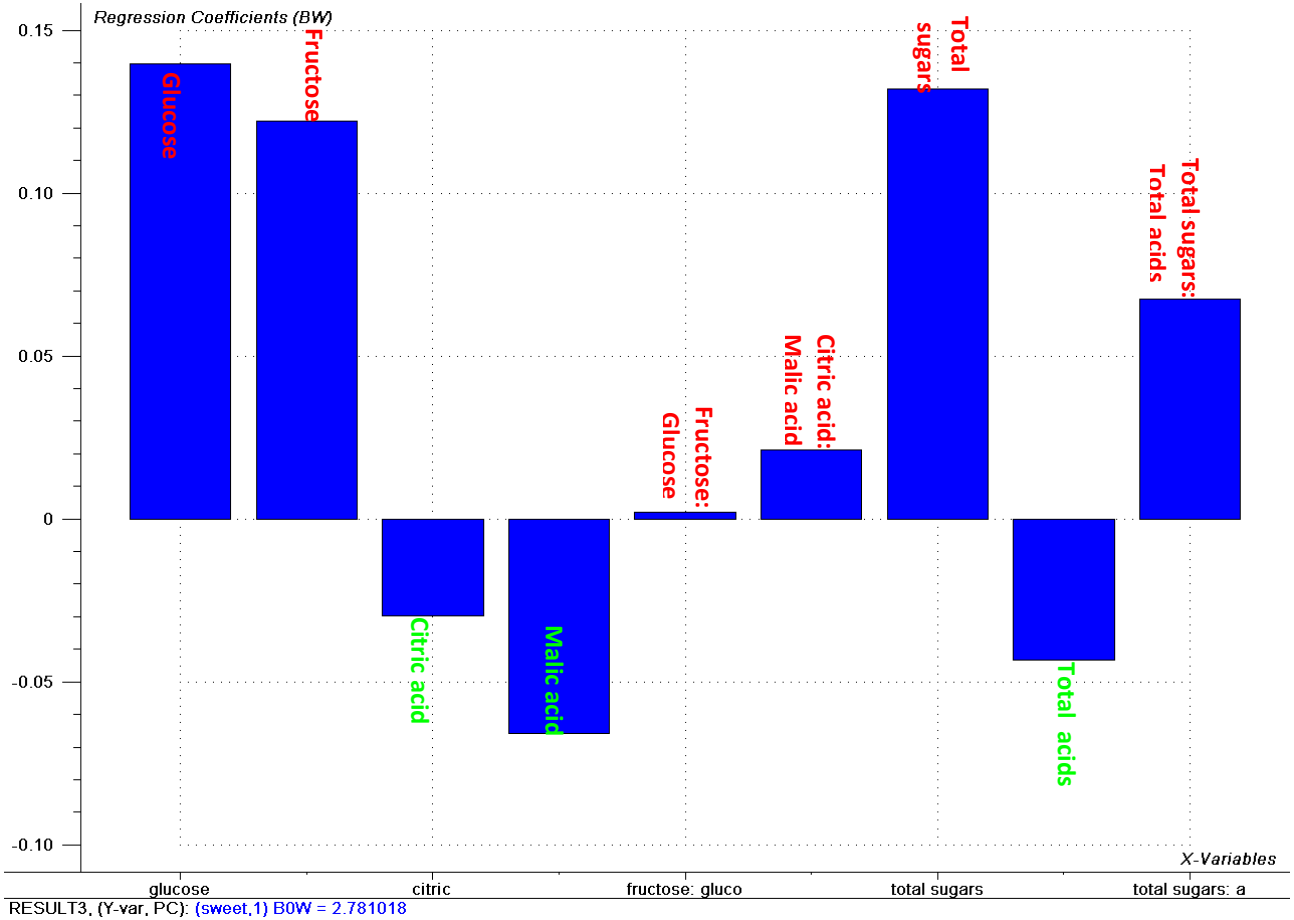


Figure 4.23 PLS1 model for prediction of Y sweetness from X-variables in *sugars* and *acids* content: β-coefficients.

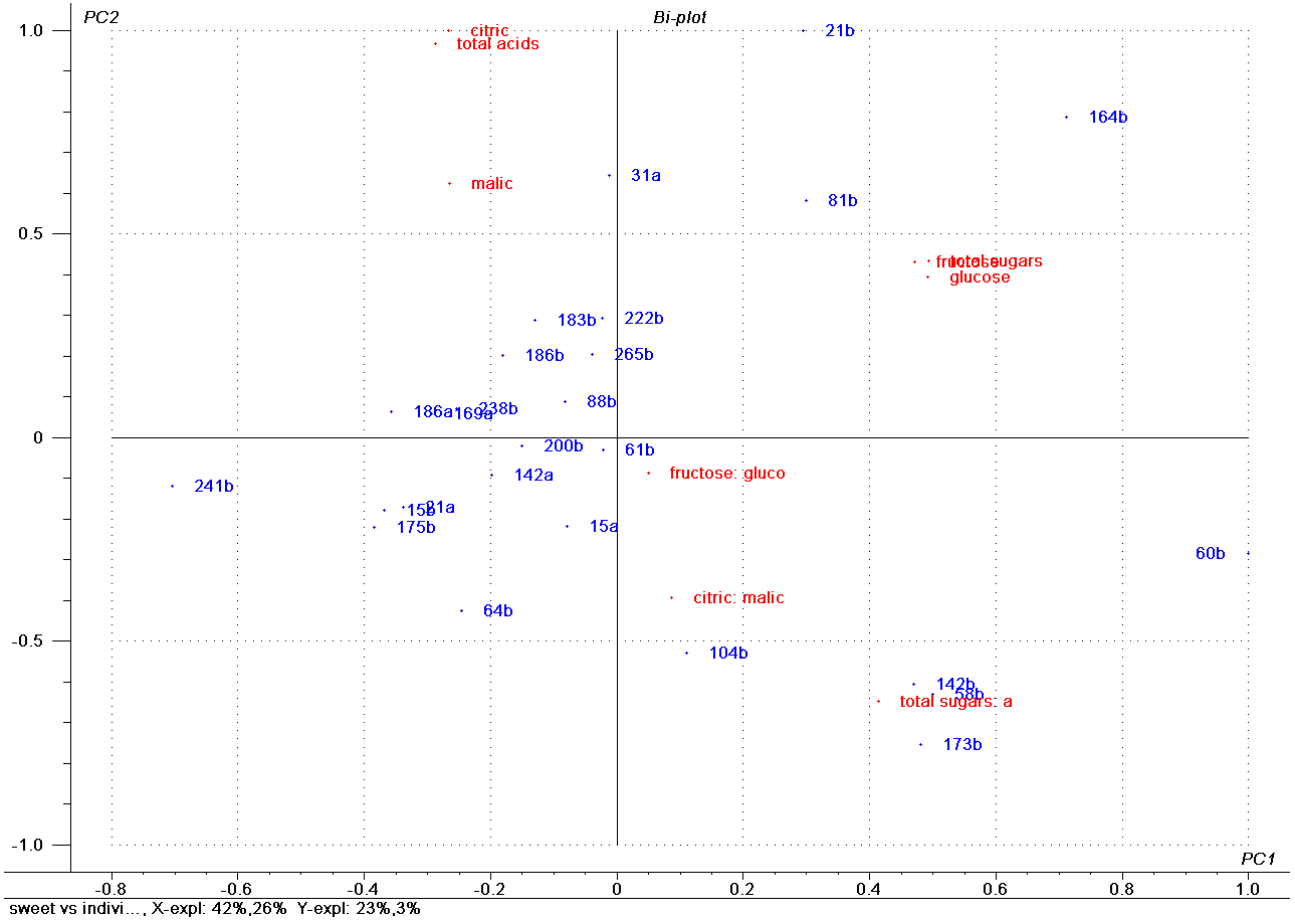


Figure 4.24 PLS1 model for prediction of Y sweetness from X-variables in s*ugars* and *acids* content: product space.

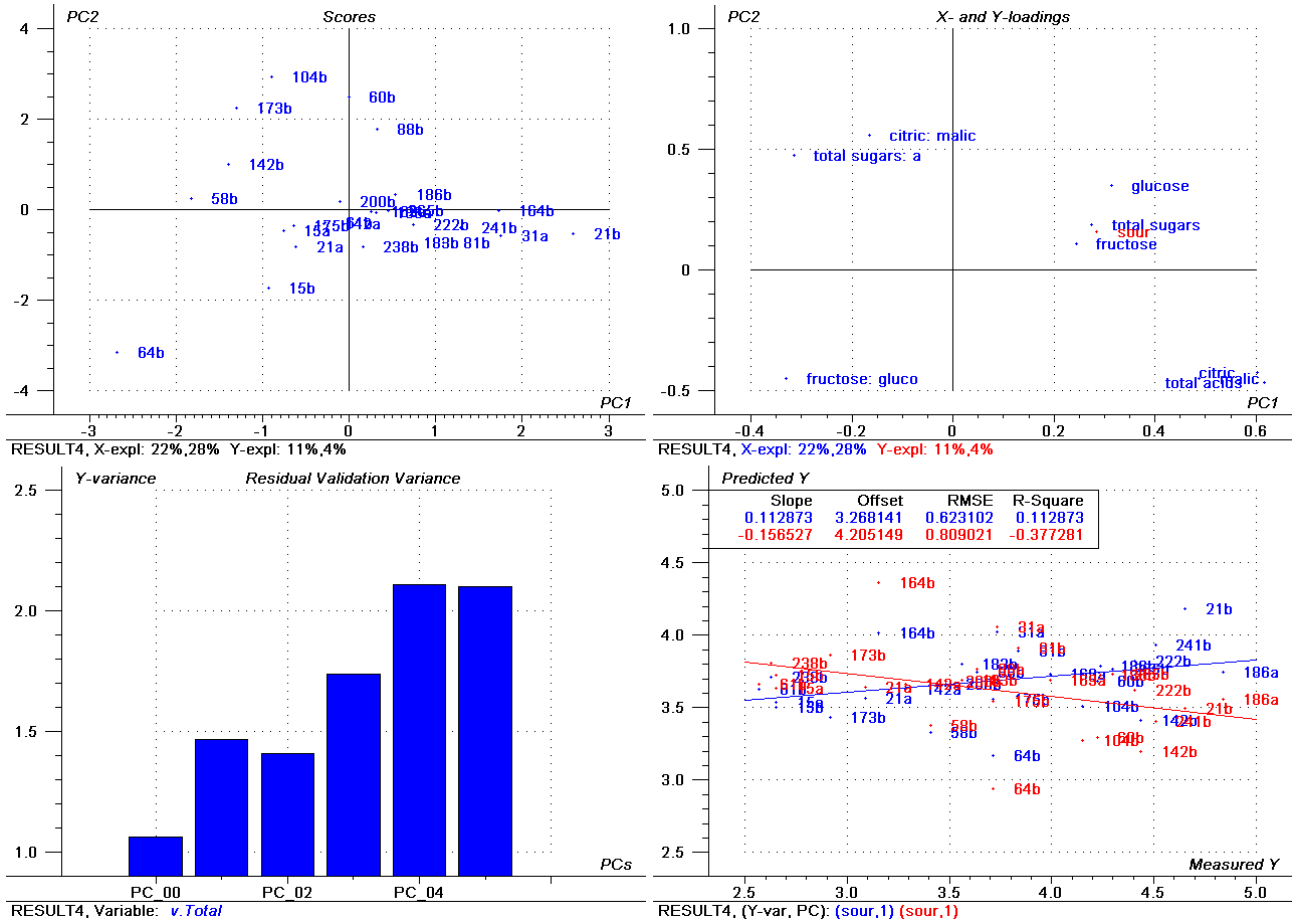


Figure 4.25 PLS1 model for prediction of Y sourness from X-variables sugars and acids content: *individual, total* and *ratios*.

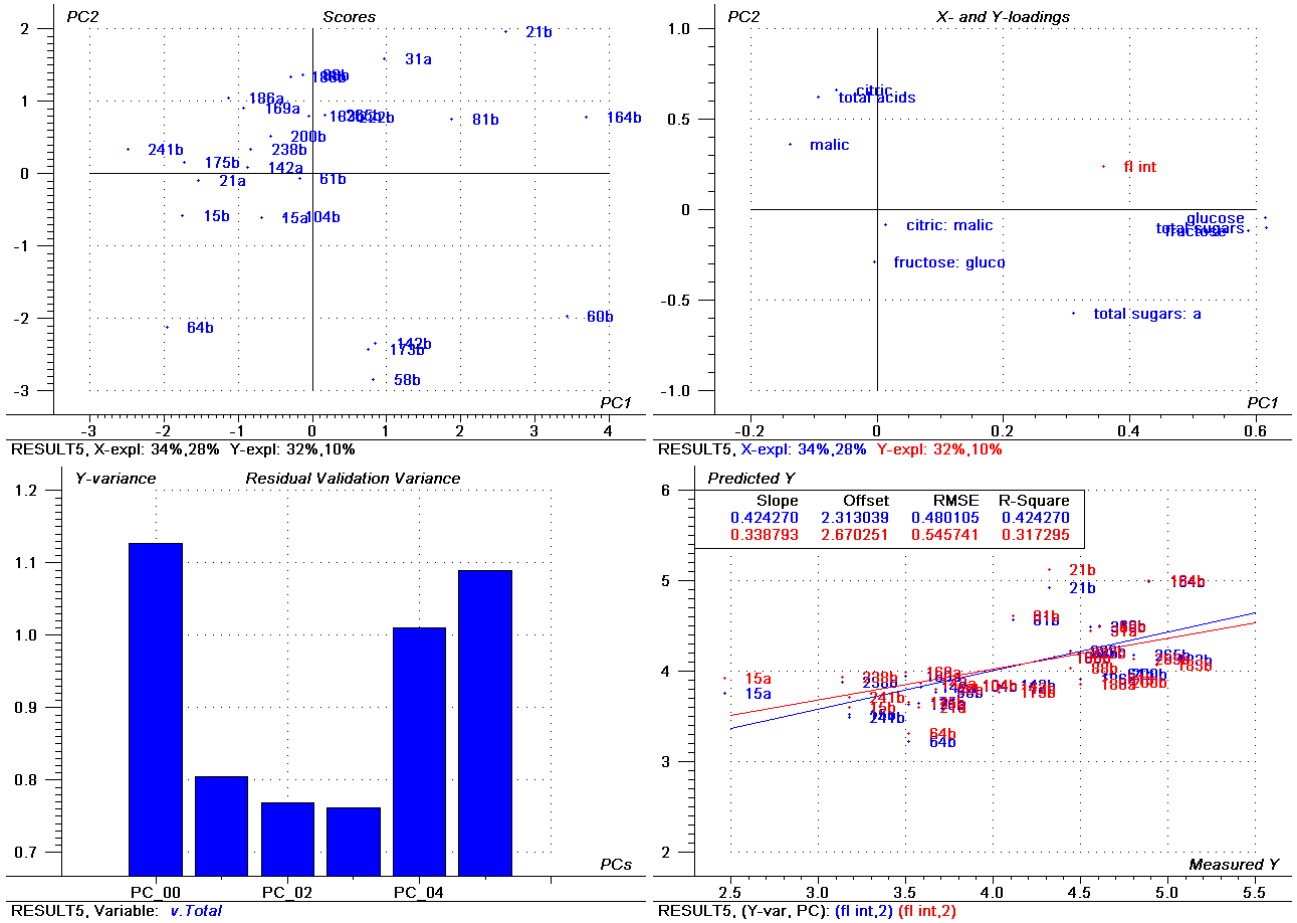


Figure 4.26 PLS1 model for prediction of Y flavour intensity from X-variables sugars and acids content: *individual, total* and *ratios*.

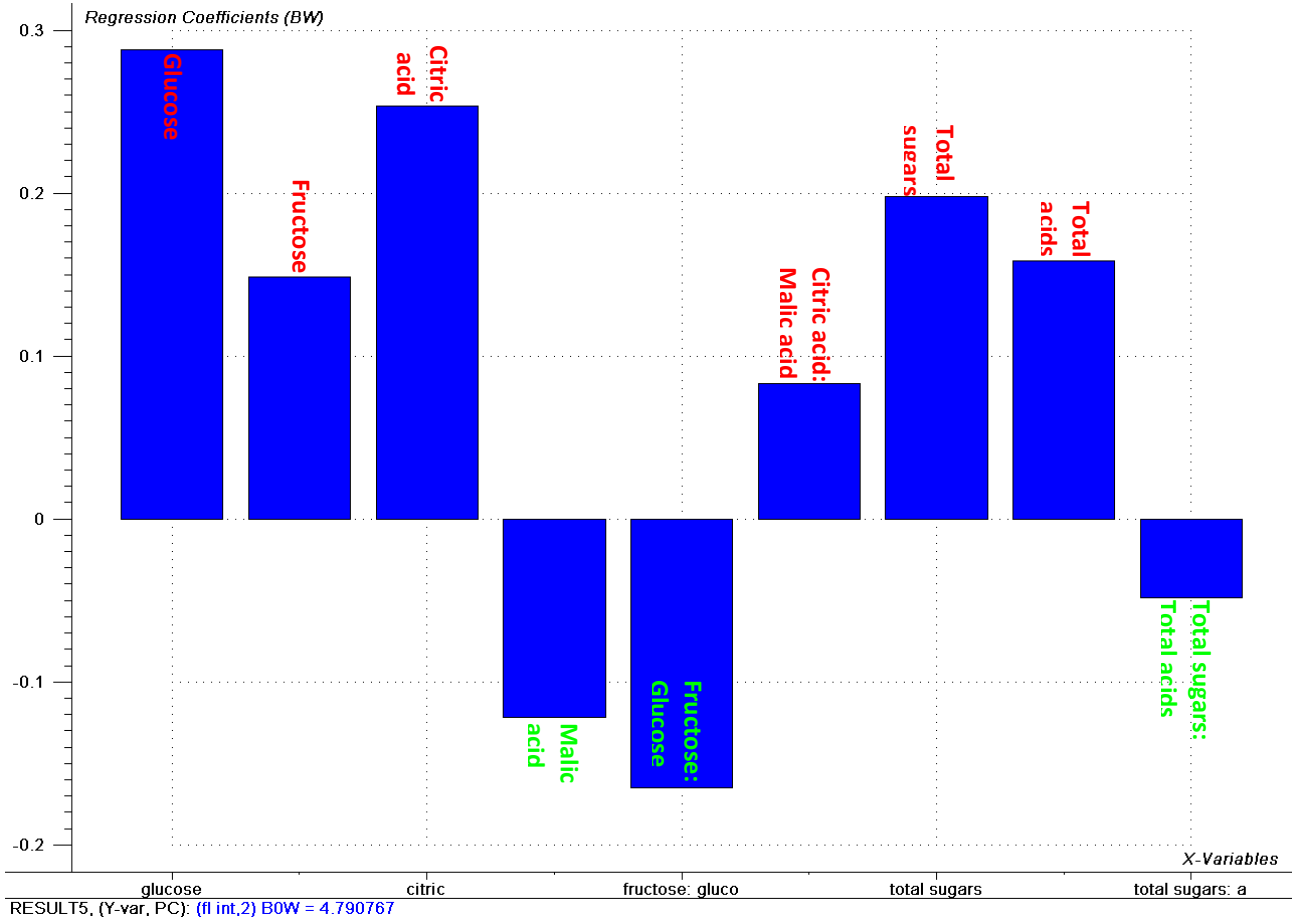


Figure 4.27 PLS1 model for prediction of Y flavour intensity from X-variables in *sugars* and *acids* content: β-coefficients.

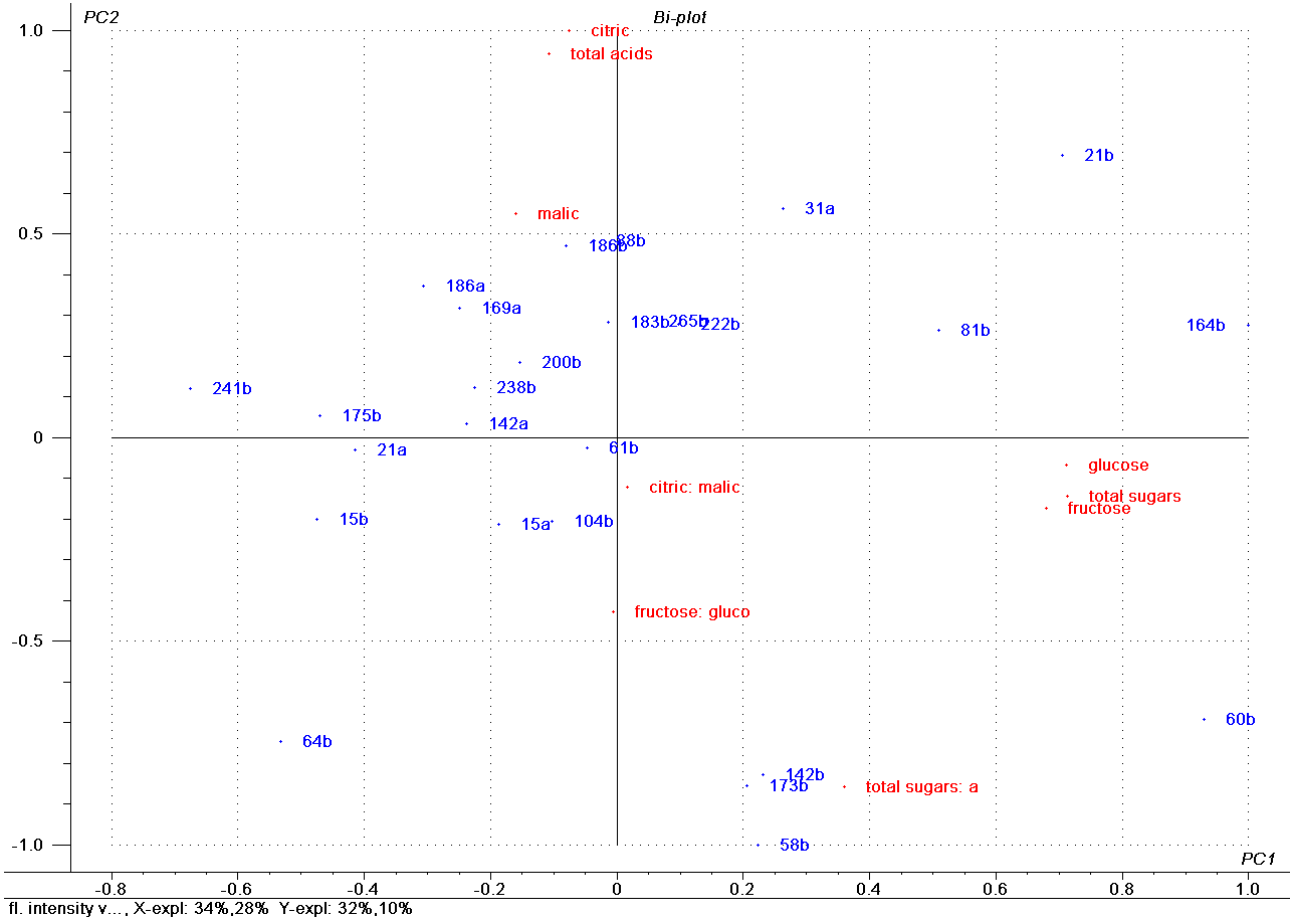


Figure 4.28 PLS1 model for prediction of Y flavour intensity from X-variables in *sugars* and *acids* content: product space.

4.5 Role of raspberry ketone in flavour quality

4.5.1 PLS-1 Model: Sweetness (Y) from X sugars, acids, weight, ^{o}Brix and raspberry ketone contents

Previous models demonstrated positive and negative contributions by aroma volatiles to flavour traits sweetness and flavour intensity. Correlations between total soluble solids and volatile contents in explaining flavour trait variance were also evident, possibly due to interrelatedness between taste and aroma perceptions, demonstrated in other foods. In raspberry, its characteristic aroma has been attributed to the volatile raspberry ketone (Pabst et al., 1990; Larsen and Poll, 1990; Wysocki et al., 1992; Roberts and Acree, 1996). Raspberry ketone exists as both free and bound forms, therefore varied quantities are reported from quantification studies; 0.9 - 420 μg per 100g fresh fruit (fw) and values reported are very much dependent on extraction methods used and more importantly, whether it liberates bound raspberry ketone or not. Raspberry ketone also has a very low aroma threshold value, 0.1 - 1.0 μg / 100g fw fruit (Larsen and Poll, 1990) and besides its contribution to 'raspberry' aroma, it may also impact on other flavour traits, possibly those studied here.

To establish what effects raspberry ketone has on flavour traits studied here, difference in model performance was assessed with and without inclusion of raspberry ketone in X-variables. As mentioned previously, raspberry ketone was only quantified in SCRI polytunnel crop. Inclusion of raspberry ketone (RK) into X-sugars, organic acids and physicochemical variables in models increased total Y-sweetness variance by 1% (Y-variance = 41% to 42%) (Figures 4.29 & 4.30). The model without raspberry ketone as an X-variable has one additional factor compared to model with raspberry ketone, yielding a 4-factor model that accounted for 73.4%, 67.9%, 66.6% and 66.2% in Y-variance of sweetness. This model was better fitted to calibration (R^2_{cal} = 0.43) than validation (R^2_{val} = 0.34) datasets (Figure 4.29). Inclusion of raspberry ketone as an X-variable resulted in a three-factor model (Y-sweetness variance = 76.5%, 69.0% and 68.4%) with similar R^2-values (0.42) for both calibration and validation datasets (Figure 4.30). Along Factor 1, all X-variables

except L*, a* and b* colourmeter values were positively loaded without inclusion of raspberry ketone, but with its inclusion, malic, citric and total acids contents were grouped together. On Factor 2, with exception of ΔE colourmeter value, clear separation between variables relating to metabolite contents and physicochemical traits was evident; colourmeter values (L*, a* and b*), 10-berry weight and ^{o}Brix were negatively loaded along Factor 1 without inclusion of raspberry ketone. Its inclusion into model separated acids contents to the intersection of Factor 1 and Factor 2. Multivariate product space yielded no distinctive sample groupings.

Raspberry ketone had enhancing effects on sugars and acids contents, which consequently increased its effects on sweetness variance. In modelling sweetness without inclusion of raspberry ketone, factors that had highest influences were 10-berry weight and total soluble solids (^{o}Brix) (β-coefficient values = 1.17, 0.55) (Table 4.20). In model with inclusion of raspberry ketone, β-coefficient values for ^{o}Brix and 10-berry weight reduced, considerably more for 10-berry weight. Its inclusion also increased β-coefficient values for sugars and acids contents and colourmeter values. Raspberry ketone itself had low β-coefficient value (0.255), but its importance is implicated based on its enhancing effects on other variables and that its inclusion into models increase total variance in sweetness by 1%, partially explained by its enhancing effects on sugars and acids content and other physicochemical traits.

Key points:

- Inclusion of raspberry ketone as an X-variable data enhanced effects of sugars and acids contents on sweetness and increased sweetness variance explained by 1%.
- Models with included raspberry ketone had increased β-coefficients values for sugars, acids contents and colourmeter values.
- Models without raspberry ketone yielded 10-berry weight and ^{o}Brix as main positive drivers of sweetness.
- Importance of raspberry ketone to flavour quality is implicated from its associations to other variables.

Table 4.20 Effect of raspberry ketone X-variable on β-coefficients of individual and total sugars, acids, °Brix, weight and colour parameters in partial least square regression (PLS1) analysis of Y-variable sweetness scores in 2007 SCRI polytunnel fruit.

Variables	β-coefficients	
	Without RK	With RK
Fructose	-0.432	0.574
Glucose	0.138	0.152
Total sugars	0.400	0.957
Citric Acid	-0.485	-0.214
Malic Acid	0.101	1.062
Total Acids	0.149	0.291
°Brix	0.555	0.433
10-berry weight	1.173	0.164
L*	-0.596	-0.528
a*	0.155	0.462
b*	-0.232	0.383
ΔE	0.753	0.879
RK	-n/a-	0.255

No suitable models corrrelated sweetness with volatile contents with inclusion of raspberry ketone as X-variables. However in previous PLS1 models that included crops of all cultivation sites, volatiles were shown to be important contributors to Y-variable sweetness.

Sourness modelled to raspberry ketone contents also did not yield informative models, with low and negative R^2-values for both calibration (R^2_{cal}) and validation (R^2_{val}) datasets (Table 4.21). Therefore, such models were not explored further.

Table 4.21 Model suitability of Ysourness PLS1 models with and without raspberry ketone X-variable in polytunnel fruits. Legend: Cal.= Calibration, Valid.= Validation, var. exp.= variance explained, # = number.

	Sugars, acids, weight, °Brix, colour values		Sugars, acids, volatiles content	
Model	Sourness	Sourness/ **RK**	Sourness	Sourness/**RK**
R^2- Cal.	0.09	0.09	0.12	0.07
R^2- Valid.	-0.07	-0.01	-0.04	-0.05
Y var. exp.	9%	9%	12%	7%
# factor	-nil-	-nil-	-nil	-nil

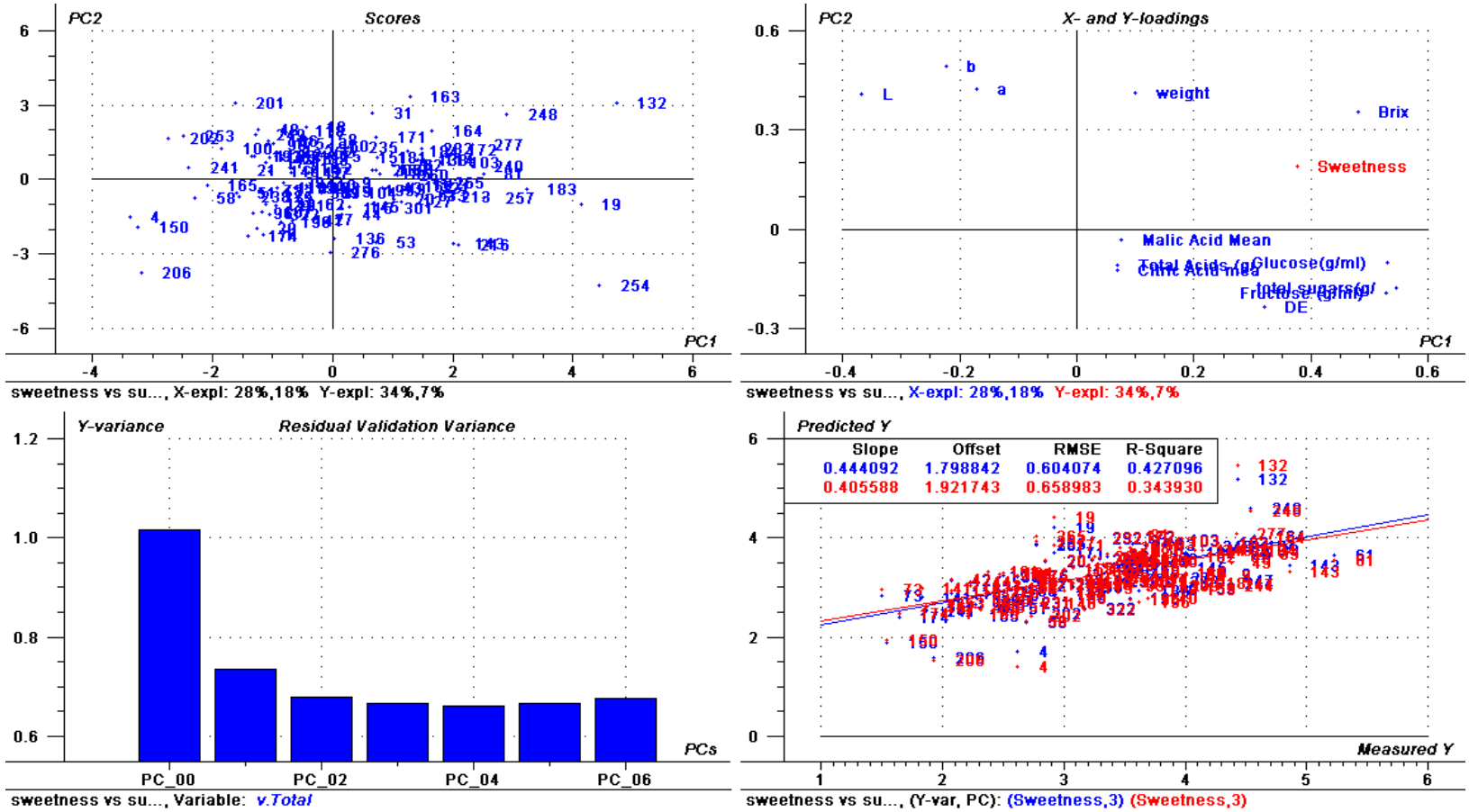


Figure 4.29 PLS1 model for prediction of Y sweetness from X-variables *sugars, acids, °Brix, weight* and *colour* parameters in polytunnel fruit only.

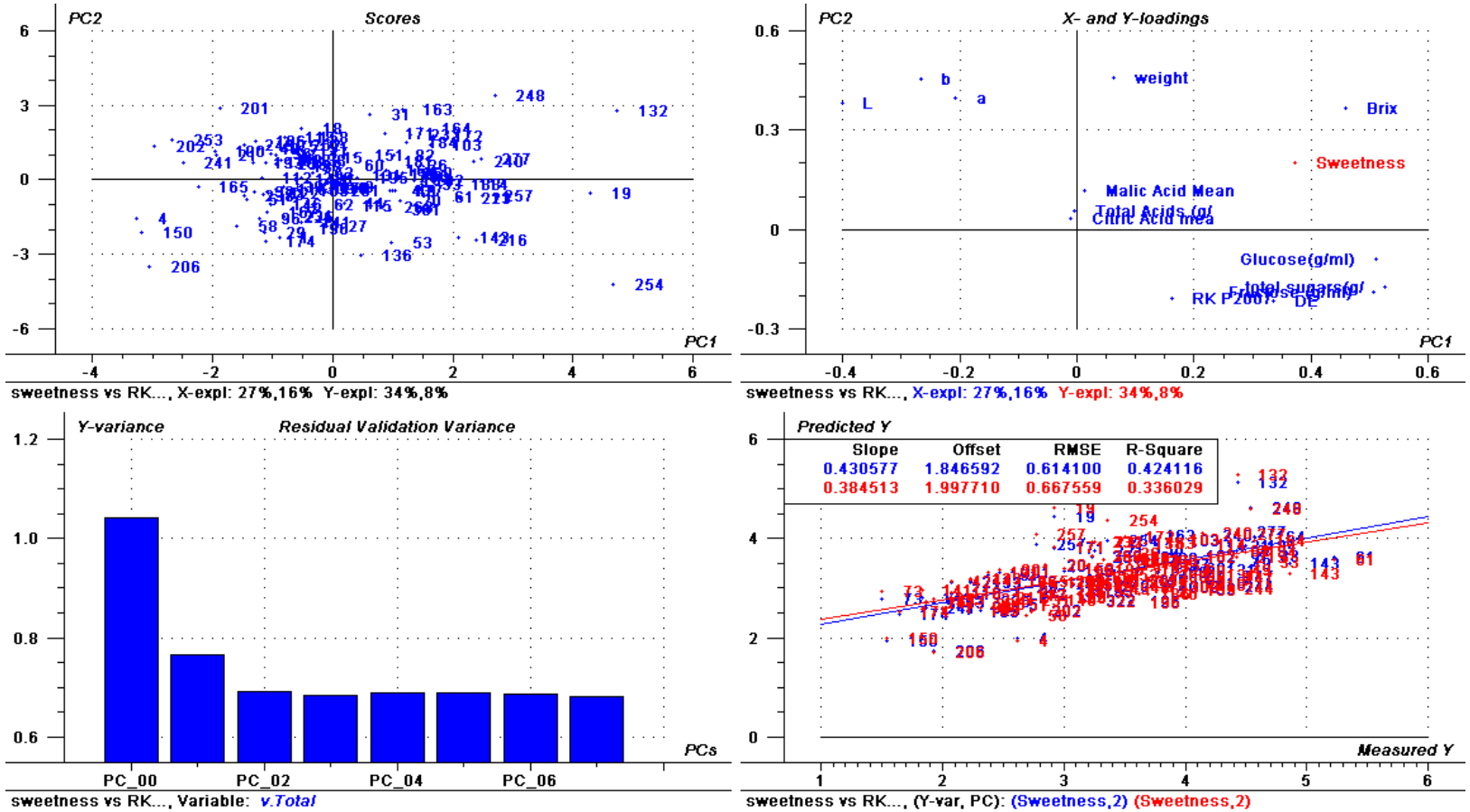


Figure 4.30 PLS1 model for prediction of Y sweetness from X-variables *sugars, acids, °Brix, weight, colour parameters* with **RK** in polytunnel fruit only.

4.5.2 PLS-1 Model: Flavour intensity (Y) from X sugars, acids, weight, ^{o}Brix and raspberry ketone contents

Similar results as in sweetness models were found in models correlating flavour intensity to X-sugars, acids contents, 10-berry weight and ^{o}Brix. Inclusion of raspberry ketone as X-variables increased total variance in flavour intensity by 3% (33% vs. 36%) (Figure 4.31). Models with and without raspberry ketone as X-variables had 2 factors, with higher percentage variance explained in flavour intensity in absence of raspberry ketone as an X-variable; Factor 1 (74.6% - without RK vs. 71.5% - with RK) and Factor 2 (69.6% - without RK vs. 63.6% - with RK). Both models were also better fitted to calibration (R^2_{cal} = 0.45 – without RK vs. 0.39 – with RK) than validated (R^2_{val} = 0.37 – without RK vs. 0.31 – with RK) datasets. All variables except L*, a* and b* colourmeter values were positively loaded along Factor 1 and with inclusion of raspberry ketone as an X-variable, this did not change. On Factor 2, as was in sweetness models, variables were discriminated based on sugars, acids content and physicochemical variables; L*, a*, b* colourmeter values, 10-berry weight and ^{o}Brix. Raspberry ketone along with sugars and acids contents were all positively correlated to flavour intensity on Factor 1. In models without raspberry ketone, main positive drivers of flavour intensity were total sugars content and ΔE colourmeter value. With inclusion of raspberry ketone, effects by total sugars content increased but decreased for ΔE. It also increased effects of other sugars, citric acid, ^{o}Brix, L* and b* colourmeter values. The β-coefficient value for raspberry ketone in these models was approximately 192% higher than in sweetness models, which implies its effects on flavour intensity perception is possibly 2-fold more than its influence on sweetness.

Key points:

- Inclusion of raspberry ketone data into models increased total variance explained in flavour intensity by 3%.
- As was in sweetness, importance of raspberry ketone to flavour intensity perceptions is implied from its effects on other variables and the consequent influences of these variables to flavour intensity.

- However, effects by raspberry ketone alone is greater on flavour intensity than on sweetness.

Table 4.22 Effect of raspberry ketone X-variable on β-coefficients of individual and total sugars, acids, °Brix, weight and colour parameters in partial least square regression (PLS1) analysis of Y-variable flavour intensity scores in 2007 SCRI polytunnel crop.

Variables	β-coefficients	
	Without RK	With RK
Fructose	0.435	0.603
Glucose	0.141	0.135
Total sugars	0.814	0.927
Citric Acid	0.262	0.375
Malic Acid	0.311	0.155
Total Acids	0.380	0.141
°Brix	0.390	0.427
10-berry weight	0.254	0.259
L*	-0.537	-0.484
a*	0.423	0.340
b*	0.152	0.223
ΔE	0.809	0.789
RK	-n/a-	0.746

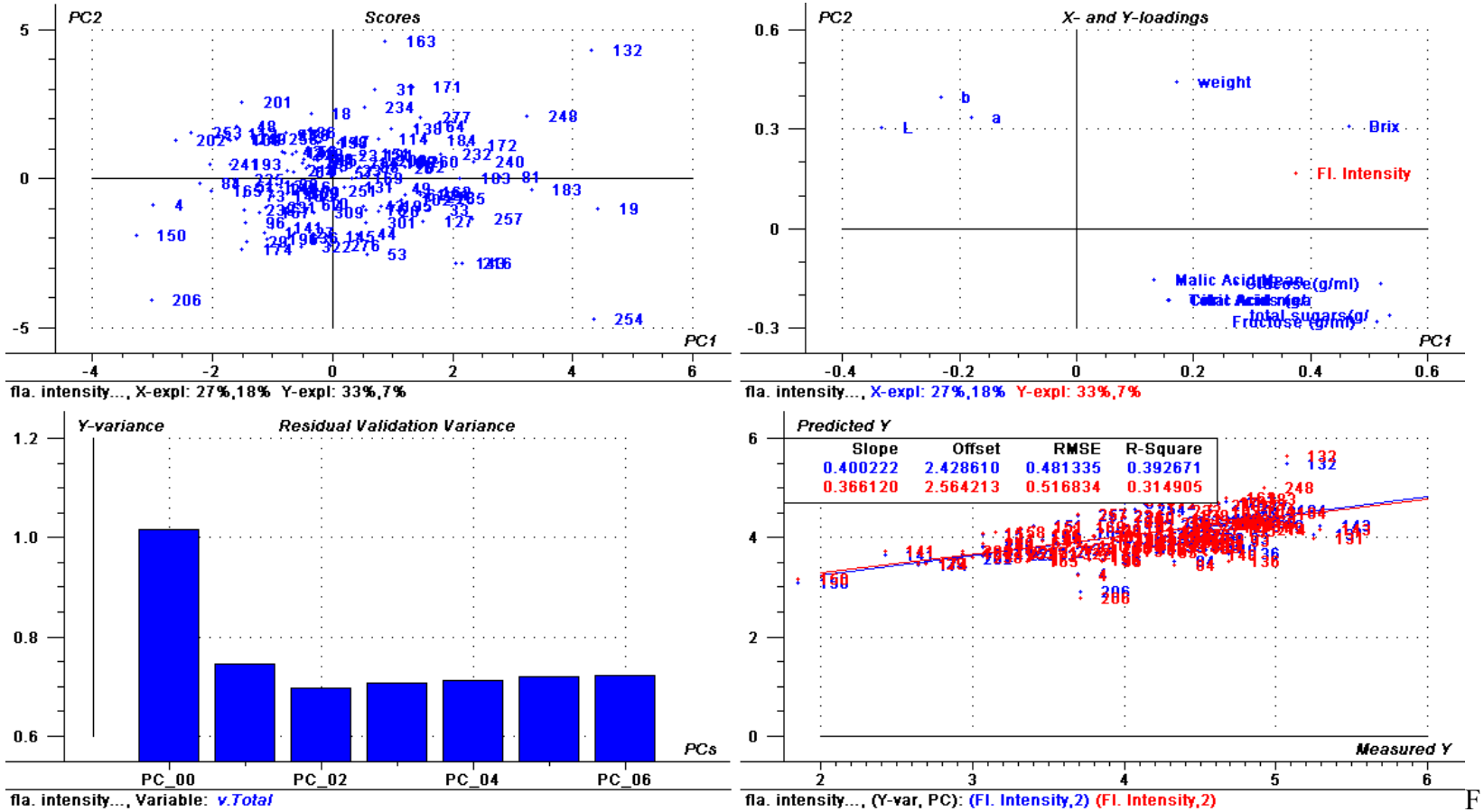


Figure 4.31 PLS1 model for prediction of Y flavour intensity from X-variables *sugars, acids, °Brix, weight, colour* parameters in polytunnel fruit only.

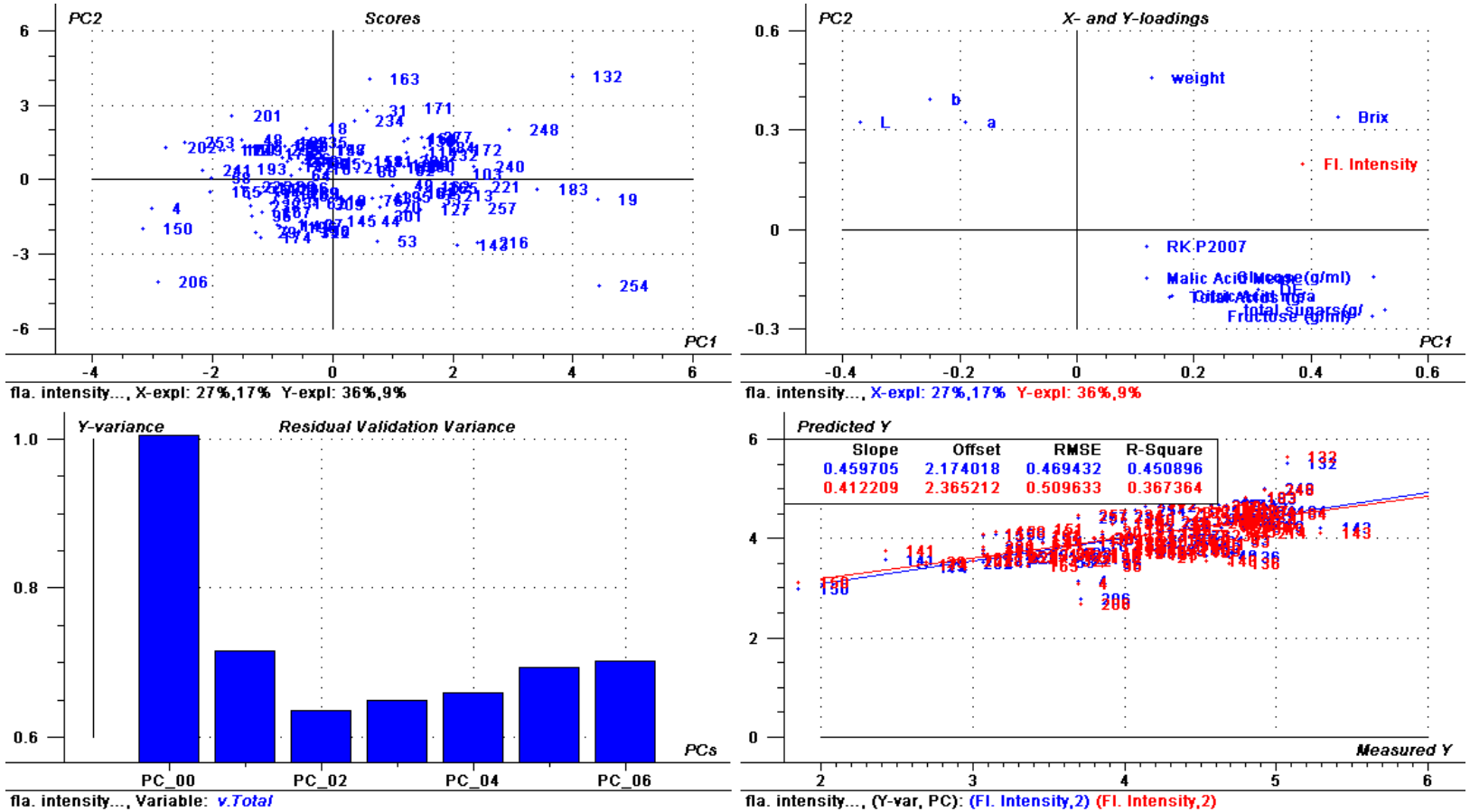


Figure 4.32 PLS1 model for prediction of Y flavour intensity from X-variables *sugars, acids, °Brix, weight, colour* parameters with **RK** in polytunnel fruit only.

4.5.3 PLS-1 Model: Flavour intensity (Y) from X volatiles and raspberry ketone contents and anthocyanin contents

The next set of models explain interactions between raspberry ketone with other volatile contents in influencing flavour intensity perceptions. Model that included raspberry ketone with other X-volatiles contents, had total variance explained reduced by 2% (21% vs. 19%) (Figure 4.33, 4.34). Compared to previous models, which correlated flavour intensity to sugars, acids contents and physicochemical variables, variance explained in these models were much less. These models also had low R^2-values for calibration (R^2_{cal} = 0.19) and validation (R^2_{val} = 0.07) datasets, which indicated ill fit and therefore these models were not considered further (Figure 4.33).

Key points:

- Raspberry ketone along with contents of other volatile compounds did not affect variance in flavour intensity perceptions in this study.

Although previous models indicated correlations between flavour intensity variance and anthocyanin contents, when anthocyanins were modelled with raspberry ketone data as X-variables, models produced low R^2-values for calibration (R^2_{cal} = 0.13) dataset and a negative value for validation (R^2_{val} = -0.12) dataset (Figure 4.34). The models did not meet criteria for goodness-of-fit and were not explored further.

Key points:

- Anthocyanins modelled with raspberry ketone contents did not adequately explain flavour intensity variance.

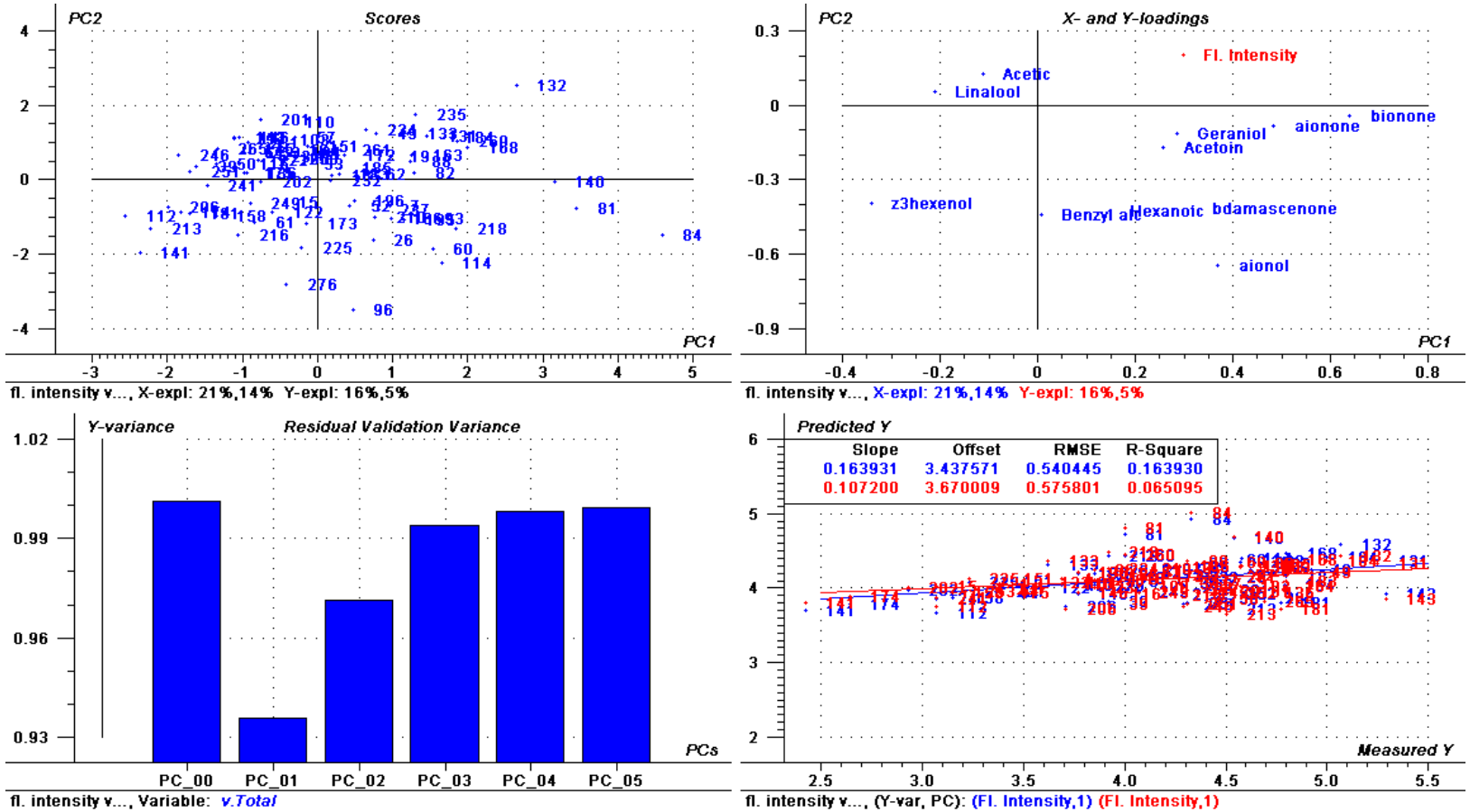


Figure 4.33 PLS1 model for prediction of Y flavour intensity from X-variables *volatiles contents* in polytunnel fruit only.

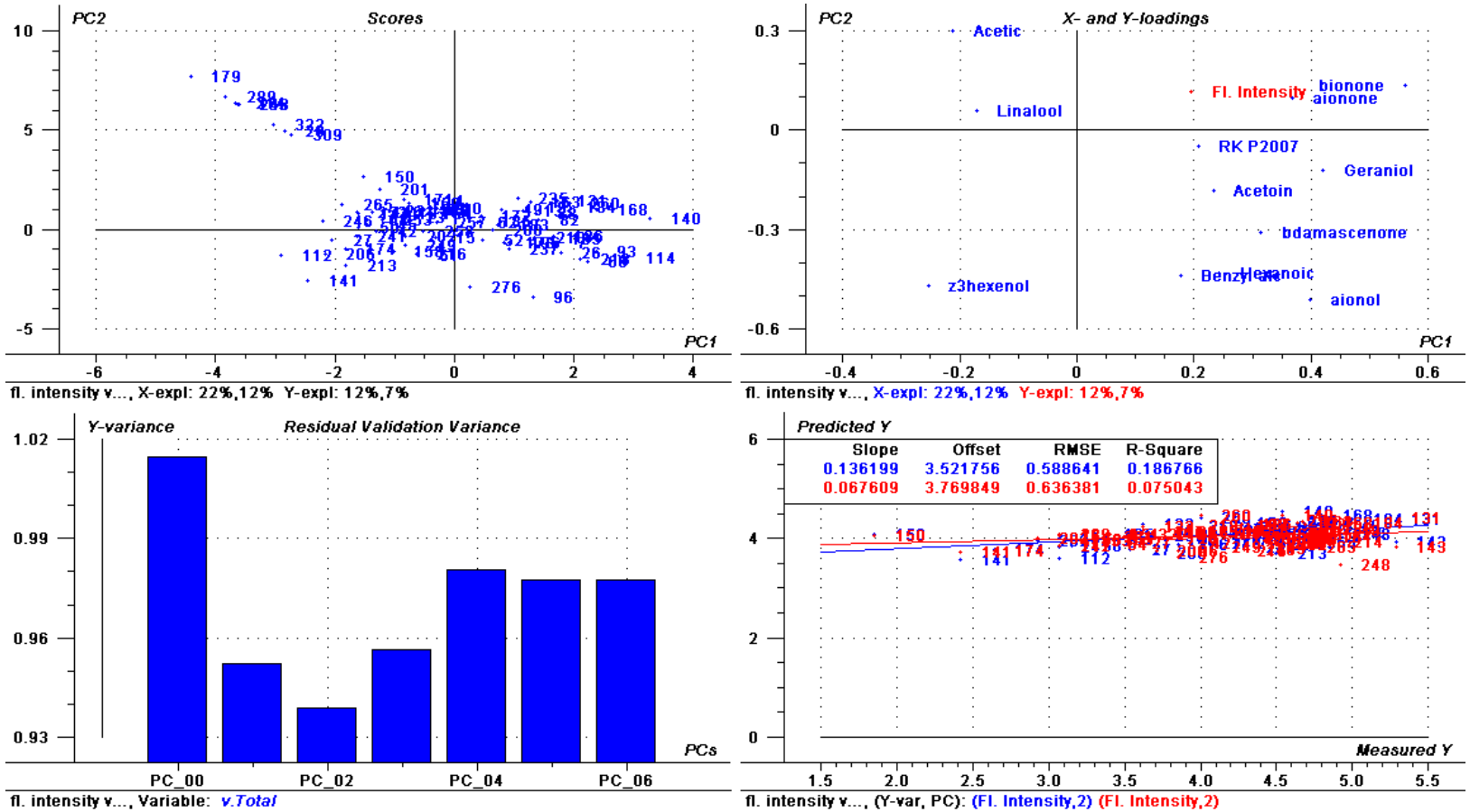


Figure 4.34 PLS1 model for prediction of Y flavour intensity from X-variables volatiles contents with **RK** in polytunnel fruit only.

4.6 Discussion

In previous experimental chapters, it was concluded variance in sensory data of this raspberry mapping population was strongly correlated to metabolites content, which consecutively is significantly determined by external environmental factors, primarily harvest year and cultivation sites. Experimental data suggested harvest year produced compounded effects of sunlight incidence, temperature and rainfall, different between 2006 and 2007. Main effects of cultivation site was polytunnel cultivation compared to open field cultivation, and possibly other factors associated with it, such as site management, fertigation, microclimate, terrestrial and site management at SCRI, Invergowrie, and in commercial fruit production site in Blairgowrie. These factors significantly influenced intensity of sweetness, sourness and flavour intensity, as rated by selected sensory panellists.

4.6.1 Univariate correlations of flavour and metabolites content

Key points:

- Flavour traits were significantly affected by cultivation practice.
- Seasonal differences were observed in sourness and flavour intensity of field crops.
- In 2006 field crop, flavour intensity was significantly correlated to sweetness and was inversely related to sugars content.
- In 2007 field crop, flavour intensity was also significantly linked to sourness and correlated to acids content in 2007.
- Positive links between sweetness, flavour intensity and sugars content remained in SCRI polytunnel crop but was lost in Commercial polytunnel crop.
- Sourness did not correlate to any metabolite variable but was inversely linked to sweetness in field crops.
- ^{o}Brix was a reliable predictor of sweetness and flavour intensity in both field crops and only for flavour intensity in Commercial polytunnel crop.

- Correlations of ^{o}Brix with metabolites contents were not consistent through harvest years or cultivation sites.
- Univariate analyses produced limited explanations of flavour traits.

The flavour traits assessed in this study were much affected by panel related factors; variation due to different interpretation and sensitivities of panellists, assessment environment amongst other factors. Training was performed previous to experimental scoring but undoubtedly scoring varied between panellists. However the sensory analyses strategy adopted in this study generated robust data for analyses, as evident from linear and multivariate regressions of flavour traits and metabolites content pervasive throughout harvest years and cultivation sites. Sweetness and flavour intensity were significantly correlated in field crops of 2006 and 2007 and in all cultivation sites ($p < 0.01$, $r > 0.500$), which made this correlation robust.

Sweetness and sourness had varying relationships. In both polytunnel and 2006 field crops sourness was inversely correlated to sweetness. In the two field crops, sourness was correlated with flavour intensity, with a lower correlation coefficient value than the value with sweetness. Hence, growth and certainlys that result in overall flavour quality perceptions, factors viable for atiBest practice in management of the fruiting environment seems the short-term strategy of choice for better raspberry flavour quality. In this study, polytunnel cultivation yielded raspberries higher in metabolites contents and scores for flavour traits. Enhanced fruit sugar contents produced benefits in sweetness and flavour intensity, traits rated here.

Univariate correlation analyses revealed fruit sugars and acids were not consistent drivers of sweetness, sourness or flavour intensity scorings. For example, sugars correlated to sweetness only in 2006 SCRI field and 2007 SCRI polytunnel crops. Higher sweetness scores in polytunnel crop correlated to higher sugars content, but lower acids content in 2006 field crop compared to 2007 did not translate to lower sourness scores, indicating correlations of metabolites contents to sensory profile is not

a simple association. Another example of this complex relationship is how despite varying conditions in different cultivation sites, flavour intensity was strongly associated with sugars contents; individual, total contents and its ratios.

Soluble solids content measurement value, ^{o}Brix, provided a reasonable estimate of sweetness and flavour intensity but only for the field crops and in polytunnel crop, it was a reliable indicator for flavour intensity. ^{o}Brix was also a good indicator of sugars and acids contents, demonstrated in multivariate models. These correlations were consistent in both univariate and multivariate regression analysespolytunnel crops however, loss of correlations of flavour traits and ^{o}Brix could possibly be due to contributions to sweetness and flavour intensity by fruit volatiles contents, not measured by ^{o}Brix and generally in greater quantities in these crops compared to field crops. Therefore, sugars and acids content partially explain sweetness, sourness and flavour intensity scoring, but other metabolites contribute to a compound effect, with sugars and acids content, to these flavour traits.

Of flavour traits assessed here, only sweetness had multivariate links with raspberry ketone contents, but the model was not well fitted. From univariate correlations, raspberry ketone had significant links with brightness, L*, and volatiles α-ionol, benzyl alcohol, acetoin and hexanoic acid but its influence on flavour traits was not clear. But in multivariate regression it appeared to enhance effects of sugars and acids content on sweetness variance.

4.6.2 Multivariate correlations of flavour and metabolites content: Regression (PLS1) models of flavour quality with metabolites content

Key points:

- Sweetness and flavour intensity had strong correlations with volatiles contents.
- Contributions by non-volatiles (sugars, acids and other total soluble solids) to flavour traits were complemented by fruit volatiles content.

- Colour traits were correlated with flavour trait variance, especially sourness, but there were no correlations to colour pigments (anthocyanins).
- Brightness, L*, a colourmeter value has potential to be an indicator of high sourness and low sweetness and flavour intensity profiles of field crops.

PLS-1 regression analyses of sweetness and flavour intensity to sugars, acids and volatiles contents revealed common factors that affected both flavour traits. Sugars and hexenol contents influenced both flavour traits to a great extent. Greater number of volatiles affected sweetness more than flavour intensity, and when raspberry ketone was included in models, it had greater enhancing influence on other volatiles and variables, which resulted in greater impact on sweetness variance. Although increase in total variance was greater for flavour intensity with the inclusion of raspberry ketone, overall variance due to volatiles content was much lower compared to sweetness variance. For both flavour attributes, non-volatiles and other physicochemical attributes explained highest percentage of trait variance. This was also for sourness, although correlations with sugars and acids content were inconsistent and not validated in multivariate regression analyses. Volatiles appeared to have minimal or no contribution to sourness at all. Of physicochemical factors, ^{o}Brix had univariate and multivariate links to flavour traits and associated metabolites. However, in multivariate regression, its associations were lost in polytunnel crops, possibly due to greater contributions by fruit volatiles to flavour traits, resulting from higher volatile contents in these crops. From raw data, progeny fruits with high ^{o}Brix values also scored high for sweetness and flavour intensity (average score > median score, 3.5), but only true for polytunnel crops, not field. As was demonstrated from univariate correlations sweetness and flavour intensity were correlated to ^{o}Brix in field crops, but only to flavour intensity in Commercial polytunnel crop. Therefore, care must be taken when using ^{o}Brix to predict flavour qualities. From results, it could be a better predictor of flavour intensity than for sweetness, across cultivation environments. However, it should not replace comprehensive sensory evaluations.

Sourness had univariate links with brightness denoted by L* colourmeter value, but this was not apparent in multivariate regression. However, L* was a negative influence for sweetness and flavour intensity, and from this it was inferred L* could be an indicator for undesirable flavour quality; fruits with high sourness, low sweetness and flavour intensity profiles. For sweetness and flavour intensity only colour difference, ΔE measured by colourmeter, was linked in models with raspberry ketone included. But colour pigments anthocyanins were not convincingly modelled to these flavour traits.

From models, volatiles were demonstrated to be important contributors to sweetness and flavour intensity, particularly hexenol. Similar contributions by volatiles to flavour traits have been reported in *Rubus* fruit. Thornless blackberry variety NZ 9351-4 had highest contents of furaneol, linalool, geraniol, ethyl hexanoate, trans-2-hexanol and β-ionone, which contributed aroma notes *fresh fruit*, *raspberry*, *floral*, *strawberry* and *citrus* (Du et al., 2010, Du and Qian, 2010). This variety also had highest fructose and glucose contents. In this study, fruits with highest content of geraniol and hexenol scored higher than median score (3.5) for sweetness and flavour intensity in both field and polytunnel crops, but did not have highest contents of sugars. Linalool in this study, unlike the finding of Du and others, had a negative impact on sweetness and flavour intensity. Hence volatiles had a compounded effect with non-volatiles content on flavour trait variance. In a study on effects of abiotic conditions on apple volatile production, rainfall, temperature and humidity were shown to significantly influence production (Vallat et al*.,* 2005). These effects were lessened with protected cultivation and reduced differences in fruit flavour quality. In this study, volatiles contents were higher in polytunnel crops compared to field grown crops, which partially explains its increased contribution to flavour traits.

Colourmeter measurement values were significantly associated with flavour trait variances. All values had positive links with sweetness and flavour intensity, except L* (brightness), which was significantly linked to sourness (Table 4.24). From this, it is inferred bright and light coloured fruit could be low in sweetness and flavour intensity

but high in sourness. From raw sensory scores, progeny fruits with high L* values were scored below median score (< 3.5) for sweetness and higher (> 3.5) for sourness and flavour intensity. Therefore with exception of flavour intensity, this hypothesis is likely and colourmeter measurements have potential to be a mechanical indicator of flavour quality prior harvest. Although fruit colour could be used as an indicator of a good flavour quality fruit, fruit pigment anthocyanins did not have significant correlations to any flavour traits. As all berries were collected at similar maturity stages, berry colour in this study is possibly a hereditary factor, as Glen Moy is the lighter coloured berry compared to Latham.

Table 4.24 Colourmeter values and its representations.

Parameter	Measuring
L*	Brightness to darkness Value (0=Black, 100=White)
a*	Green to red spectrum Value (-a =green, +a=red)
b*	Blue to yellow spectrum Value (-b=blue, +b=yellow)
ΔE	Total colour difference Equation: $[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{\frac{1}{2}}$

4.6.3 Multivariate correlations of flavour and metabolites content: Regression models of raspberry ketone contents on flavour quality

Key points:

- Raspberry ketone (RK) has enhancing effects on other variables that positively affected sweetness and flavour intensity.

- Raspberry ketone increased influence of sugars on sweetness and flavour intensity.
- Importance of raspberry ketone is implied based on its enhancing effects on other variables and therefore indirect influence on flavour traits.

In this study, models revealed positive contributions of raspberry ketone content to sweetness and flavour intensity variance. Most important were its compound effects with sugars, acids and volatiles contents on these flavour traits. Correlations between aroma and taste affected by raspberry ketone was demonstrated with heated raspberries, where higher RK content enhanced '*raspberry*' odour characters, '*strong raspberry, ripe raspberry, candy and preserves*' and increased perception of sweet notes, absent from unheated fresh raspberries (Roberts and Acree, 1996). Therefore, RK contents in this study could, released during mastication, could result in increased sweet aroma note perceptions, which in turn increase sweetness perceptions.

Flavour trait variances may also result from influences by RK on other volatiles. Specific volatiles in this study were positive drivers of sweetness and flavour intensity, particularly hexenol and geraniol. An explanation for indirect contribution of RK to raspberry flavour character is because much of RK in fresh berries exists as bound glycosides, liberated with heat treatment (Roberts and Acree, 1996). Therefore other volatiles are reported to have more impact on raspberry flavour character than RK, for example as suggested in another study that 'real raspberry' flavour trait correlated to α- and β-ionone contents in fresh raspberries, not raspberry ketone (Aprea et al., 2009). These volatiles were also shown to contribute to fresh raspberry aroma by Poll and others (Larsen et al., 1991). In this study, α- and β–ionone were influential to flavour traits, positive impacts by α-ionone and negative influences from β–ionone. Therefore, although contribution by RK contents to flavour traits is demonstrated in this study, its roles in overall flavour development process remains complex and unclear.

4.7 Conclusion

The chapter aimed to explore influences by fruit metabolite compositions and other physicochemical variables; 10-berry weight, ^{o}Brix, colourmeter measurements, underlying variances in three key flavour traits; sweetness, sourness and flavour intensity in the raspberry mapping population. Univariate and multivariate regression analyses identified factors that impacted significantly on flavour traits, with some factors persistent over harvest years and cultivation sites and some factors specific to these conditions. Non-volatile sugars, acids and other total soluble solids had significant linear correlations to sweetness and flavour intensity, validated in multivariate models. Sourness however was not convincingly correlated to any metabolite variable, but had significant links to brightness colourmeter value, L*. Multivariate regression revealed that influence of non-volatiles was enhanced by volatiles contents on sweetness and flavour intensity. In particular, raspberry ketone enhanced effects of sugars and acids contents on sweetness and flavour intensity. A potentially useful outcome from this exercise was the demonstration that instrumental measurements of total soluble solids (^{o}Brix) and colour have potential to be a reliable indicator of flavour intensity and sourness traits. Overall, preliminary factors identified here, which have most impact on raspberry flavour, can be a focus for future research on developing control measures on raspberry flavour quality.

CHAPTER 5

Discussions and Outlooks

Chapter 5 Discussions and Outlooks

5.1 Preamble

A principal hypothesis in this work was that quantitative differences in three key raspberry sensory flavour traits; sweetness, sourness, flavour intensity, in fruits of a mapping population would, as complex traits, show effects by genetic and environmental influences. The genetic influence would be inherited as alleles of quantitative trait loci (QTLs) and the environmental influences would be effects from seasonal weather variance, geography and method of cultivation. Both influences would be observable through differences in fruit metabolite contents and its consequent flavour quality traits. At the start of the study, the working hypothesis was non-volatile metabolite contents of fruit, i.e. sugars and acids contents would be the primary determinant of sensory flavour trait intensity. A key studied environmental factor was influences from open field and polytunnel cultivation, on fruiting plants and its sensory ratings of key flavour traits.

Calculations of heritability for sensory flavour and metabolite (Table 5.1) showed a range of trait variances explained by environmental factors. Of these polytunnel cultivation was notably important (Table 5.2) with increased variance explained in quantitative trait loci (QTL) for all metabolite contents and sourness. In a recent study, it was demonstrated these raspberry QTLs showed greater variability related to environment for sensory flavour and non-volatile metabolite traits than for flavour volatiles (Paterson et al., 2012). Multivariate modelling however suggested both non-volatiles and volatiles contributed to variance in at least two of the three flavour traits; sweetness and flavour intensity. Sensory flavour and non-volatile metabolites traits also showed clear relationships with fruit physicochemical traits; colourmeter values, 10-berry weight and total soluble solids content (^{o}Brix). Mapping QTLs on to the raspberry genetic linkage map resulted in common or adjacent loci to candidate genes and / or molecular markers for transcription factors and regulators of other plant traits; anthocyanin production, plant water potential and presence or absence of cane hairs.

Hence, from these associations, fruit flavour traits development could be viewed as a component of plant tissue differentiation and product of wider complex of mechanisms that control overall development. Such findings are further discussed and implications for other *Rosaceae* fruits explored.

5.2 Non-volatile sugars and organic acids content of raspberries

5.2.1 Environment: Effects of different harvest years and cultivation practices

Key findings:

- Increased sun exposure but reduced water availability was related to higher contents of fruit sugars and acids contents in field crops.
- Transport protein candidate genes were within QTL regions, therefore links of sugars and acids contents with whole plant metabolite transport systems likely.
- Polytunnel (protected) cultivation yielded crops with higher fruit sugars and acids contents, with highest amounts recorded in Commercial polytunnel crop.
- Cultivation method influenced genetic x environment interactions and these effects were considerable on metabolite transport and accumulation in crops.

From univariate analyses, field crops from 2006 and 2007 differed significantly in sugars and acids content, with greater variations in acids content. Reduced photosynthesis due to less light availability could be a possible factor as there was less available (sun) light in 2007 compared to 2006 (**Chapter 2)**. Reduced photosynthesis rate was also proposed as possible cause for reduced anthocyanin contents in 2007 field crop from this mapping population (Kassim et al*.,* 2009). Overlap of sugar and anthocyanin QTLs on LG 1 and 3 suggested existence of common genetic regulation factors but modelling of theses datasets yielded no causal relationship. Anthocyanin production in other *Rosaceae* crops was shown to be regulated by MYB-**T**ranscription **F**actors (TF), notably as responsible for flesh colour intensity in apples (Lin-Wang et al*.,* 2010). In *Arabidopsis* mutant *pho3* high in sugar contents, sugars and anthocyanin

Table 5.1 Variance components and heritability estimates for fruit metabolite content and sensory traits in 2006 and 2007. Legend: F 2007 = 2007 SCRI field, P 2007 = 2007 SCRI polytunnel and C 2007 = 2007 Commercial polytunnel crop.

	2006	2007							
Metabolite contents	h^2 (%)	σ^2_G			σ^2_{GS}			h^2 (%)	
		F 2007	*P 2007*	*C 2007*	*F 2007*	*P 2007*	*C 2007*	*F 2007*	*P 2007*	*C 2007*
Fructose	**46.0**	0.0555	0.2868	0.5377		0.8801		**5.9**	**24.6**	**37.9**
Glucose	**47.1**	0.0088	0.0274	0.0439		0.0801		**9.9**	**25.5**	**35.4**
Malic acid	**46.6**	0.0016	0.0013	0.0027		0.0056		**22.6**	**19.2**	**32.0**
Citric acid	**46.7**	0.0357	0.0115	0.0074		0.0546		**39.5**	**17.4**	**12.0**
Total sugars	**46.6**	0.0857	0.4522	0.7492		1.2872		**6.2**	**26.0**	**36.8**
Total acids	**47.0**	0.0476	0.0181	0.0125		0.0783		**37.8**	**18.8**	**13.8**
Raspberry ketone	**-**	-	-	-		-		**-**	**50.4**	**-**
Flavour trait										
Sweetness	**53.7**	0.3507	0.5896	0.3698		1.3102		**21.1**	**31.0**	**22.0**
Sourness	**50.0**	0.2619	0.3176	0.3499		0.9294		**35.9**	**25.5**	**27.3**
Flavour intensity	**50.0**	0.2659	0.3506	0.1893		0.8058		**24.8**	**30.3**	**19.0**

Table 5.2 Summary of metabolite and sensory QTLs: Legend: F = SCRI field, P = SCRI polytunnel and C = commercial polytunnel. Values in italics are highest in trait explained variance.

Metabolites	**Crop**	**Site**	**LG**	**Locus (cM)**	**LOD score**	**% variance explained**
Fructose	2006	F	1	90.21- 109.34	3.32	11.1
Glucose	2006	F	1	90.21- 106.25	3.46	11.8
	2007	C		*56.39- 58.88*	*5.58*	*40.9*
Citric acid	2006	F	3	89.14- 91.27	2.47	9.9
	2007	P	3	9.09- 13.60	2.64	8.7
		C	4	*59.70- 62.20*	*2.32*	*14.3*
Malic acid	2007	P	2	99.10- 106.85	2.76	8.8
		C	2	*76.12- 82.20*	*2.68*	*16.8*
Total sugars	2006	F	1	90.21- 109.34	3.38	11.5
	2007	C	3	*58.88- 84.68*	*3.55*	*32.1*
Total acids	2006	F	3	89.14- 91.27	2.57	10.7
	2007	F	5	53.20- 55.80	2.12	10.4
		C	5	*10.90- 11.90*	*2.33*	*14.2*
Raspberry ketone	2007	P	2	70.8- 82.2	3.14	12.6
			2	82.2- 95.3	3.49	13.3
			2	*82.2- 99.1*	*3.06*	*13.5*
Flavour	**Year**	**Site**	**LG**	**Location (cM)**	**LOD score**	**% variance explained**
Sweetness	2007	F	5	52.8- 65.3	3.06	13.5
		P	2	136.0-136.6	3.05	10.5
Sourness	2006	F	5	0.0- 24.7	2.50	10.1
	2007	F	4	121.9- 128.3	3.03	11.6
		C	5	24.1-49.1	3.26	27.1
Flavour intensity	2006	F	4	50.8- 56.6	2.79	12.7
	2007	F	3	52.6- 84.4	4.48	18.8
		P	2	12.8- 148.6	3.61	13.2

production were highly correlated to expression levels of MYB-TF (Lloyd and Zakhleniuk, 2004). In another *Arabidopsis* study, high glucose content increased expression of bHLH-TF, but not MYB-TF (Price et al*.,* 2004). In this study, transcription factors were candidate genes under Commercial polytunnel QTL for total sugars contents, similar to findings of Lloyd and Zakhleniul (2004), but also for malic acid QTLs and QTLs for other acids contents in 2006 field crop.

In this study increased water availability correlated to reduced sugars and acids contents. Underlying sugars and acids QTLs were candidate genes for **M**embrane and (**T**onoplast) **T**ransport **P**roteins (MIP and TIP) suggesting metabolite accumulation and final contents in fruits could be linked to osmoregulation and transport of intermediates (such as sucrose) into fruit cells. In other *Rosaceae* species, best researched in peach, QTLs for sucrose, soluble solids content and fresh weights mapped to same linkage group as markers for MIP and TIP genes:*PRUpe;MIP3* and *PRUpe;gTip* (Etienne et al*.,* 2002). As part of this study undertaken by associates, these markers mapped to pigment-related QTLs; total anthocyanins and colourmeter values, on the raspberry genetic linkage map (McCallum et al*.,* 2010). This suggested aquaporins and other MIPs and TIPs have important roles in metabolite accumulation, which determine, at least flavour character if not its quality. Transport efficiency of aquaporins was demonstrated to be affected by abiotic factors, for example light source type. In *Arabidopsis thaliana*, blue and white light activated aquaporin gene *Ath*H2(PIP1b) and light intensity reduction or absence eliminated transcription / accumulation of reporter gene product in a gene construct (Kaldenhoff et al*.,* 1996). In peach, aquaporins that regulate water uptake affected sucrose dilution in peach fruit cells (Génard et al*.,* 2003). From these examples, environmental factors affecting cell and tissue transport systems have effects on metabolite accumulation in fruit cells and have consequent impact on final flavour character. From this study, environmental effects on metabolite accumulations were influenced by cultivation method, in particular polytunnel cultivation, which increased sugars and acids content in crops. In contrast field cultivation produced crops lesser in sugars but higher in acids contents. Apart from increasing contents of sugars and acids,

polytunnel cultivation also increased yield, berry size (10-berry weight) and total soluble solids content (^{o}Brix) (McCallum, 2009). Similar results were reported in strawberry (Atkinson et al*.,* 2006; Voća et al*.,* 2006, 2009) and loquat (Polat et al*.,* 2005). In this study, different polytunnel cultivation sites produced crops also significantly different in sugars and acids content, with Commercial site producing a crop with quantities of sugar and acids. The key difference between polytunnel cultivation sites was in its fertilisation regime, with Commercial site using potassium-enriched fertiliser (**Chapter 2**). Positive effects of potassium use on fruit quality were observed in tomato, with increased transport of assimilated carbon into fruits (Mengel and Viro, 1974). In muskmelon (Lester et al*.,* 2005) fruit quality increased when potassium was applied to whole plants rather than roots before maturity. Therefore, a strategy of polytunnel cultivation with potassium-enriched fertiliser may create ideal conditions for development of fruits with abundant flavour metabolites.

5.2.2 Genetic: Co-localisation of sugars and acid with other flavour QTLs

Key findings:

- QTLs for sugars and acids content in fruit crops co-located to transcription factors for metabolite transport proteins, implying possible links.
- QTLs identified in this study were relatively large necessitating fine mapping strategies such as use of single nucleotide polymorphism (SNP) arrays with Expectation-Maximization algorithms under Fixed effect models.

Sugar and acid contents QTLs co-localised to genetic markers for transcription factors (TFs) involved in regulation of pigment and volatile compound biosyntheses, namely anthocyanin and phenylpropanoid pathways. From a sensory perspective, anthocyanins and sugars impact on two fruit quality traits, colour and taste. Co-regulation would imply linked expression of parallel traits in fruit ripening. Acids content were shown to impact on anthocyanin stability, important in strawberry colour and essential for consumer appeal (Garzón and Wrolstad 2002).

Other links were QTLs for malic acid in Commercial polytunnel and raspberry ketone with Gene *H* region, responsible for cane hair presence. Associations between these metabolites and cane hair phenotype merit further study. Other QTLs in this study had links to genetic markers of whole plant development. Notable were RiMYB (82.5 cM) and Ri4CL1SNP (83.6 cM) on LG 3 with QTLs for fruit ripening, colour and taste development (Figure 5.2). Co-location of QTLs and markers related to plant physicochemical traits, development and metabolite transport, suggest common regulation and consequent development of fruit flavour quality is complex, determined not just by metabolites but also by various other plant traits.

5.3 Sweetness, sourness and flavour intensity in raspberry

5.3.1 Environment: Effects of seasons and cultivation practices

Key findings:

- Increased water availability in 2007 produced field crop that was rated sourer and more intensely flavoured, but less sweet.
- Polytunnel cultivation increased sensory scores for all studied flavour traits.

Hotter and drier summer in 2006 produced a crop higher in sugars and acids contents and also scored higher for sweetness and flavour intensity, but lower in sourness, both desirable outcomes. The cooler and wetter 2007 summer yielded field crop with significantly increased sourness and flavour intensity scores but with no significant difference in sweetness (**Chapter 3)**. Univariate correlations between flavour traits were also less clear in 2007 field crop. Therefore, for unprotected field plants, a hotter, drier season was more favourable in producing fruits of desirable flavour character.

However in this study, of interest was effects on and relationship between data on flavour trait intensity and fruit contents of metabolites, volatile and non-volatile. The prevailing weather and cultivation strategy were found to have significant effects on both flavour traits and sugar and acids contents. Polytunnel cultivation produced fruits

that scored higher for desirable flavour traits, sweetness and flavour intensity, which showed a strong (univariate) correlation. Sourness showed a weaker but still significant correlation with flavour intensity and was inversely related to sweetness. From Pearson's analyses, total soluble solids (i.e. ^{o}Brix) was significantly correlated with sweetness and flavour intensity of polytunnel crop and also with total sugars contents of SCRI field crops. In kiwifruit, ^{o}Brix was correlated with sweetness and flavour acceptability but not sourness or acidity (Rossiter et al., 2000). However increased ^{o}Brix values in these kiwifruits were not reflected in enhanced scoring of 'flavour intensity', suggesting greater contributions by interactions of sugars and organic acids, with or without contributions by volatile flavour compounds and /or interactions of these metabolites to this trait (Rossiter et al*.,* 2000). From univariate correlations in this study, flavour character was contributed significantly by both sugars (non-volatile) and hexenol (volatile) contents. Raspberry ketone content alone did not significantly impact flavour traits in berries, but from models of this study (**Chapter 4)**, its influence is possibly compounded with collective impact of other volatiles and non-volatiles on sensory perceptions. This factor, coupled with its low odour threshold value, make raspberry ketone an important contributor to characteristic raspberry aroma. Therefore, contributions by volatiles to development of good flavour berries merit further examination. From this study, polytunnel cultivation had positive effects on metabolite accumulation and flavour trait development, shown to be significant linked from univariately correlated and also linked to a number of other variables from regression models.

Sourness is an important sensory trait correlated both with metabolites contents and for consumer acceptance in other fruit and vegetable crops; chicory, common bean and pomegranate (Bunning et al*.,* 2010, Florez et al*.,* 2009; Borochov-Neori et al*.,* 2009). In this study, sourness was important only in the field crops; organic acids content and sourness both rose significantly in 2007. This could be explained from both increased acids contents due to greater water uptake (with greater water availability) and due to dilution of other flavour-active metabolites. However, effects of acids content on flavour

traits are unclear. In kiwifruit and mango, low dry matter pulps scored higher for sweetness and sourness with addition of sugars, except when pulps were already perceived sour prior to sugar addition (Malundo et al., 2001, Marsh et al., 2003, 2006). Sugars content, more than acids, was important for release of flavour-active volatiles (i.e. aldehydes and alcohols), which contribute to sweetness, and *banana* and *lemon* notes in kiwifruit (Marsh et al., 2003), and also in mango (Malundo et al., 2001). Therefore, sugars participate in not only taste, but aroma perceptions as well. However, acids content possibly temper sweetness from sugars, and therein is its importance, because consumers judge fruit flavour quality based on balance of sweetness and sourness and not just on sweetness.

In tomato, relationships were established between specific metabolite contents and flavour characters; a '*sweet*' trait was linked with multiple metabolite QTLs for sucrose, soluble solids content and volatiles eugenol and hex-3-en-1-ol (Saliba-Colombani et al., 2001). Similar links of total soluble solids (^{o}Brix) with sweetness and flavour intensity were found in this study, in 2006 field and 2007 polytunnel crops. From multivariate models, volatiles contributed to sweetness and flavour intensity variances, possibly by a combination rather than a single volatile species, and also from its interactions with non-volatiles (**Chapter 4**). Links of metabolite contents to sensory traits were also supported by overlap of flavour trait and volatile QTLs on LG 2, 3 and 5. It is thus apparent that flavour development in these crops is complex resulting from a culmination of influences beginning with abiotic factors exerting significant effects on fruit metabolites accumulation. These factors ultimately determine final contents and consequent profiles of flavour-active volatile and non-volatile compounds. At consumption, all these factors culminate and determine sensory ratings of mature (ripe) raspberries and assessment of final flavour quality by multiple retailer buyers.

5.3.2 Genetic: Co-localisation of flavour QTLs with other raspberry traits

Key findings:

- Preliminary associations of flavour traits and candidate genes / markers are:
    - sourness QTL and *F3'H* gene for anthocyanin biosynthesis,
    - flavour intensity QTL and RiD4R1 and RiD4R2 transcription factors,
    - sourness QTL with raspberry TIP (metabolite and water transport), MYB-transcription factor and 4-coumarate ligase (anthocyanin biosynthesis).
- At present proposed QTLs for flavour traits are simple linkages, which could be better defined by further fine QTL mapping and other molecular strategies, e.g. identifying SNPs in other *Rosaceae* crops.

Overlapping QTLs for raspberry sensory flavour traits and metabolites contents suggest common relationships. Co-localisations of flavour traits QTLs with QTLs of specific volatile compounds, colour and plant development processes (Figure 5.1- 5.4) offered insight into factors determining flavour character and also possible strategies for control and manipulation of flavour quality. For example, genes *H* and *F3'H* linked with flavour QTLs, which suggests regulation of cane hair presence and pigment development phenotypes may also impact on flavour development. Associations between regulatory and metabolite transport systems markers; MYB-transcription factor (LG 3), MIP and TIP aquaporins (LG 2) and QTLs for sweetness, sourness and flavour intensity (Figure 5.2) also imply common regulation. These preliminary findings are encouraging results for development of plant breeding strategies to develop good flavour berry crops, because preliminary QTLs and links to genetic markers will serve as starting point for further investigations, to validate these associations and assess the possibility of using plant phenotypes; colour, size and weight, as flavour character indicators. Furthermore, with refined QTLs from modern strategies, marker assisted pre-selection of progenies at seedling level for premium flavour characters will become possible. From evident impact of polytunnel cultivation on metabolites contents and sensory flavour trait intensities, plants genetically pre-selected for specific flavour attributes could have

enhanced flavour development under this cultivation strategy, possibly due to reduced genetic x environment interactions.

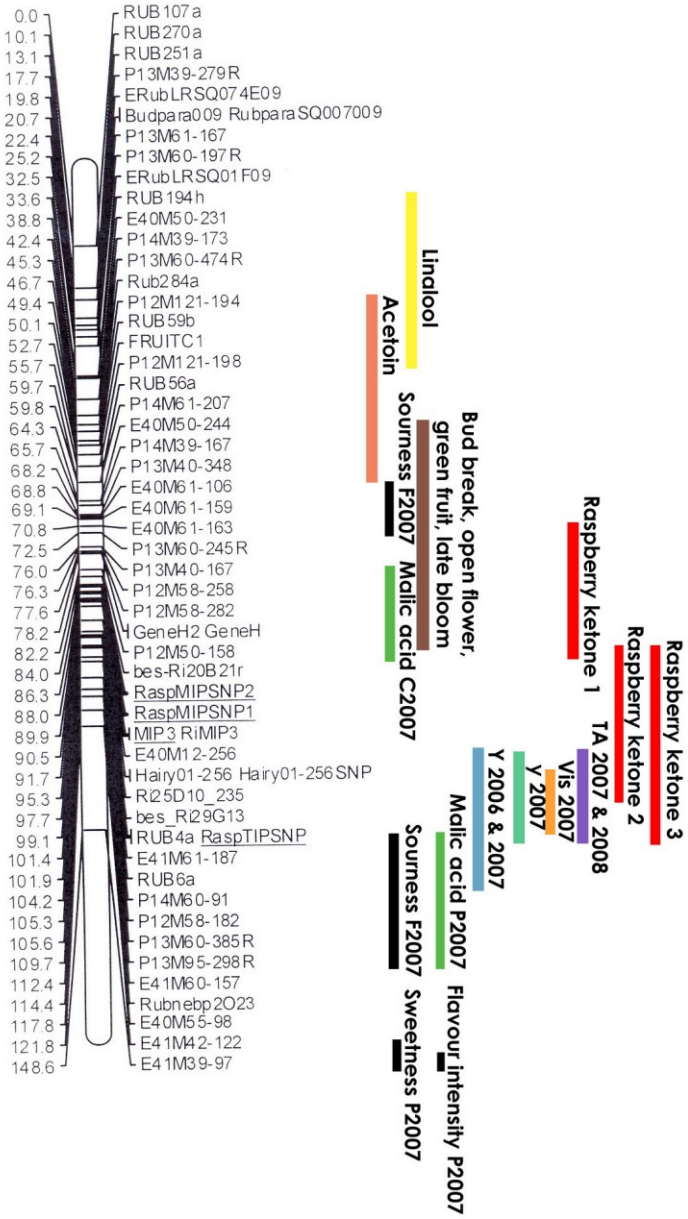


Figure 5.1: Fruit quality QTLs on LG2

Variables	Source
Flavour, sugars and acids	This study
Plant development	Graham et al., 2009
Colour parameters and titratable acidity (Ta)	McCallum et al., 2010
Volatiles	Kassim, 2009 (PhD thesis)

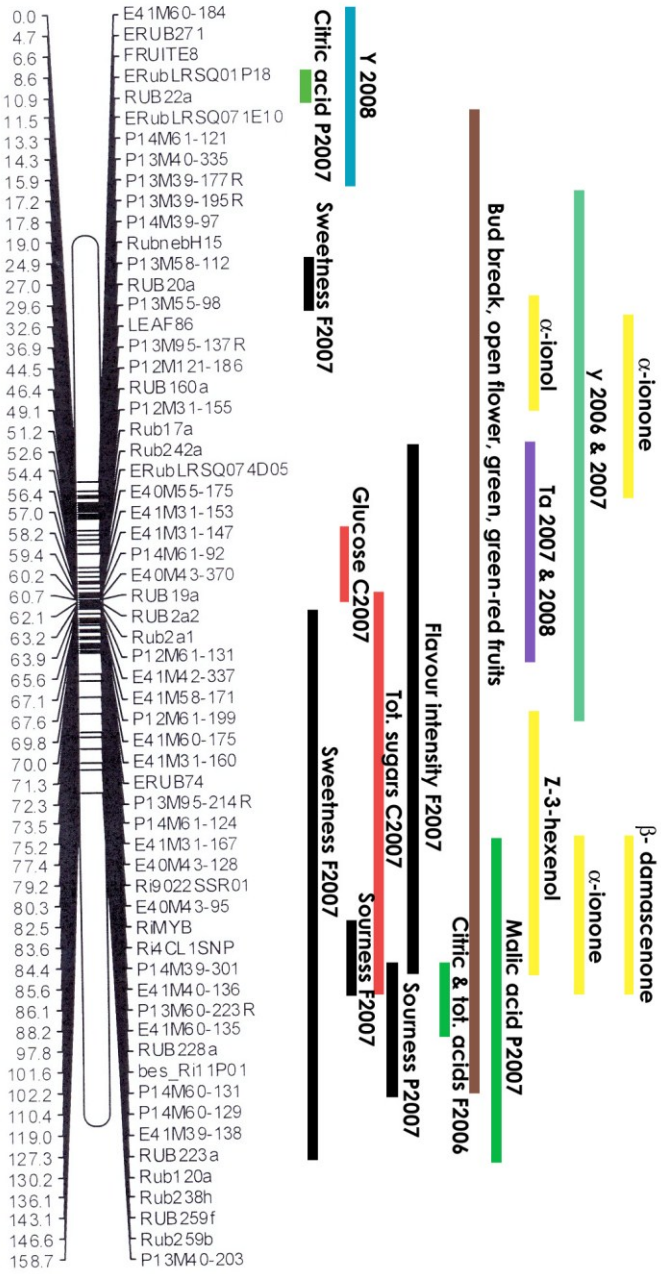


Figure 5.2: Fruit quality QTLs on LG3

Variables	Source
Flavour, sugars and acids	This study
Plant development	Graham et al., 2009
Colour parameters and titratable acidity (Ta)	McCallum et al., 2010
Volatiles	Kassim, 2009 (PhD thesis)

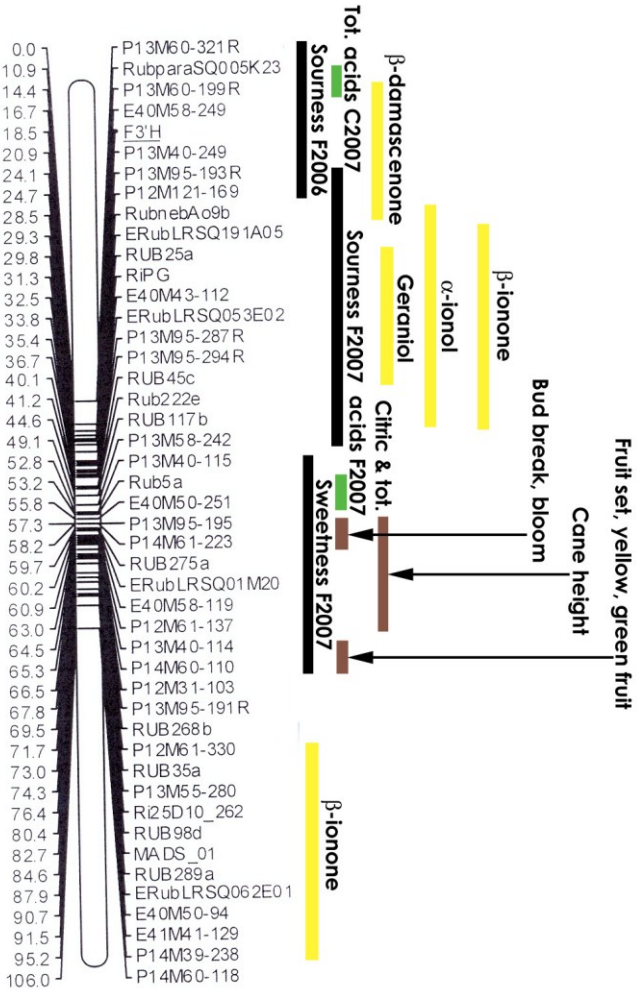


Figure 5.3: Fruit quality QTLs on LG4

Variables	Source
Flavour, sugars and acids	This study
Plant development	Graham et al., 2009
Colour parameters and titratable acidity (Ta)	McCallum et al., 2010
Anthocynins	Kassim et al., 2009
Volatiles	Kassim, 2009 (PhD thesis)

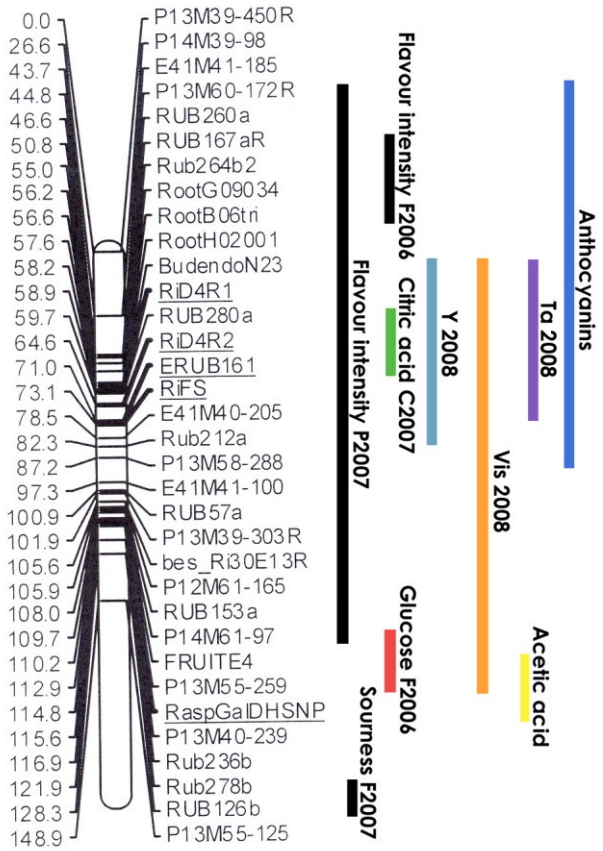


Figure 5.4: Fruit quality QTLs on LG5

Variables	Source
Flavour, sugars and acids	This study
Plant development	Graham et al., 2009
Colour parameters and titratable acidity (Ta)	McCallum et al., 2010
Volatiles	Kasssim, 2009 (PhD thesis)

5.4 Modelling flavour characters in raspberry

5.4.1 Sweetness

Key findings:

- Total soluble solids contents (^{o}Brix) correlated well to variance in sweetness.
- Volatiles also contributed to variance in sweetness.
- Hexenol contributed to positive explanation of sweetness variance and linalool negatively.
- Links between sweetness and colour traits are implied from fruit groupings.
- Berry weight also affected sweetness; heavier berries scored higher for sweetness.

In PLS1 models for Y-sweetness (**Chapter 4**), variance explained increased when X-volatiles data was included in the model based on X-non-volatiles (62% to 71%). This suggested sweetness scoring was based on both aroma and oral (gustation) stimuli. Importance of volatiles contents to sweetness perceptions was further demonstrated by effects of raspberry ketone, indirectly from its compounded effects with sugars and acids contents, shown in models. Although β-coefficient values that represented effects by raspberry ketone on sweetness perceptions were low, its importance was implied from increased explained variance with its inclusion into models. As mentioned previously, other studies also concluded similar results in fresh and processed raspberries, where heating increased flavour traits specific to raspberries and 'sweet' aroma notes, presumably from liberation of glycosidically-bound raspberry ketone (Larsen and Poll, 1990; Klesk et al., 2004). From findings here and in other studies, effects by volatiles contents on flavour traits is likely, but when coupled with effects by other metabolites, e.g. sugars and acids.

From univariate correlations, TSS as estimated by ^{o}Brix had significant links with sweetness. This link was further substantiated by its multivariate links to sweetness in regression models. However, it was observed univariate links were not sustained in all

crops across harvest years and cultivation sites. Interactions with volatiles contents, as mentioned previously, and other abiotic factors possibly influenced these links, indicated in PLS-1 models. Thus, though the attraction of using TSS values, measured by ^{o}Brix as an instrumental measurement of flavour quality is great, it may mislead researchers, as models revealed sweetness to be affected by not only non-volatile sugars and acids but also by volatiles contents, berry weight and colour parameters, making it a multi-factorial trait.

5.5.2 Sourness

Key findings:

- Sourness was affected greater by fruit physical traits; berry weight and colour brightness, than metabolite contents, from PLS1 models
- Sourness variance was correlated to total soluble solids content, indicated by ^{o}Brix and not consistently with acids contents.
- Pigment-related variables had significant links with sourness; colour brightness was the most positive factor, but sourness only had weak correlations with anthocyanin pigment contents.
- Fructose and malic acid negatively impacted sourness, but citric acid positively.

Modelling sensory sourness from metabolite and / or physicochemical data prove problematic. Neither univariate nor multivariate regression analyses produced coherent explanations of variance. Multivariate PLS1 models which best explained Y sourness were those with fruit physical traits as independent X-variables; ^{o}Brix, 10-berry weight and colourmeter parameters. This suggested berry colour, juice content and total soluble solids contents influenced sourness scoring at time of consumption (**Chapter 4**). Another possible factor for lack of sourness variance explanations may be individual assessor variation creating noise in Y-data (dependent variable). Wide variations in sourness detection sensitivity have been attributed to differences in assessor saliva flow rate and anion detection on the tongue (Norris et al*.,* 1984; Christensen et al*.,* 1987; DeSimone et al*,*. 2001; Neta et al*.,* 2007). Physiological changes in assessors, natural or

disease-caused, can compromise acuteness of sourness detection (Grzegorczyk et al., 1979; Mojet et al., 2001; Hershkovich and Nagler, 2004). At the time of sensory evaluations, all assessors were deemed of good health, but varied age range and sensory experiences could contribute to noise in scoring that culminated in limited explanations of total variance in models. Nonetheless, well correlated univariate factors affecting this flavour trait here are worthy of further study, notably links between sourness and other fruit variables, for example fruit acidity factors and colour.

5.5.3 Flavour intensity

Key findings:

- More flavour intensity variance was explained by fruit volatiles than non-volatiles.
- Fruit sugars content increased influence of volatiles and acids content decreased it.
- Sugars and volatiles contents had more impact on flavour intensity variance, rather than sweetness perceptions identified from univariate correlations,
- Of independent variables, total soluble solids contents (^{o}Brix), berry size and water content (10-berry weight) explained highest variance in flavour intensity.
- In colour variables, all colourmeter readings, except brightness, contributed positively to explanations of flavour intensity variance.

Initial univariate correlations between sweetness and flavour intensity could be partially explained by common effects from flavour metabolites in volatiles and non-volatiles contents. From this study, flavour intensity was not a product of additive and subtractive effects by sugars and acids respectively, or parallel increases in level sensory stimuli, as suggested by others (Schifferstein and Frijters, 1990; Stevens, 1957). Scoring could be better explained by an alternative sensory perception hypothesis (Reed et al*.,* 2006), which takes into account more independent X-variables influencing flavour character scorings than just effects by metabolite contents on taste and aroma perceptions. The links between flavour intensity and

colour-related variables, i.e. anthocyanin pigments, though modest, could be due to common biosynthetic links, for example to carotenoid degradation, which contribute precursors for both aroma and pigment colour biosyntheses, reported also in tomato, watermelon (Lewinsohn et al., 2001, 2005) and melon (Ibdah et al., 2006). This hypothesis is also supported by co-location of flavour intensity with anthocyanin and visual colour score QTLs. As increased aroma is often related to increased colour during ripening in fruits, common regulations for the relevant metabolites is likely. Such variance explanations in flavour intensity perceptions can be validated through gene expression and metabolomics studies.

5.5 Raspberry ketone and flavour volatiles contribution to sensory character

5.5.1 Sweetness and flavour intensity

Key findings:

- Modest volatiles influences were more pronounced on flavour intensity than on sweetness.
- Of volatiles the ionones were most influential in explanations of flavour intensity variance.
- From multivariate models, hexenol reduced influence of raspberry ketone on both sweetness and flavour intensity.
- From models, it was particularly the joint effects of raspberry ketone with other metabolites (i.e. sugars and other volatiles), which explained sensory variance.

Sweetness and flavour intensity had significant correlations, shown in both univariate and multivariate regression analyses. As mentioned previously, links of sweetness and raspberry ketone was demonstrated in other raspberry studies, through characteristic aroma notes from raspberry ketone (RK) and other volatiles, notably ‘perfume’, ‘hot tea’ and ‘woody’. In fresh raspberries, it was shown that sweet aroma notes from RK contents increased sweetness perceptions and consequently influence flavour intensity scorings (Klesk et al., 2004). These enhanced effects by sweet aroma notes also

modified or negated aromas produced by undesirable volatiles, for example pungency from acetic acid in wine, which reduces its flavour quality (Rapp and Mandery 1986). In this study, RK inclusion into models increased β-coefficient values of acetic acid but also values for benzyl alcohol, α- and β-ionone, all responsible for producing 'sweet' aroma notes which possibly mitigated sour notes from acetic acid (Table 5.3. Taste-aroma interactions were reviewed extensively by Nobel (Noble, 1996), and it was concluded contributions by aroma to taste was of more consequence than taste to aroma. This was shown in apples, where volatile contents, notably interactions between butanol with hexyl and 2-methybutyl acetates, significantly increased apple flavour perceptions (Young et al*.,* 1996). However, contradictory evidence was found in time-intensity experiments of aroma and taste interactions, where sugars (sucrose) was more influential than the volatile isoamyl acetate, in increasing fruit flavour perceptions, from a multichannel flavour delivery system (Dynataste), but it was also shown scoring was susceptible to inter-panel variation (Hort and Hollowood, 2004). Apart from RK and other metabolites contents and its interaction as a factor in flavour perceptions, plant traits determining final contents of flavour metabolites in fruits could indirectly influence. In this study, there were co-locations of sweetness, sourness and flavour intensity QTLs with volatile QTLs (β-damascenone, α-ionone and Z-3-hexenol) on LG3. These QTLs also co-located to acids contents QTLs and the MYB-TF marker, important in anthocyanin development (Kassim et al., 2009). A review (Delwiche, 2004) identified importance of colour to flavour perceptions but supporting data was based on reference solutions rather than real food matrices. In strawberry, for example, aroma and not the characteristic red colour impacted more on flavour scorings (Frank et al*.,* 1989). In this study, it was brightness and but not overall berry colour, which accounted for higher percentages of flavour trait variance in PLS1 models. This finding suggests parameters other than absolute sugars, acids and volatile contents affect flavour scorings and therefore flavour quality in raspberries is part of multi-factorial trait, which possibly has shared regulation systems and common metabolites; a finding similar to other fruit crops.

Table 5.3 Volatiles identified in American red raspberry cv. Meeker (Klesk et al., 2004)

Volatiles	Aroma notes
Benzyl alcohol	*Floral, perfume, raspberry*
Acetic acid	*Pungent, sour, vinegar*
α- ionone	*Rose, floral, sweet, perfume*
β- ionone	*Floral, perfume, raspberry*

5.6 Implications for other *Rosaceae* crops

5.6.1 QTL Mapping

To facilitate marker-assisted breeding for premium quality raspberries, mapping of sensory and biochemical datasets onto nucleotide polymorphisms produced, as preliminary results from this study, not only QTLs but also chromosomal regions and candidate genes potentially important to flavour development. Co-location of QTLs to molecular markers for metabolite transport proteins, transcription factors and other metabolic structural genes suggested flavour development may share common regulation pathways as other plant development traits. These preliminary findings advance our understanding of which factors are important to fruit quality development and help focus efforts by breeders on developing genetic tools to select seedlings with the right genetic structural basis for enhanced metabolite accumulation, which will expedite production of new raspberry varieties of premium flavour quality to fulfil market demand. To date, QTL mapping for fruit flavour quality has been documented in other *Rosaceae* species; in peach (Dirlewanger et al., 1999; Etienne et al., 2002; Quilot et al., 2004; Ogundiwin et al., 2009) and apple (King et al., 2001; Kenis et al., 2008; Dunemann et al., 2009). Peach is a reference fruit in *Rosaceae* marker-assisted breeding, partly due to its small haploid genome (300 Mbp). There were comparable genetic findings from this study and in peach as co-locations of sweetness and acid content QTLs. Considering genome sizes of the two crops are similar (red raspberry: 275 Mbp; peach: 300 Mbp) genetic regulations involved in fruit trait expression may be

comparable in both crops (Quilot et al., 2004, Jung et al., 2004). Other genomic efforts in red raspberry have identified genomic regions important in colour-related variables; scored colour and pigment production, and other fruit trait QTLs; berry yield and berry weight (Kassim et al., 2009; Graham et al., 2009; McCallum et al., 2010) and most recently in volatiles contents (Paterson et al., 2012), as results of parallel studies conducted alongside this research.

Findings in this study of environmental influences and possibly genetic x environmental interactions on metabolites contents in raspberry were parallel to those reported in peach. Metabolite QTLs were affected by environment x genotypes interactions; demonstrated by physiological model experiments that showed water flux was a crucial factor in determining fruit flesh sugar contents (Quilot et al., 2005). Here, similar relationships with fruit water contents were identified from univariate and multivariate regressions and QTL mapping analyses. Parallel findings in peach and from this study could benefit broader marker-assisted breeding efforts in *Rosaceae*, by accelerating production of molecular markers for factors relevant to flavour quality development, widely applicable to a large number of related species.

5.6.2 Flavour quality PLS1 models

Flavour quality models specific to *Rosaceae* crops are scarce, compared to other fruit crops notably the model tomato (Jones and Scott, 1983; Malundo et al., 1995; Bucheli et al., 1999; Tandon et al., 2003; Abegaz et al., 2004) and kiwifruit (Ball et al., 1998; Wismer et al., 2005; Marsh et al., 2006; Harker et al., 2009). Models in *Rosaceae* crops relating metabolites content to flavour quality indicate ^{o}Brix as a good predictor of flavour and other sensory qualities, found in peach and nectarine, despite significantly varying contents of sugars and acids (Crisosto et al., 2006). The contrary finding of this study could be due to small size of raspberries compared to peach and nectarine, indicated by significant correlations with the variable berry weight in models. In apple, where flavour quality modelling efforts have been extensive, identified post harvest physical changes also affected changes in volatile profiles (Natale et al., 2001; Saevels

et al., 2004). Although this was not investigated in this study, it is well known red raspberry has a short shelf-life after harvest and prone to bruising. Therefore, this presumably would also affect its flavour traits resulting from volatiles contents. In parallel studies of red raspberry aroma profiles (Kassim et al., 2009; Paterson et al., 2012), approximately 80% of identified volatiles responsible for raspberry flavour quality were common to blackberries, a close relative (Du et al., 2010). Therefore, preliminary findings from models are possibly transferable to other *Rosaceae* crops, with more likely success in closely related species.

Market success for any edible fruit hinges on good flavour quality. Therefore identifying crucial contributing factors to its development is an important effort. Breeding efforts to produce commercial varieties may cause reduced or lack of sensory performance; demonstrated from flavour quality modelling in kiwifruit. In this fruit crop flavour traits 'sweetness, 'honest cooked sugars' and 'blackcurrant' found in non-commercial varieties were more significant in driving sensory panellists' preference than flavour characters of commercially important kiwifruit (Wismer et al., 2005). In this study it was apparent different sensory and biochemical profiles were found in different commercial varieties, including parent cultivars of the mapping population. Although preference was not scored in this study, there was concurrence amongst sensory panellists that some commercial varieties and progeny berries performed better than others in sensory tests. A proposed explanation for reduced flavour quality in commercial fruit crops is the narrow genetic diversity resulting from repeated inbreeding, as explained previously in **Chapter 1**.

Differences in cultivation method and sites significantly affected metabolites content and flavour profiles in this study, indicated from univariate and multivariate regression analyses. Flavour quality models showed sour field crops were attributed to acids content and berry colour brightness, while in polytunnel crops, which were sweeter, it was driven by ^{o}Brix and all colourmeter values except brightness. A few of these factors were also mapped QTLs identified in this or parallel studies. However, as QTLs

identified in this study were relatively large and varied between crops, these can only serve, at best, as preliminary indicators of factors with most affect flavour quality. Further advanced genetic approaches like SNP arrays and further fine QTL mapping of these populations would yield more robust QTLs to utilise in marker-assisted breeding. Possibly from indicated correlations, QTLs developed to select seedlings for physicochemical traits; e.g. berry weight and increased pigment production, could also result in parallel improved flavour quality traits. However, from effects of polytunnel cultivation identified here, genotype x environment interactions is possibly more influential in flavour quality determination, than just genotype alone.

5.7 Principal findings from this study

Of factors affecting flavour quality examined here, polytunnel cultivation had increased metabolites contents and sensory scores for key flavour traits. In field crop, different weather conditions in the two harvest years resulted in lower sugars content and higher sourness scorings in 2007. Therefore, conditions most favourable for flavour quality developments were achieved under polytunnel cultivation, with possibility of further improvement by application of potassium-enriched fertilisers, as shown in the Commercial polytunnel fruit.

There were correlations identified in this study between sensory traits and metabolites contents; total soluble solids (^{o}Brix) and to other plant or fruit trait; colourmeter values and 10-berry weight. Preliminary flavour quality QTLs (sensory flavour traits, sugars and acids contents) were also identified with linkage to markers associated to plant metabolites / traits; anthocyanin pigment content, cane hair presence and metabolite transport systems. These multivariate and genetic correlations, demonstrated how flavour quality in raspberry is a complex multi-factorial trait, possibly controlled by a common regulation with plant morphogenesis and developmental processes in red raspberry.

Multivariate regression models in this study also yielded similar correlations between flavour traits with metabolites content as those found in univariate correlation and QTL mapping analyses. In these models, sweetness and flavour intensity were better explained by independent variables than sourness. Most interestingly models indicated relationship between sugars, acids and specific volatiles contents, where sensory explained variance was attributed to combined rather than singular effects, for example volatiles data added into sugars and acids models increased variance explained in sweetness. Raspberry ketone did not have significant univariate correlations with flavour traits but in models, effects by raspberry ketone on sensory traits were compounded with contributions from sugar and acids contents. Although these correlations are preliminary and require validation, early indications of factors affecting flavour quality development identified here provide focus for future research efforts into red raspberry flavour quality, for fruit breeders and food scientists alike, with the common aim to create new cultivars of premium quality red raspberries demanded by stakeholders.

5.8 Applications/ Future work

5.8.1 Flavour quality QTLs: Metabolite contents and flavour traits

Preliminary flavour quality QTLs identified had simple genetic links with metabolite-related markers, involved in its biosynthesis and transport, and with gene regulation factors affecting other plant traits. Firstly, these QTLs must be validated through more advanced molecular approaches. For example, by developing SNP arrays specific to each flavour trait and its affecting variables. These arrays should be used not only with raspberry DNA but genetic material of other *Rosaceae* species, to ensure QTLs are robust and applicable for breeding efforts in other *Rosaceae* crops.

As most QTLs identified in this study was cultivation site specific, effects from genotype x environment interactions are likely. These QTLs had co-localised to markers or genes responsible for regulation of other plant traits, notably to phenotypic specific genes (gene *H* for cane hair presence) and enzymes affecting colour pigment development. Further expression studies should be conducted to see if increased flavour

trait scores corresponds to parallel gene expression or increased enzyme productions for these specific phenotypes; cane hair presence and colour. Positive correlations can yield strategies for flavour quality monitoring during plant development and prior to harvest.

5.8.2 Flavour models

From models, sweetness and flavour intensity in red raspberry were driven by effects of sugars and acids contents, and from its associations to volatiles contents. Similar compounded effects were found essential to 'flavour intensity' in kiwifruit and affected characteristic aroma in apple, where specific volatiles had different contributions to apple aroma and some volatile species reduced aroma intensities (Bult et al., 2002). Modifying effects by volatiles contents were also indicated in this study, where raspberry ketone and hexenol contents enhanced or muted effects of sugars and acids on flavour traits. Because of these correlations, a plant breeding strategy aimed at increasing sugars, acids and volatiles contents is possibly more effective in producing berry crops with premium flavour quality than just a strategy to increase sugars and acids contents alone. Furthermore, models indicated physicochemical traits not previously thought to impact on sweetness and flavour intensity of raspberry crops; colour associated variables and berry weight. Validation of these correlations should be performed and if links are reliable, present an easy instrumental method to assess flavour quality of berry crops during plant growth and prior to harvest. Therefore, similar to genetic links, preliminary variables identified in models affecting flavour quality traits could provide focus for future research efforts on which factors most impact on red raspberry flavour quality and help to develop methods to control and manipulate final flavour quality.

5.9 Conclusion

Flavour quality, in red raspberry, is a complex trait determined by a number of variables and factors. Polytunnel cultivation was shown to influence key variables, possibly from reduced environmental effects and increased genetic x environment interactions on flavour quality development. Preliminary QTL mapping yielded links with markers to

other plant trait development pathways. Validation of these links could help molecular breeders develop new markers for plant / fruit traits that could also indirectly select seedlings for good flavour quality. Genetic links of flavour traits with plant phenotypes, like cane hair presence and enzyme expression for fruit pigment production, merit further validation study and could offer potential methods to monitor pre-harvest flavour development in crops. This will further progress efforts in developing new raspberry varieties with good flavour quality for the UK fresh fruit market. However, genetic seedling pre-selection for elevated metabolites contents and premium sensory flavour traits is only a part of the breeding strategy, because effects by genetic x environment interactions is possibly stronger than genotype alone; demonstrated in polytunnel crops from this study. Therefore, the combined strategy of genetic pre-selection with polytunnel cultivation would appeal to the UK fresh fruit market, because it provides an alternative to genetic manipulation (GM) methods, as there is great consumer and retailer concern over GM safety and its effects on crop vigour.

CHAPTER 6

References

Chapter 6 References

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Appendices

Appendices

Appendix 1 QTLs and genes of fruit quality traits in other important fruit crops: Melon

Fruit/Cross	Trait	Gene/QTL, LG	Associated sensory traits	Gene/QTL,LG	Reference
MELON					
C. *melo* L. cv. Piel de Sapo x Accession PI161375	Fruit size	QTL fs1.1, fs3.1, fs5.1, fs6.1, fs6.2, fs7.1, fs9.1, fs11.1, LG1, 3, 5-7, 9, 11	-n/a-	-n/a-	Montforte et al., 2004
	Soluble solids	QTL ssc1.1, ssc2.1, ssc4.1, ssc4.2, ssc8.1, LG1, 2, 4, 8	-n/a-	-n/a-	
	External colour	QTL ecol3.1, ecol7.1, ecol9.1, ecol10.1, LG3, 7, 9, 10	-n/a-	-n/a-	
	Flesh colour	QTL gfc1.1, ofc2.1, ofc3.1, ofc12.1, LG1, 2, 3, 12	-n/a-	-n/a-	
C. *melo* var. inodorous H. Jacq. x Accession PI161375	Glucose	QTL on LG3 QTL on LG3, 8 QTL on LG55, 8, 9	Sourness Bitterness Sweetness	QTL on LG3 QTL on LG3, 8 QTLs on LG5, 8, 9	Obando-Ulloa et al., 2009
	Fructose	QTL on LG5, 8 QTL on LG5, 8 QTL on LG5, 9, 11	Taste Hedonics Sweetness	QTL on LG5, 8 QTL on LG5, 8 QTL on LG5, 9, 11	

Appendix 1 QTLs and genes of fruit quality traits in other important fruit crops: Melon (cont.)

Fruit/Cross	Trait	Gene/QTL, LG	Associated sensory traits	Gene/QTL,LG	Reference
C. *melo* var. inodorous H. Jacq. x Accession PI161375	Fructose	QTL on LG5, 12	Taste	QTL on LG5, 12	Obando-Ulloa et al., 2009 (continued)
	Sucrose	QTL on LG 5	Hedonics	QTL on LG5	
		QTL on LG3, 10	Sweetness	LG 3, 10	
		QTL on LG3, 8, 10	Taste	QTL on LG3, 8, 10	
	Sucrose equivalents	QTL on LG8, 10	Hedonics	QTL on LG8, 10	
		QTL on LG8	Bitterness	QTL on LG8	
		QTL on LG10	Sweetness	QTL on LG10	
		QTL on LG8, 10	Taste	QTL on LG8, 10	
	Total sugars	QTL on LG5	Bitterness	QTL on LG5	
		QTL on LG3, 10	Hedonics	QTL on LG3, 10	
		QTL on LG10	Sweetness	QTL on LG10	
		QTL on LG5, 8	Bitterness	QTL on LG 5, 8	
		QTL on LG8, 10	Taste	QTL on LG8, 10	
	Glutamic acid	QTL onLG3, 8, 10	Hedonics	QTL on LG 3, 8, 10	
		QTL on LG8	Bitterness	QTL on LG8	

Appendix 1 QTLs and genes of fruit quality traits in other important fruit crops: Melon (cont.)

Fruit/Cross	Trait	Gene/QTL, LG	Associated sensory traits	Gene/QTL,LG	Reference
C. *melo* var. inodorous H. Jacq. x Accession PI161375	Glutamic acid	QTL on LG8	Taste	QTL on LG8	Obando-Ulloa et al., 2009 (continued)
		QTL on LG8	Hedonics	QTL on LG8	
	Oxalacetic acid	QTL on LG7	Sweetness	QTL on LG7	
		QTL on LG3	Sourness	QTL on LG3	
		QTL on LG3	Bitterness	QTL on LG3	
		QTL on LG7, 8	Taste	QTL on LG7, 8	
	Ascorbic acid	QTL on LG4, 7	Hedonics	QTL on LG4, 7	
	Citric acid	QTL on LG10	Sweetness	QTL on LG10	
		QTL on LG10	Taste	QTL on LG10	
		QTL on LG10	Hedonics	QTL on LG10	
	Fumaric acid	QTL on LG3, 8	Sweetness	QTL on LG3, 8	
		QTL on LG3	Taste	QTL on LG3	
		QTL on LG8	Hedonics	QTL on LG8	
	Succinic acid	QTL on LG8	Taste	QTL on LG8	
		QTL on LG10	Sweetness	QTL on LG10	
		QTL on LG8, 10	Taste	QTL on LG 8, 10	
		QTL on LG3, 4, 10	Hedonics	QTL on LG3, 4, 10	
C. *melo* subsp. *agrestis* var. momordica x subsp. *melo* var. Reticulatus	Sucrose	QTL suc2.1, suc2.2, suc063.1, suc073.1, suc5.1, suc8.1, LG2-3, 5, 8	Flesh softness -n/a-	QTL flc2.1, flc6.1, LG2, 6	Harel-Beja et al., 2010

Appendix 1 QTLs and genes of fruit quality traits in other important fruit crops: Melon (cont.)

Fruit/Cross	Trait	Gene/QTL, LG	Associated sensory traits	Gene/QTL,LG	Reference
C. *melo* subsp. *agrestis* var. momordica x subsp. *melo* var. reticulatus	Glucose	QTL glu4.1, LG4	-n/a-		Harel-Beja et al., 2010
	Total soluble solids	QTL tss2.1, tss2.2, tss5.1, LG2, 5	Flesh colour	QTL flc6.1, LG6	
	Total carotenoids	QTL car6.1, car8.1, LG 6, 8	Flesh colour	QTL flc8.1, LG8	
	β-carotene	QTL βcr2.1, βcr6.1, LG2, 6	Flesh colour	QTL flc8.1, LG8	
	Phytoene	QTL phy6.1, phy6.2, LG6	Flesh colour	QTL flc8.1, LG8	
	α-carotene	QTL αcr8.1, LG8	Flesh colour	QTL flc8.1, LG8	
	Lutein	QTL lut8.1, LG8	**Flesh colour**	**QTL flc8.1, LG8**	

List of publications

List of Publications

I.

Plant and Animal Genomes XVI Conference
January 12-16, 2008
Town & Country Convention Centre
San Diego, CA

Progress In Marker Assisted Breeding In Red Raspberry For Flavour Character

Dzeti Zait[1], Angzzas Kassim[1], Julie Graham[2], Susan McCallum[2] & Alistair Paterson[1]

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Red raspberry (R. ideaus) is a profitable temperate soft-fruit crop (1). Increased consumption would benefit consumer health (2). However current retail purchase may be prejudiced by inconsistent quality. Consumers assess berries visually at purchase and subsequently on flavour character, and together these determine repeat purchase. Identification of *quantitative trait loci* (QTLs) associated with development of berry sweetness and flavour intensity would yield markers for favourable alleles and a toolkit for marker assisted breeding.

An early fruiting Glen Moy was crossed with late-cropping, North American Latham and progeny canes were established on an open field and two locations of covered sites. Trained assessors scored berries for sweetness, sourness and flavour intensity; sugars and organic acid contents were quantified by HPLC. QTLs were mapped on to a revised genetic linkage map for red raspberry (2). Appearance was studied by quantifying pigment anthocyanins, instrumental measurement and visual colour scoring and 10-berry weight. Preliminary data showed correlation between 3 raspberry flavour attributes was preserved over two seasons, indicating genetic control is greater than environmental influences on flavour development. *Single nucleotide polymorphisms* (SNPs) differentiating parental genomes were identified through PCR of candidate genes with primers designed on the basis of sequence data from other *Rosaceae* members.

(1) http://faostat.fao.org/
(2) Ross et al (2007), "Antiproliferative activity is predominantly associated with ellagitannins in raspberry extracts" Phytochemistry 68, 218 - 228
(3) Graham et al (2004), "Construction of a genetic linkage map of red raspberry (Rubus idaeus subsp. idaeus) based on AFLPs, genomic-SSR and EST-SSR markers" Theor. Applied Gen. 109, 740-749.

II.

II.

Oral and poster presentation
10th Sensometrics Conference
26th - 28th July 2010
Rotterdam, Netherlands

P0020:

Modelling to understand sweetness, sourness and flavour intensity scoring of red raspberries to facilitate marker assisted breeding

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This study explored the basis of three key attributes of raspberry flavour: sweetness, sourness and flavour intensity. Fruits were available from progeny (<188) from a cross of a North American subspecies (Latham) with a Scottish variety (Glen Moy) that had been planted in three environments: field (2006 & 2007) and covered polytunnels (2007) at SCRI; and polytunnel at a commercial site near Blairgowrie (2007). For sensory data, two panels (2006; 2007) of semi-trained assessors scored fruit purees for sweetness, sourness and flavour intensity. Metabolite data on sugars and organic acids, and for a subset of fruits raspberry ketone contents, were obtained from HPLC. Other data was available: e.g. flavour volatiles contents, oBrix, and 10-berry weights.

The first aim was examine the interrelationships between the three crucial flavour attributes. A second was to relate sensory scoring data to metabolite contents. The third was identifying fruit components that contributed to intensity of each flavour attribute.

Univariate modelling, explored correlations between sensory scorings showing sweetness directly correlated with flavour intensity, inversely to sourness. Sweet:Sour ratio was not significantly correlated to any attribute. ^{o}Brix was a good predictor of both sweetness and flavour intensity.

Partial least square regression related scoring to metabolite contents. Explanation of variance in sweetness and flavour intensity from sugars and organic acids data was encouraging (62%< R2<80%) and enhanced by inclusion of volatiles data. Interestingly raspberry ketone made only marginal contributions. Sourness was not adequately predicted from non-volatiles data. Specific fruit volatiles were significantly correlated with both sweetness and sourness.

III.
Contributing author
Research Article: Molecular Nutrition and Research

Environmental and seasonal influences on red raspberry anthocyanin antioxidant contents and identification of quantitative traits loci (QTL)

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Keywords:
bHLH;bZIP;Marker assisted breeding;NAM;PKS1 (CHS)

Abstract
Consumption of raspberries promotes human health through intake of pharmaceutically active antioxidants, including cyanidin and pelargonidin anthocyanins; products of flavonoid metabolism and also pigments conferring colour to fruit. Raspberry anthocyanin contents could be enhanced for nutritional health and quality benefits utilising DNA polymorphisms in modern marker assisted breeding. The objective was to elucidate factors determing anthocyanin production in these fruits. HPLC quantified eight anthocyanin cyanidin and pelargonidin glycosides: -3-sophoroside, -3-glucoside, -3-rutinoside and -3-glucosylrutinoside across two seasons and two environments in progeny from a cross between two Rubus subspecies, Rubus idaeus (cv. Glen Moy)×Rubus strigosus (cv. Latham). Significant seasonal variation was detected across pigments less for different growing environments within seasons. Eight antioxidants mapped to the same chromosome region on linkage group (LG) 1, across both years and from fruits grown in field and under protected cultivation. Seven antioxidants also mapped to a region on LG 4 across years and for both growing sites. A chalcone synthase (PKS 1) gene sequence mapped to LG 7 but did not underlie the anthocyanin quantitative traits loci (QTL) identified. Other candidate genes including basic-helix-loop-helix (bHLH), NAM/CUC2-like protein and bZIP transcription factor underlying the mapped anthocyanins were identified.

IV.
Contributing author
Research Article: TAG Theoretical and Applied Genetics

Environmental and seasonal influences on red raspberry flavour volatiles and identification of quantitative trait loci (QTL) and candidate genes

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Abstract
Raspberry volatiles are important for perceptions of sensory quality, mould resistance and some have nutraceutical activities. Twelve raspberry character volatiles were quantified, 11 of them in fruit from two seasons, from plants from the Glen Moy 9 Latham mapping pop- ulation growing in both open field and under cover (poly-tunnels). Effects of season and environment were examined for their impact on the content of a-ionone, a-ionol, b-ionone, b-damascenone, linalool, geraniol, benzyl alcohol, (Z)-3-hexenol, acetoin, acetic and hexanoic acids, whilst raspberry ketone was measured in one season. A significant variation was observed in fruit volatiles in all progeny between seasons and method of cultivation. Quantitative trait loci were determined and mapped to six of the seven linkage groups, as were candidate genes in the volatiles pathways.